Contemporary Clinical Neuroscience

S. Hossein Fatemi *Editor*

The Molecular Basis of Autism

Springer

Contemporary Clinical Neuroscience

For further volumes:
http://www.springer.com/series/7678

S. Hossein Fatemi
Editor

The Molecular Basis of Autism

Springer

Editor
S. Hossein Fatemi M.D., Ph.D., FACPsych
Professor, Departments of Psychiatry and
Neuroscience
MMC 392
University of Minnesota Medical School
Minneapolis
Minnesota
USA

Contemporary Clinical Neuroscience
ISBN 978-1-4939-2189-8 ISBN 978-1-4939-2190-4 (eBook)
DOI 10.1007/978-1-4939-2190-4
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2015931385

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

To my father, S. Mehdi Fatemi, and to my family, S. Ali Fatemi, M.D., Naheed Fatemi, Parvin Fatemi, S. Mohammad Fatemi, Neelufaar Fatemi, Maryam Jalali-Mousavi, and my mother, Fatemeh Fatemi

Foreword

The book entitled "The Molecular Basis of Autism" has brought together over 40 autism experts to combine their expertise in defining the biological basis of this severe neurodevelopmental disorder. The book comprehensively describes the latest research on the roles of potential biomarkers (i.e., oxytocin, neurexins, and neuroligins) and classical neurotransmitter systems (i.e., γ-aminobutyric acid (GABA), glutamate, dopamine, acetylcholine, and serotonin) as well as the epidemiology, genetics and epigenetics of autism. Its overall importance may lie in the description of emerging findings related to potential novel treatments and biologically based diagnostic methods. For example, deficits in GABAergic and glutamatergic signaling have been replicated in human and animal experiments. Aberrations in glutamatergic transmission are also described, for instance the increased levels of metabotropic glutamate receptor 5 (mGluR5) that have been observed in subjects with idiopathic autism as well as fragile X syndrome (FXS), and also in a mouse model of FXS. These findings might open new avenues for therapeutic intervention such as the use of modulators for mGluR5. In conclusion, I believe that this collection of chapters will be important to the field of psychiatry as well as neurology, neuroscience, and pharmacology.

Arvid Carlsson, MD, PhD
Göteborg, Sweden

Preface

Autism is a major, debilitating neurodevelopmental disorder with shared genetic and environmental etiologies and a rising prevalence. Since the initial discovery of this disorder by Leo Kanner, there has been an explosion of new knowledge about its etiopathogenesis. *The Molecular Basis of Autism* provides the current state of knowledge about the etiology and treatment of this disorder. In the following twenty chapters, various contributors present up-to-date discussions of the state of knowledge on important aspects of autism. In part one of the book, chapters cover clinical topics of interest such as history of discovery of autism, diagnostic features, epidemiology, genetics, epigenetics, immunology, neuroimaging, neuropathology, pharmacology and behavioral treatment modalities. In part two, chapters deal with involvement of a number of neurotransmitters, proteins, and brain markers that may influence etiopathogenesis of autism such as dopamine, glutamate, serotonin, oxytocin, vasopressin, acetylcholine, Reelin, gamma-aminobutyric acid (GABA), fragile X mental retardation protein (FMRP), neurexins and neuroligins, neurotrophins, as well as novel mechanisms of disease production such as cognition and motor control and oxidative stress and mitochondrial dysfunction in causation of autism. It is hoped that this timely collection of chapters will provide a comprehensive guide for scientists and clinicians regarding the biology of this disorder.

S. Hossein Fatemi, MD, PhD, FACPsych
Minneapolis, Minnesota

Acknowledgments

I am most grateful to Professor Mario Manto for the original proposal to edit this book. I am especially indebted to Mr. Timothy D. Folsom and Ms. Jennifer M. Bredemeier for help with various aspects of editing this book. I am also grateful to the authors who have generously given their valuable time to write the various chapters in this book. Finally, I am thankful to Ms. Simina Calin, Mr. Kevin Wright, Mr. Jacob Rosati, and Ms. Nikita Dhiwar of Springer for the excellent job in publishing this book.

S. Hossein Fatemi, MD, PhD, FACPsych
Minneapolis, Minnesota

Contents

Part II Basic Science Aspects of Autism

Contributors

Schahram Akbarian, M.D., Ph.D., Professor, Department of Psychiatry, Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, Hess Center for Science and Medicine, New York, NY, USA

Evdokia Anagnostou, M.D., Associate Professor, Department of Pediatrics, Bloorview Research Institute, University of Toronto, Toronto, ON, Canada

Paul Ashwood, Ph.D., Professor, Department of Medical Microbiology and Immunology and M.I.N.D. Institute, University of California, Davis, Sacramento, CA, USA

Danielle Baribeau, M.D., Resident, Department of Psychiatry, University of Toronto, Toronto, ON, Canada

Natalia Benza, M.D., Physician, Department of Pediatrics, Children's Hospital of Michigan, Detroit, MI, USA

Charles D. Blaha, Ph.D., Professor, Director of the Division of Experimental Psychology, Department of Psychology, University of Memphis, Memphis, TN, USA

Maria L. Carlsson, Ph.D., Associate Professor, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

C. Sue Carter, Ph.D., Professor, Department of Psychiatry, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Manuel F. Casanova, M.D., Gottfried and Gisela Kolb Endowed Chair in Psychiatry, Vice Chair for Research, Department of Psychiatry and Behavioral Sciences, University of Louisville School of Medicine, Louisville, KY, USA

Abha Chauhan, Ph.D., Head, Developmental Neuroscience Laboratory, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY, USA

Ved Chauhan , Ph.D., Head, Cellular Neurochemistry Laboratory, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY, USA

Sau W. Cheung, Ph.D., M.B.A., Professor, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

Diane C. Chugani, Ph.D., Professor, Department of Pediatrics, Wayne State University School of Medicine; Director, Translational Imaging Laboratory, Division of Clinical Pharmacology and Toxicology, Children's Hospital of Michigan, Detroit, MI, USA

James Dachtler, Ph.D., Visiting Research Fellow, The Leeds Psychosis Research Group, School of Biomedical Sciences, University of Leeds, Leeds, UK

V. Mark Durand, Ph.D., Professor, Department of Psychology, University of South Florida, St. Petersburg, St. Petersburg, FL, USA

S. Hossein Fatemi, M.D., Ph.D., FACPsych, Professor, Associate Chair for Neuroscience and Translational Research, Bernstein Endowed Chair in Adult Psychiatry, Departments of Psychiatry and Neuroscience, University of Minnesota Medical School, Minneapolis, MN, USA

Timothy D. Folsom, M.S., Junior Scientist, Department of Psychiatry, University of Minnesota School of Medicine, Minneapolis, MN, USA

Sunday M. Francis, Ph.D., Postdoctoral Associate, Department of Psychiatry, University of Minnesota, School of Medicine, Minneapolis, MN, USA

Jennifer Goldstein, Ph.D., C.G.C., Clinical Coordinator, Department of Pediatrics, Duke University School of Medicine, Durham, NC, USA

Donald E. Greydanus, M.D., Professor and Founding Chair, Department of Pediatric & Adolescent Medicine, Western Michigan University, School of Medicine, Kalamazoo, MI, USA

Detlef H. Heck, Ph.D., Associate Professor, Department of Anatomy and Neurobiology, University of Tennessee Health Science Center, Memphis, TN, USA

James W. Howell, Ph.D., Assistant Professor, The Urban Child Institute and the Department of Psychiatry, University of Tennessee Health Science Center, Memphis, TN, USA

Suma Jacob, M.D., Ph.D., Associate Professor, Department of Psychiatry, University of Minnesota, School of Medicine, Minneapolis, MN, USA

Yong-hui Jiang, M.D., Ph.D., Assistant Professor, Departments of Pediatrics and Neurology, Duke University School of Medicine, Durham, NC, USA; Department of Child Health Care, The Children's Hospital of Fudan University, Shanghai, P. R. China

Gabriel Kaplan, M.D., D.F.A.P.A., Clinical Associate Professor, Rutgers New Jersey Medical School, Springfield, NJ, USA

Russell S. Kirby, Ph.D., M.S., F.A.C.E., USF Distinguished Professor and Marrell Endowed Chair, Department of Community and Family Health, College of Public Health, University of South Florida, Tampa, FL, USA

Tal Levin-Decanini, Ph.D., Research Fellow, Department of Psychiatry, University of Minnesota, School of Medicine, Minneapolis, MN, USA

Wensheng Liu, M.D., Senior Microscopist, Senior Research Associate, Center for Advanced Microscopy, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

Allyn McConkie, Ph.D., Associate Professor, Department of Pediatrics, Duke University School of Medicine, Durham, NC, USA

Guy Mittleman, Ph.D., Chairman of the Department of Psychological Science, Ball State University, Muncie, IN, USA

Department of Psychological Science, Ball State University, Muncie, IN, USA

Elizabeta B. Mukaetova-Ladinska, M.D., Ph.D., M.R.C.Psych., Clinical Senior Lecturer/Consultant, Institute of Neuroscience, Campus for Ageing and Vitality, Newcastle University, Newcastle upon Tyne, UK

Dilip R. Patel, M.D., M.B.A., Professor, Department of Pediatric & Adolescent Medicine, Western Michigan University School of Medicine, Kalamazoo, MI, USA

Elaine K. Perry, B.Sc., Ph.D., Emerita Professor, Institute of Neuroscience, Campus for Ageing and Vitality, Newcastle University, Newcastle upon Tyne, UK

Cyril J. Peter, Ph.D., Assistant Professor, Department of Psychiatry, Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, Hess Center for Science and Medicine, New York, NY, USA

Goerge M. Realmuto, M.D., Professor, Department of Psychiatry, University of Minnesota School of Medicine, Minneapolis, MN, USA

Amy C. Reichelt, Ph.D., Postdoctoral Research Associate, Department of Psychology, School of Medical Sciences, University of New South Wales, Kensington, Sydney, NSW, Australia

Abraham Reichenberg, Ph.D., Professor, Department of Psychiatry, Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, Hess Center for Science and Medicine, New York, NY, USA

Destanie R. Rose, B.S., Graduate Student, Department of Medical Microbiology and Immunology and M.I.N.D. Institute, University of California, Davis, Sacramento, CA, USA

Angela Sagar, M.D., Psychiatrist, UCLA Health, Los Angeles, CA, USA

Elizabeth M. Sajdel-Sulkowska, D.Sc., Assistant Professor, Department of Psychiatry, Harvard Medical School and Brigham Womens Hospital, Boston, MA, USA

Fred R. Volkmar, M.D., Irving B. Harris Professor, Director, Child Study Center, Department of Psychiatry, Yale University, New Haven, CT, USA

Ping Wang, Ph.D., Postdoctoral Fellow, Department of Pediatrics, Duke University School of Medicine, Durham, NC, USA

Yi Wang, M.D., Ph.D., Professor, Department of Neurology, The Children's Hospital of Fudan University, Shanghai, P. R. China

Xiu Xu, M.D., Ph.D., Associate Professor, Departments of Child Health Care and Neurology, The Children's Hospital of Fudan University, Shanghai, P. R. China

Part I
Clinical Aspects of Autism

Chapter 1
A Brief History of Autism

Fred R. Volkmar

Abstract In the 70 years that have elapsed since the first description of infantile autism considerable progress has been made. The official recognition of the condition in 1980 stimulated further research and around the same time the strong neurobiological and genetic aspects of autism began to emerge. Subsequent research has focused on issues of early diagnosis and intervention, understanding the neural mechanisms of autism, and approach to treatment. Unfortunately the research base varies considerably by age group with much research on younger individuals and rather little on adults—particularly older adults. Recent changes in diagnostic practice may complicate the interpretation of past, and future, research.

Keywords Autism · Autism spectrum · DSM-IV · DSM-5 · Diagnosis

1.1 A Brief History of Autism

Since its initial description in 1943 (Kanner 1943) interest in, and research on, autism has increased dramatically. In the 20 years following Kanner's classic description fewer than 50 peer reviewed papers on the topic appeared, but they increased gradually and then dramatically between 1973 and 1982 over 1000 papers appeared, and between 2003 and 2012 with well over 10,000 papers were published. This dramatic rise reflects several factors including early confusion about the diagnostic validity of autism (particularly apart from childhood schizophrenia), confusion about social class association and etiology and confusion about basic aspects of phenomenology. In this chapter I succinctly review the development of autism as a diagnostic concept, advances in research and treatment, and social and public policy issues. It is, of course, important to understand that all these issues are inter-connected, e.g., advances in treatment may have important policy implications which in term may impact research. As noted subsequently a range of materials on autism are now available (including on line reference works and lectures); the interested reader should review the original sources. An excellent review of the history of autism is provided by Adam Feinstein in his recent book (Feinstein 2010).

F. R. Volkmar (✉)
Child Study Center, Yale University, PO Box 207900, New Haven, CT 06520, USA
e-mail: fred.volkmar@yale.edu

S. Hossein Fatemi (ed.), *The Molecular Basis of Autism,*
Contemporary Clinical Neuroscience, DOI 10.1007/978-1-4939-2190-4_1

1.2 Development of Autism as a Diagnostic Concept

Kanner's work was a remarkably accurate description of the condition we know today as autism (or autism spectrum disorder) and continues to impact approaches to diagnosis as well as to research and treatment. As the first child psychiatrist in the U.S. Kanner reported 11 cases where the child seemed to come into the world with an inability to relate to other people. Kanner regarded this as one of two essential features of the condition and emphasized the child's lack of social interest in his use of Bleuler's (1911) earlier term for autistic (self-centered) thinking in schizophrenia (the use of this term also, unfortunately immediately introduced considerable confusion about the relationship of infantile autism and schizophrenia). Kanner was careful to site the work of Arnold Gesell (Gesell and Ilg 1943) on normative social development to highlight the significance of the social isolation. In addition to 'autistic aloneness' Kanner emphasized a second core feature which he termed insistence on sameness (or resistance to change). This referred to the often marked difficulties children with autism had in dealing with change in the environment. Over time the concept has come to include stereotyped motor mannerisms (presumed to help the child maintain sameness) as well as the unusual interests and idiosyncratic responses observed. In retrospect it was, and remains, quite striking that in many ways the child's lack of interest in the social world was so very different from the over engagement in the nonsocial world (a topic that has become an increasing focus of research).

In addition to the confusion caused by Kanner's use of the word autism his otherwise excellent initial description also included a few observations that mislead early investigators. For example, he suggested that there was apparently normal intellectual potential (given performance on some—usually nonverbal—parts of IQ tests), that other medical conditions were not apparently present (given the lack of obvious physical stigmata and attractive appearance of the children), and, in his original report, there appeared to be a strong association with social class (most cases coming from families where a parent was highly successful). A considerable amount of time was spent in the 1950's and 1960's correcting these misimpressions.

It became apparent that the profile on psychological testing in autism was unusual for marked scatter with nonverbal skills being relatively preserved but verbal abilities are often highly deficient and overall IQ scores frequently in the intellectually deficient range (Goldstein et al. 2009). As children with autism were followed over time it became clear that they were at markedly increased risk for epilepsy (Volkmar and Nelson 1990) and that autism was associated with some medical disorders particularly Fragile X syndrome and tuberous sclerosis (Rutter et al. 1994). Finally the association with socio-economic status of families came to be viewed as an aspect of referral bias (Wing 1980) and today there is recognition that apparent ethnic/racial disparities are likely to reflect under-diagnosis in disadvantaged populations bias against use of the label (Mandell et al. 2009). Probably most importantly a series of studies in the U.K. (Kolvin 1971; Rutter 1972) made it clear that autism was a distinctive disorder and differed from childhood schizophrenia in

terms of its onset, clinical features, and associated family history. By the late 1970s it was clear that autism was a distinctive disorder and attempts were made to develop better approaches to diagnosis. Rutter's approach (Rutter 1978) emphasized delayed social and language development (not just due to any overall developmental delay) along with unusual interests/behavior with an onset before 30 months of age. The National Society for Autistic Children (NSAC) NSAC definition (NSAC 1978) emphasized other aspects of the condition (disturbances in rates/sequences of development, hyper/hyposensitivity).

As a result of these advances a decision was made to include autism (as "infantile autism") in the landmark 3rd edition of the Diagnostic and Statistical Manual (DSM-III) (APA 1980). In DSM-III a new term, Pervasive Developmental Disorder, was coined for the class of disorder to which autism was assigned. In addition to autism other concepts were also included, e.g., 'residual' infantile autism for individuals who once met criteria but no longer did so. While including autism as an officially recognized condition was a distinct advance many aspects of the approach chosen were problematic (see Volkmar and Klin 2005), e.g., the definition lacked a strong developmental orientation and the notion that people 'grew out' of infantile autism to 'residual' autism was problematic. As a result many changes were adopted in DSM-III-R including a stronger developmental orientation (Volkmar and Klin 2005). This approach had its own problems—particularly with over-diagnosis of autism among the more cognitively impaired (Volkmar et al. 1992).

DSM-IV appeared in 1994 and for autism a large international field trial was conducted in conjunction with the pending revision of the international classification of diseases (ICD-10) (Volkmar et al. 1994). The DSM-IV approach included social, communication, and restricted interests/behaviors criteria, was polythetic, and did a better job of diagnosis autism regardless of IQ. The DSM-IV/ICD-10 approach also recognized explicitly a number of other PDD subtypes (Asperger's, Rett's, Childhood Disintegrative Disorder, and subthreshold autism). The convergence of DSM-IV with ICD-10 likely significantly advanced research.

DSM-5 has appeared in 2013 (APA 2013). It has adopted a number of changes including a new overarching diagnostic concept—autism spectrum disorder, eliminating previous subtypes such as Asperger's, and using criteria derived from research diagnostic assessment instruments. DSM-5 has proven unusually controversial as it appears that some individuals will 'lose' their diagnosis complicating both service provision and research (Volkmar and Reichow 2013). The final impact of DSM-5 remains unclear although a potential lack of convergence with ICD-10 seems problematic.

1.3 Related Diagnostic Concepts

Both before, and after, Kanner's classic description of autism in 1943, other clinician-researchers described diagnostic constructs with some points of similarity to autism. Indeed it is likely that cases of autism may first have appeared in some of

the descriptions of so-called "feralwild" or "feral" children (thought to have been raised by animals) and indeed it has now been recognized that in addition severe deprivation can also be associated with 'autistic like' behaviors (Rutter et al. 1999; Wolff 2004). Some of these disorders have been officially recognized while the validity of others remains debated. In general their major point of similarity to Kanner's autism has to do with the presence of severe social disability.

In 1908 the Viennese special educator Theodor Heller described children who developed normally for some years (typically 3 to 4) but then had a marked regression with the development of what now would be viewed as an autistic-like clinical syndrome (see Heller 1908, 1930) and for an English translation see (Westphal et al. 2013). His original term for the condition "dementia infantilis" reflected the then common notion of regression being a form of dementia; subsequently the term "disintegrative psychosis" was used. We now know (Volkmar et al. 2005) that the condition differs from dementia in that the loss of skills is not progressive and that it is not a psychosis (the latter term arose in the context of broad views of childhood psychosis current in the first decades of the twentieth century (Volkmar and Tsatsanis 2002). Although it resembles autism once it is established the regression is highly unusual, much later than is typical in autism, and the outcome, sadly, is worse (see Volkmar et al. 2005). While included in DSM-IV the condition has been dropped from DSM-5 complicating potential research.

In 1966 the neurologist Andreas Rett (1966) observed a group of girls who exhibited some autistic features as well as unusual motor mannerisms (peculiar hand washing stereotypies), marked loss of skills, breathing difficulties, and orthopedic problems. Although he originally speculated that this condition was an autism variant in fact the 'autistic-like period' (of relative social withdrawal) is relatively brief and confined largely to the preschool years (Van Acker et al. 2005). This condition, recognized in DSM-IV but dropped from DSM-5 has now been shown to be due, in general, to the presence of a specific gene defect (Van Acker et al. 2005). Apart from its clinical significance the research significance of the condition has arisen from the potential for animal models (via knock-out approaches) for this disorder.

In 1944 Hans Asperger, then a Viennese medical student, described a group of boys with marked social vulnerabilities and motor problems who also exhibited unusual specialized interest (that interfered with functioning); well-developed language (though not good social-communication) skills (Asperger 1944). Unaware of Kanner's 1943 report, Asperger termed the condition autistic psychopathy (or, in probably a better translation, autistic personality disorder) using the word autism as had Kanner to convey marked social vulnerability. This condition received little attention until Lorna Wing's (1981) influential review and case series. Subsequently interest increased and data from the DSM-IV field trial was used, in part, to support inclusion of the condition as one of the pervasive developmental disorders.

Unfortunately the definition adopted was not ideal and problems were quickly noted (Miller and Ozonoff 1997). Subsequent work has noted the importance of including features like circumscribed interests and motor delays if robust patterns of difference are to be observed (Klin et al. 2005). The unfortunate decision in DSM-IV to adopt a precedence rule (i.e. that a diagnosis of autism should take

precedence) also created problems (Miller and Ozonoff 1997) and could have been remedied had clinicians been allowed to choose the more appropriate diagnosis. Although including Asperger's in DSM-IV markedly increased research in the area, the lack of consensus on definition has led to a proliferation of views on diagnosis (Sharma et al. 2012) leading to difficulties in interpretation of research. These difficulties also have contributed to the decision not to include Asperger's in DSM-5 see (Lord and Jones 2012). This seems unfortunate given the potential for significant research and clinical implications of the condition, e.g., relative to differences from autism in neuropsychological profile (with implications for treatment; Klin et al. 1995; Lincoln et al. 1998), associations with other psychiatric problems and differences in family psychiatric history (Klin et al. 2005) and in outcome (Szatmari et al. 2003).

Atypical Autism/PDD-NOS: Despite the change in name from Pervasive developmental disorder to Autism Spectrum Disorder and despite the marked increase in the 'broader autism spectrum' (reflected in the media and epidemiological studies) research on this condition (or group of conditions) has remained limited (Towbin 2005).

1.4 Advances in Research

The mistaken belief in psychological causation of autism (and the confusion with childhood schizophrenia) during the 1950's and 1960's significantly impeded research. This situation began to change in the 1970's. Work on the phenomenology of autism in England by Kolvin (1971) and Rutter (1972) strongly supported the validity of the category. In addition the strong brain basis of autism began to be appreciated, e.g., given the high rates of seizure disorder observed with onset both early in life and in adolescence (Volkmar and Nelson 1990) and the very strong genetic basis of the condition (revealed in the first twin study (Folstein and Rutter 1977). Parents, some of whom also became early researchers, were critical of this effort. For example, Bernard Rimland authored an influential early book (1964) arguing for a neurobiological approach to understanding (Rimland 1964) and studies using his first diagnostic instrument (Rimland 1971) also supported the validity of autism as different from schizophrenia. Early work on intervention began to show the importance of highly structured educational program (Bartak and Rutter 1973). Early research on intervention also revealed the importance of structured treatment programs (Bartak and Rutter 1973) and the role of behavioral, rather than psychodynamic, interventions (Ferster 1989; Lovass and Smith 1988). Early research on the neurobiology of autism centered on different systems including various brain systems (Ornitz and Ritvo 1976) and neurochemical correlates, particularly relative to the neurotransmitter Serotonin (Hanley et al. 1977). In addition a range of pharmacological interventions were evaluated by Campbell and colleagues at NYU (Campbell 1975). The first Journal, in the field the Journal of Autism and Developmental Disorders, was established in 1971 with Kanner as the initial editor. Over

time research interest has dramatically increased with over 2000 peer reviewed publications on an annual basis at present and with a number of other journals now focused on autism.

1.5 Treatments

The earliest efforts to employ a psychotherapeutic intervention model for autism (Bettelheim 1967) gave way to more structured educational intervention programs (Bartak and Rutter 1973). It became clear that structured behaviorally based intervention had a particular role in building skills and helping the child generalize and maintain skills learned. Over the last several decades a range of approaches have been developed (see National Research Council 2001 for an excellent summary). These include more behaviorally based programs using applied behavior analysis, to more developmentally modeled programs, e.g., the Denver model developed by Rogers (1996) as well as other programs that combine aspects of both the developmental and behavioral approaches (Koegel et al 2001). Still other programs are more eclectic and designed to address the specific learning styles seen in individuals with autism (Schopler et al. 1995). Many of these programs have now become strongly evidence based (Reichow et al. 2011).

To briefly summarize a complicated literature on the theoretical basis of treatment, it has become clear that, in essence, lacking the social 'frame' that most infants have, those with autism develop idiosyncratic and nonsocially focused ways of learning about the world. As a result they have very different styles of learning reflected, early in life, by difficulties in early emerging language skills (prosody, social language, rule taking) and then by delayed vocabulary and language acquisition with an unusual 'gestalt' style of learning (reflected in the tendency to echo heard language and in the use of idiosyncratic language (Schoen et al. 2011; Volkmar and Wiesner 2009). Similarly unusual interests and preoccupations (presumably also arising because of lack of primary social orientation) can pose obstacles for learning and are reflected in other differences in learning style (Volkmar and Wiesner 2009). There has been an increased awareness of the importance both of early diagnosis for ultimate outcome (National Research Council 2001) and of peer exposure and the importance of a focus on social skills (Prendeville et al. 2006) and in mainstreaming in the acquisition of social skills as well as supporting teachers and parents as they, in turn, support the child's learning (Venn et al. 1993). On balance, with earlier intervention and more sustained and targeted intervention outcome apparently has gradually improved with more individuals with autism now able to be independent and self-sufficient (Volkmar and Wiesner 2009) although sadly research on older individuals and appropriate treatment programs to assist them have been minimal. For some individuals, even with good programs from early in life, outcome remains poor (Howlin 2005). It should also be noted that although more interventions can now be regarded as evidence based there are many complementary and alternative treatments in frequent use (Volkmar and Wiesner 2009). Issues in the use of such

treatments become particularly complex when there is some risk to the child (either directly in terms of safety or in terms of removing the child from treatments shown to be effective).

1.6 Research Directions

As noted previously early research efforts were impeded by a lack of consensus on diagnosis. As this began to change in the late 1970's more useable research began to appear. Parents and advocacy groups also formed to support research. As with other areas of medicine and behaviour much of the significant research conducted has been in the context of college/university settings with their strong commitment both to research and training. As noted above there has been a major increase in the research enterprise within the U.S. and abroad based both on an increased governmental commitment, strong support from advocacy groups, and, particularly in the U.S., the engagement of philanthropy (Autism Speaks, the Autism Science Foundation, and the Simons foundation have been notable contributors in this regard). Important obstacles and gaps in research remain, e.g., the more or less total absence of work on aging in autism and the minimal amounts of work on intervention for adolescents and adults. Treatment research has been particularly challenging to fund. On the other hand advances in the basic research on genetic factors (Abrahams and Geschwind 2008) and in our understanding of the social brain (McPartland and Pelphrey 2012) have been very significant. This basic research is beginning to be joined with treatment research, e.g., in studies showing brain changes in response to treatments (Voos et al. 2012). Research on pharmacological interventions has also significantly advanced including new work focused on the core social disability (Scahill and Martin In Press). The multisite study of risperidone has served as an excellent example of collaborative work that established the efficacy of this agent (McCracken et al. 2002).

1.7 Social Policy Issues

The predominant model of intervention in the 1950's was psychodynamically based. Public schools often declined to provide educational programs for children with autism despite the growing body of work on the impact of special education and behavioral treatments. This situation markedly changed with advent of Public Law 94–142 which mandated, in the U.S., that schools receiving federal funding had to provide services to students with disabilities—including autism (see National Research Council 2001). Other important social policy advances included the organization in the U.S. and U.K. of national parent advocacy groups. These groups advocated for research as well as for treatment programs. This work culminated in the 1990's with the establishment of a number of federal programs supporting network

of investigators. These included the Centers of Excellence for Programs in Autism (CPEA) led through the National Institute of Child Health and Human Development by then Director Dwayne Alexander and program officer Marie Bristol-Power and were then succeeded by support from multiple NIH institutes. Subsequent efforts have included the Studies to Advance Autism Research and Treatment (STAART) and Autism Center of Excellence Programs. The impact of these initiatives both on research and well trained investigators has been enormous.

Despite the clear role of legal entitlements to education, policy issues regarding school intervention program and appropriate practice continue to be addressed. In the U.S. the entitlement to education is now codified as IDEA (Individuals with Disabilities Education Act) which mandates free appropriate public education (FAPE) to children with a range of disabilities (Mandlawitz 2005). Follow-up federal legislation and judicial decisions have impacted the ways the IDEA is implemented. In addition another statute, the Americans with Disabilities Act of 1990, can apply to children as well as to adolescents and adults (particularly for young adult in college where it mandates against discrimination). On the other hand a source of confusion is that this law mandates supports when appropriate but does not establish program participation as an absolute right, e.g., in college (Wolff et al. 2009). It is important to note that decisions about intervention also have important economic implications with major expenditures in the U.S. and U.K. which might be minimized by improved diagnosis and treatment and better functional outcome (Knapp et al. 2009).

Interestingly some areas of work relevant to policy have received comparatively much less attention in the literature. Cultural aspects of autism remain an area where little work has been done. The issues here seem less to have to do with the presentation of autism (which likely is rather similar around the world) but with philosophies about intervention (Brown and Rogers 2003); study of ethnic and cultural issues is a priority as is the study of potential differences in presentation based on gender and socio-economic status. There is some suggestion that, in the U.S., children living in poverty, may be more likely under-diagnosed. (Mandell et al. 2009)

1.8 Training and Dissemination of Knowledge

As noted above the strong connection of research (and to some extent treatment) efforts to University settings has had a fortunate impact in developed countries of increasing the number of individuals trained in methods of research and knowledge about autism. Over time this work has been disseminated in various ways including new approaches in recent years including the internet (http://www.youtube.com/course?list=EC27FAF837577D180A) with some publications now including hypertext links to more rapidly update information and expand the range of knowledge rapidly available (Volkmar 2012).

1.9 Summary and Challenges for the Future

Although much progress has been made, many areas of challenge remain. From the research side the potential for connecting genetic and brain mechanisms is now on the horizon (Kaiser et al. 2010) and has the potential to significantly advance treatment research. The advent of specific genetic mechanisms will also allow for development of much more sophisticated animal models. New approaches to early diagnosis may facilitate more rapid screening—relevant particularly to at risk siblings. Similarly the ability to observe brain and other changes in response to treatment programs may help clarify the important issue of understanding which children do, and do not respond to various treatments.

Challenges remain. Particularly in the current economic climate, research funds are limited. Much of the research work has focused on young children with adolescent and adult research receiving short shrift. Given the potential for helping many young people become productive and self-sufficient adults this is especially unfortunate. Even for school age children important questions remain about best approaches to adopting treatments relevant to the specific child. Considerable variability exists in the U.S. between (and sometimes within) states despite overall Federal mandates.

Acknowledgements The author acknowledges the support of the Simons Foundation, the Associates of the Child Study Center, and the Yale Clinical and Tranlational Science Award (CTSA).

References

Abrahams BS, Geschwind DH (2008) Advances in autism genetics: on the threshold of a new neurobiology. Nat Rev Genet 9:341–355

Asperger H (1944) Die “autistichen Psychopathen” im Kindersalter. Arch Psychiatr Nervenkrankh 117:76–136

American Psychiatric Association (1980) Diagnostic and statistical manual, 3rd edn. American Psychiatric Association, Washington, D.C

American Psychiatric Association (2013) Diagnostic and statistical manual of mental disorders, 5th edition: DSM-5. American Psychiatric Association, Washington, D.C.

Bartak L, Rutter M (1973) Special educational treatment of autistic children: a comparative study. 1. Design of study and characteristics of units. J Child Psychol Psychiatry 14:161–179

Bettelheim B (1967) The empty fortress: infantile autism and the birth of the self. Free Press of Glencoe, Oxford, England

Bleuler E (1911) Dementia praecox oder Gruppe der Schizophrenien. (trans: Zinkin J) International Universities Press, New York

Brown JR, Rogers SJ (2003) Cultural Issues in autism. In: Hendren RL, Ozonoff S, Rogers S (eds) Autism spectrum disorders. American Psychiatric Press, Washington, DC, pp 209–226

Campbell M (1975) Pharmacotherapy in early infantile autism. Biol Psychiatry 10:399–423

Feinstein A (2010) A history of autism. John C. Wiley, Hoboken

Ferster CB (1989) Positive reinforcement and behavioral deficits of young children. Focus Autistic Behav 4:1–12

Folstein S, Rutter M (1977) Infantile autism: a genetic study of 21 twin pairs. J Child Psychol Psychiatry 18:297–321
Gesell A, Ilg FG, Learned J, Ames LB (1943) The infant and child in the culture of today: the guidance of development in home and nusery school. Harper & Brothers, New York
Goldstein S, Naglieri JA, Ozonoff S (2009) Assessment of autism spectrum disorders. Guilford Press, New York
Hanley HG, Stahl SM, Freedman DX (1977) Hyperserotonemia and amine metabolites in autistic and retarded children. Arch Gen Psychiatry 34:521–531
Heller T. (1908) Dementia Infantilis. Z Erforsch Behandl Jugenlichen, Schwachsinns 2:141–165
Heller, T. (1930) Uber Dementia infantalis. Z Kinderforsch 37:661–667
Howlin P (2005) Outcomes in autism spectrum disorders. In: Volkmar FR, Klin A, Paul R, Cohen DJ (eds) Handbook of autism and pervasive developmental disorders, 3rd edn. Wiley, Hoboken, pp 201–222
Kaiser MD, Hudac CM, Shultz S, Lee SM, Cheung C, Berken AM, Deen B, Pitskel NB, Sugrue DR, Voos AC, Saulnier CA, Ventola P, Wolf JM, Klin A, Vander Wyk BC, Pelphrey KA (2010) Neural signatures of autism. Proc Natl Acad Sci U S A 107:21223–21228
Kanner L (1943) Autistic disturbances of affective contact. Nerv Child 2:217–250
Klin A, Volkmar FR, Sparrow S, Cicchetti D, Rourke BF (1995) Validity and neuropsychological characterization of Asperger syndrome: convergence with nonverbal learning disabilities syndrome. J Child Psychol Psychiatry 36:1127–1140
Klin A, Pauls D, Schultz R, Volkmar FR (2005) Three diagnostic approaches to asperger syndrome: implications for research. J Autism Dev Disord 35:221–234
Knapp M, Romeo RE, Beecham J (2009) Economic cost of autism in the UK. Autism 13:317–336
Koegel RL, Koegel LK, McNerney EK (2001) Pivotal areas in intervention for autism. J Clin Child Psychol 30:19–32
Kolvin I (1971) Studies in the childhood psychoses. I. Diagnostic criteria and classification. Br J Psychiatry 118:381–384
Lincoln A, Courchesne E, Allen M, Hanson E, Ene M (1998) Neurobiology of Asperger Syndrome: seven case studies and quantitative magnetic resonance imaging findings. In: Schopler E, Mesibov GB, Kunc LJ (eds) Asperger syndrome or high functioning autism? Plenum, New York, pp 145–166
Lord C, Jones RM (2012) Annual research review: re-thinking the classification of autism spectrum disorders. J Child Psychol Psychiatry 53:490–509
Lovass O, Smith T (1988) Intensive behavioral treatment for young autistic children In: Lahey BB, Kazdin AE (eds) Advances in clinical child psychology, vol 11. Springer US, New York, pp 285–324
Mandell DS, Wiggins LD, Carpenter LA, Daniels J, DiGuiseppi C, Durkin MS, Giarelli E, Morrier MJ, Nicholas JS, Pinto-Martin JA, Shattuck PT, Thomas KC, Yeargin-Allsopp M, Kirby RS (2009) Racial/ethnic disparities in the identification of children with autism spectrum disorders. Am J Public Health 99:493–498
Mandlawitz MR (2005) Educating children with autism: current legal issues. In: Volkmar FR, Klin A, Paul R, Cohen DJ (eds) Handbook of autism and pervasive developmental disorders, 3rd edn. Wiley, Hoboken, pp 1161–1173
McCracken JT, McGough J, Shah B, Cronin P, Hong D, Aman MG, Arnold LE, Lindsay R, Nash P, Hollway J, McDougle CJ, Posey D, Swiezy N, Kohn A, Scahill L, Martin A, Koenig K, Volkmar F, Carroll D, Lancor A, Tierney E, Ghuman J, Gonzalez NM, Grados M, Vitiello B, Ritz L, Davies M, Robinson J, McMahon D, Research Units on Pediatric Psychopharmacology Autism Network (2002) Risperidone in children with autism and serious behavioral problems. N Engl J Med 347:314–321
McPartland JC, Pelphrey KA (2012) The implications of social neuroscience for social disability. J Autism Dev Disord 42:1256–1262
Miller JN, Ozonoff S (1997) Did Asperger's cases have Asperger disorder? A research note. J Child Psychol Psychiatry 38:247–251

National Research Council (2001) Educating young children with autism. National Academy Press, Washington, DC
NSAC (1978) National society for autistic children definition of the syndrome of autism. J Autism Child Schizophr 8:162–169
Ornitz EM, Ritvo ER (1976) The syndrome of autism: a critical review. Am J Psychiatry 133:609–621
Prendeville JA, Prelock PA, Unwin G (2006) Peer play interventions to support the social competence of children with autism spectrum disorders. Semin Speech Lang 27:32–46
Reichow B, Doehring P, Cicchetti DV, Volkmar FR (2011) Evidence-based practices and treatments for children with autism evidence-based practices and treatments for children with autism. Springer Science, New York
Rett A (1966) Uber ein eigenartiges hirntophisces Syndroem bei hyperammonie im Kindersalter. Wein Med Wochenschr 118:723–726
Rimland B (1964) Infantile autism: the syndrome and its implications for a neural theory of behavior. Appleton-Century-Crofts, New York
Rimland B (1971) The differentiation of childhood psychoses: an analysis of checklists for 2218 psychotic children. J Autism Child Schizophr 1:161–174
Rogers SJ (1996) Brief report: early intervention in autism. J Autism Dev Disord 26:243–246
Rutter M (1978) Diagnosis and definition of childhood autism. J Autism Child Schizophr 8:139–161
Rutter M, Bailey A, Bolton P, Le Couteur A (1994) Autism and known medical conditions: myth and substance. J Child Psychol Psychiatry 35:311–322
Rutter M, Andersen-Wood L, Beckett C, Bredenkamp D, Castle J, Groothues C, Kreppner J, Keaveney L, Lord C, O'Connor TG (1999) Quasi-autistic patterns following severe early global privation. English and Romanian Adoptees (ERA) Study Team. J Child Psychol Psychiatry 40:537–549
Rutter M (1972) Childhood schizophrenia reconsidered. J Autism Child Schizophr 2:315–337
Scahill L, Martin A (In Press) Psychopharmacology In: Volkmar FR, Pelphrey K, Paul R, Rogers S (eds) Handbook of autism, 4th edn., vol 2. John C. Wiley, Hoboken
Schoen E, Paul R, Chawarska K (2011) Phonology and vocal behavior in toddlers with autism spectrum disorders. Autism Res 4:177–188
Schopler E, Mesibov GB, Hearsey K (1995) Structured teaching in the TEACCH system In: Schopler E, Mesibov GB (eds) Learning and cognition in autism. Springer US, New York, pp 243–268
Sharma S, Woolfson LM, Hunter SC (2012) Confusion and inconsistency in diagnosis of Asperger syndrome: a review of studies from 1981 to 2010. Autism 16:465–486
Szatmari P, Bryson S, Boyle M, Streiner D, Duku E (2003) Predictors of outcome among high functioning children with autism and Asperger syndrome. J Child Psychol Psychiatry 44:520–528
Towbin KE (2005) Pervasive developmental disorder not otherwise specified. In: Volkmar FR, Klin A, Paul R, Cohen DJ (eds) Handbook of autism and pervasive developmental disorders, 3rd edn., vol 1. Wiley, Hoboken, pp 165–200
Van Acker R Loncola JA Van Acker EY (2005) Rett syndrome: a pervasive developmental disorder. In Volkmar FR, Klin A, Paul R, Cohen DJ (eds) Handbook of autism and pervasive developmental disorders, 3rd edn., vol 1. Wiley, Hoboken, pp 126–164
Venn ML, Wolery M, Werts MG, Morris A, DeCesare LD, Cuffs MS (1993) Embedding instruction in art activities to teach preschoolers with disabilities to imitate their peers. Early Child Res Q 8:277–294
Volkmar, FR (2012) Encyclopedia of autism. Springer, New York
Volkmar FR, Klin A (2005) Issues in the classification of autism and related conditions. In: Volkmar FR, Klin A, Paul R, Cohen DJ (eds) Handbook of autism and pervasive developmental disorders, 3rd edn., vol 1. Wiley, Hoboken, pp 5–41
Volkmar FR, Nelson DS (1990) Seizure disorders in autism. J Am Acad Child Adolesc Psychiatry 29:127–129
Volkmar FR, Reichow B (2013) Autism in DSM-5: progress and challenges. Mol Autism 4:13

Volkmar FR, Tsatsanis K (2002) Psychosis and psychotic conditions in childhood and adolescence. In: Marsh DT, Fristad MA (eds) Handbook of serious emotional disturbance in children and adolescents. Wiley, New York, pp 266–283
Volkmar FR, Wiesner L (2009) A practical guide to autism. Wiley, Hoboken
Volkmar FR, Cicchetti DV, Bregman J, Cohen DJ (1992) Three diagnostic systems for autism: DSM-III, DSM-III-R, and ICD-10. J Autism Dev Disord 22:483–492
Volkmar FR, Klin A, Siegel B, Szatmari P, Lord C, Campbell M, Freeman BJ, Cicchetti DV, Rutter M, Kline W, Buitelaar J, Hattab Y, Fombonne E, fuentes J, Werry J, Stone W, Kerbeshian J, Hoshino Y, Bregman J, Loveland K, Szymanski L, Towbin K (1994) Field trial for autistic disorder in DSM-IV. Am J Psychiatry 151:1361–1367
Volkmar FR, Koenig K, State M (2005) Childhood Disintegrative Disorder. In: Volkmar FR, Klin A, Paul R, Cohen DJ (eds) Handbook of autism and pervasive developmental disorders, 3rd edn, vol 1. Wiley, Hoboken, pp 70–78
Voos AC, Pelphrey KA, Tirrell J, Bolling DZ, Vander Wyk B, Kaiser MD, McPartland JC, Volkmar FR, Ventola P (2012) Neural mechanisms of improvement in social motivaiton after pivotal response treatment: two case studies. J Autism Dev Disord 43:1–10
Westphal A, Schelinsk S, Volkmar F, Pelphrey K (2013) Revisiting regression in autism: Heller's dementia infantilis. J Autism Dev Disord 43:265–271
Wing L (1980) Childhood autism and social class: a question of selection? Br J Psychiatry 137:410–417
Wing L (1981) Asperger's syndrome: a clinical account. Psychol Med 11:115–129
Wolff LE, Brown JT, Bork GR (2009) Students with Asperger syndrome: a guide for college personnel. Autism Asperger Publishing Company, Overland Park, KS
Wolff S (2004) The history of autism. Eur Child Adoles Psychiatry 13:201–208

Websites

http://www.autismsciencefoundation.org
http://www.autismspeaks.org
http://simonsfoundation.org
http://www.youtube.com/course?list=EC27FAF837577D180A

Chapter 2

Autism Spectrum Disorder: Diagnostic Considerations

Goerge M. Realmuto

Abstract DSM-5 is a departure from previous diagnostic formats. Changes in emphasis are to be found in the salience of selected key symptoms. A more significant departure are the modifiers that can be used to express severity of selected groups of symptoms. Additionally specifiers and associated disorders including medical and genetic disorders should be included as part of an overall diagnostic picture.

Keywords Diagnosis · Differential diagnosis · Symptoms

Autism as a stand-alone diagnostic entity is no more. Diagnostic and Statistical Manual 5 eliminates Autism as a discrete diagnostic entity and consistent with other neurodevelopmental disorders that exist within a severity range, DSM-5 creates a continuum. Autism spectrum Disorder (ASD) (American Psychiatric Association 2013; Volkmar and Reichow 2013). The essential ingredients of the original concept, now sixty years old, of Kanner's Autistic Disturbances of Affective Contact (Kanner 1943), deficits of social-emotional reciprocity, communication/play and the narrow interests of the individual with Autism remain as part of DSM-5. What distinguishes DSM-5 from its predecessor is the constriction of the richness of the menu of criteria (Dickerson Mayes et al. 2013). Importantly, the number of criteria was reduced from three to two, focusing upon social communication deficits and restricted, repetitive behaviors. Another change loosened the age of onset to the early developmental period from a disorder that must start before age three. Finally to better capture the focus of the range of severity the DSM-5 adds a symptom severity scale.

G. M. Realmuto (✉)
Department of Psychiatry, University of Minnesota School of Medicine, 2450 Riverside Ave., Minneapolis, MN 55454, USA
e-mail: Realm001@umn.edu

S. Hossein Fatemi (ed.), *The Molecular Basis of Autism,*
Contemporary Clinical Neuroscience, DOI 10.1007/978-1-4939-2190-4_2

2.1 DSM-IV Transitions to DSM-5

The category of pervasive developmental disorder (PDD-NOS) in DSM-IV included the previous separate disorders for which the following names were given: infantile autism, childhood autism, Kanner's autism, high-functioning autism, atypical autism, pervasive developmental disorder NOS, Childhood Disintegrative Disorder and Asperger's disorder (American Psychiatric Association 2000; Gibbs et al. 2012). The notion that these were discrete disorders began to unravel as the distinctions between PDD-NOS and for example, Asperger's eroded. Reflecting the porosity of boundaries and with the literature now replete with ASD studies, DSM-5 followed suit.

2.2 DSM-5 Criteria

DSM-5 has two main criteria sets: Diagnostic "A" criterion contains both deficits in social communication and social interaction. The disturbance in social relatedness includes marked impairment in non-verbal communication, peer relationships and social-emotional reciprocity. Impairments in communication include either a delay or total lack of spoken language (without an attempt to compensate through other means) or, for verbal individuals, a marked difficulty in the ability to sustain or initiate conversation, stereotyped and repetitive (or idiosyncratic) language and lack of developmentally-appropriate make-believe or social play. There are three items with descriptors around the items. The first of these: social-emotional reciprocity is conceived as a range from failure of the reciprocal behavior at one end of the range to a reduced quantity and quality of reciprocal behavior at the other end. The second item, nonverbal communicative behavior spans poor eye contact to the absence of nonverbal communication. Third is the area of social relationships. Here the range includes awkwardness of interaction to absence of peer interest. All three are required to fulfill "A" criterion. To capture the range of dysfunction for criterion "A" the severity scale from "1" denoting support required to and "3" substantial support such as the presence of an specialized caretaker should be applied.

Criterion B has four items that describe the behaviors of ASD. The first is repetitive motor stereotypies, repetitive use of objects, or repetitive speech. Echolalia, stereotypies and lining up toys are examples. The second focuses on routines and rituals. The behaviors could be verbal such as greeting rituals, or thinking patters such as expecting furniture to remain exactly in place or simply changing from one behavior to another as is required in transitioning from one play activity to another. The third is overvalued interest in an object, sensation or activity with a significant disinterest in other possible stimuli. Collecting every version of the same song for example shows the narrowness of the person's interest. Lastly is sensory abnormalities that can vary from hyper to hypo-reactive. The variety of behaviors associated with sensory experiences with, for example, water play that might be due to the

fascination with the temperature or the way light plays on it or the characteristics of liquids demonstrates the innumerable variations to be considered for this item. Compared to "A" criterion that requires every item to be satisfied, only two of these four items must be met for a total of six items. As with Criterion "A" a severity level should be applied that specifies whether low (level one) to high (level three) support is appropriate.

Criterion C changes the expected onset to be identified to the early developmental period as noted above. Criterion D like all disorders must be accompanied by clinically significant functional impairment. And finally, criterion E is the exclusion criterion that eliminate ASD if another disorder better describes the findings. The most common example of this would be global developmental delay which would include motor and/or sensory delays as part of the total presentation.

In addition to the specification of severity, other specifies are now part of the DSM-5 diagnostic package (McPartland et al. 2012). Instead of indicating cognitive impairment on Axis 2, the ASD diagnosis will include the presence or absence of accompanying intellectual impairment. DSM-5 has done away with the multiaxial system. The place for this common comorbid problem finds its way as a modifier of ASD. Although social communication can be severely delayed in ASD, a separate diagnostic item is no longer part of the criteria. Item 2 under criterion A is about nonverbal communication and echolalia which was part of verbal deviances in previous DSM editions and is no longer a main item and is found under repetitive movement's item 1 criterion "B". Hence it is important to specify whether ASD exists with or without a language impairment.

In the older multiaxial system a genetic or medical disorder associated with a diagnosis on axis one or two would be positioned under axis three. Where to place that information now? Another accessory to the diagnosis allows for a medical or genetic disorder or an environmental factor to be associated with ASD. ASD may be associated with Fragile X or with lead exposure or epilepsy.

When ASD is associated with other comorbidities they should be listed as associated disorders. Attention deficit hyperactivity disorder, Bipolar Disorder or Oppositional defiant disorder would be listed here.

2.3 Severity and Specificity in Practice

The consequences of adding the severity and specificity modifiers can create confusing complexity. A somewhat exaggerated example might look like: ASD accompanied by moderate intellectual disability with accompanying severe expressive language impairment and moderate receptive language impairment with very limited vocabulary with even greater limitations of communicative intent, with a genetic abnormality at 22q11.2 similar to velocardiofacial syndrome, with delivery at 28 weeks accompanied by anoxia without catatonia with level two severity of language and level 3 severity of restricted, repetitive behavior due to inability to tolerate routine transitions without extreme aggressive behavior. The extent of the

work required to obtain the information to quantify and describe all of these qualifiers is extensive and may be prohibitive in many community clinics as currently resourced.

Of special interest due to the proliferation of scientific literature on the subject, is the symptom cluster of catatonia. The specific criteria for catatonia, true for all the other associated comorbidities and conditions, must be met. If catatonic features are present, then ASD "with catatonia" as a modifier would be added.

2.4 Associated Features

Not unique to ASD but of interest diagnostically because it highlights special targets of treatment that further explains the features of the disorder in a particular patient are associated features (Volkmar et al. 2014). Associated features can be differentiated from comorbid disorders as they are aspects that are not uncommon but in and of themselves not disease states. Some associated features that have needed responses from the care delivery system include poor receptive language, and uneven IQ scores. These two deficits will need to be addressed especially in educational settings. Listing them alerts the school that special services will be necessary. A different intervention perhaps led by occupational or physical therapists may be necessary to work with motor deficits such as hypotonia or toe walking. Therefore, within the diagnostic language, these associated features could be mentioned. Finally, a behavior that influences quality of life, and perhaps impacts the restrictiveness of a community placement is self-injury. Listing this behavior as an associated feature should marshal the behavioral and medical resources that influence this problem.

2.5 Diagnostic Criteria Over Developmental Periods

While the criteria are static, the unfolding of symptoms of ASD over time is not. Development changes the picture of the prominence of the autistic features without straying from the core features of autism. For example adolescents with ASD may have very defined rituals and compulsive behaviors within their narrow range of interests. In contrast the preschool child with ASD may be notable for the failure of communicative interaction (Shattuck et al. 2007).

As ASD individuals reach legal age their care may shift to providers who are without training in the pediatric population. This has implications for diagnosis and the reliability of diagnosis in the adult age group. Some differences from the plethora of diagnostic symptoms seen in childhood will confront the adult practitioner. Older ASD individuals not only bear their own developmental symptom topologies but exhibit the impact of years of treatment interventions which may have led to compensations and skills so that their deficits may be masked by their life-long

experiences. A thorough review of history and childhood records will allow for a better understanding of the continuity of diagnosis over time.

Rett's syndrome: The reader may have noticed that Rett's syndrome, now a genetically identifiable disorder is no longer part of the ASD discussion. Rett's should be thought of perhaps like Fragile X with genetic abnormalities that affect brain function bringing it in phenotypic proximity to diagnostic criteria for ASD. For Rett's, a portion of the affected individuals life has symptoms that overlap with ASD. To capture this, the diagnosis might be stated as ASD with Rett's syndrome.

2.6 Differential Diagnoses

A number of psychiatric conditions have characteristics that overlap with symptoms of ASD. Rett's syndrome has been mentioned. Selective mutism however with significant expressive language handicap and over time social disabilities due to restricted social opportunities may bear some resemblance to ASD. Early social history and more typical behaviors in very specific settings can help to differentiate mutism from ASD.

A disorder with very pervasive effects on development, especially when manifested early in life is very early onset schizophrenia. Early onset schizophrenia is rare. When it occurs the available behaviors to analyze in the young child are few and developmental variation within a group of same age children can be wide. Obtaining evidence for abnormal perceptions such as auditory hallucinations requires extensive interactive interviewing skills and the ability to phrase questions that are relevant to the younger child. What will be clear is that the very young schizophrenic child needs help. The direction will be different if the condition is severe ASD. Gathering data from multiple sources and being open to the possibility of a diagnosis of very early onset schizophrenia rather than ASD improves the chance of proper diagnosis.

Another group of profoundly impaired children who may be mistaken for severe ASD are those with severe attachment disorder. Children exposed to a nonnurturing environment with unavailable attachment figures, experiencing sensory isolation of some duration and with little opportunity for normal social development will have characteristics of ASD. Motor stereotypies, language delay, and narrow range of interests, for example, willingness to eat only the few familiar foods that may have been available to them are prominent and similar to ASD features of early childhood. Differences however can include physical failure to thrive with significant growth curve retardation measures, rapid physical and psychological growth in resource-rich, nurturing settings and a history of neglect. Children from environments of significant deprivation present with poor language and social skills and improve as the environment facilitates these biologically hardwired potentials. The possibility that a child with ASD or other severe disability is at higher risk for neglect should be entertained.

Obsessive Compulsive Disorder (OCD) may be a differential diagnostic consideration when evaluating a child with odd behaviors. Overlap of specific compulsive behaviors such as touching stranger's body parts or certain objects, concern and agitation around changes to routines like bedtime rituals may meld with the variety of restricted and repetitive patterns of behavior such as lining up toys or flipping objects associated with ASD. Children who are well and then develop OCD symptoms that have its onset associated with Streptococcal infection should be tested for Pediatric Autoimmune Neuropsychiatric Disorder associated with Streptococcal infection (PANDAS). OCD behaviors with acute onset now called Pediatric acute-onset neuropsychiatric syndrome (PANS) should be considered as alternatives to a diagnosis of ASD.

Finally, a diagnostic category that impinges on the social deficits of ASD is Social Communication Disorder. The three main elements of this disorder are deficits in communication for social purposes, altering communication to fit the needs of the listener and some pragmatic aspects of language such as summarizing an experience, using verbal and nonverbal cues to improve communicative intent. While social communication is a major fixture for a diagnosis of ASD it is only one piece. ASD can be differentiated by examination of repetitive behaviors, narrow interests and other components of its diagnostic criteria.

2.7 Conclusion

The new emphasis for DSM-5 classification as related to diagnosis of ASD, has diminished the salience of sensory issues as well as the peculiarities of language of affected individuals. Echolalia as a symptom of social communication difficulties is now incorporated into repetitive behaviors. Language without a communicative message or issues of prosody are no longer criterion symptoms. The new DSM-5 orientation to a diagnosis of ASD is to add a method of scaling severity and connecting the disorder to the complex comorbiditites and medical and genetic conditions that are so often associated with ASD. While it will take some effort to be able to fluidly express the extent of difficulties of an individual with ASD, the template has been posted.

References

American Psychiatric Association. (2000). Diagnostic and statistical manual of mental disorders, 4th edn., text revision. American Psychiatric Association, Washington, DC

American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders, 5th edn. American Psychiatric Association, Arlington

Dickerson Mayes S, Black A, Tierney CD (2013) DSM-5 under-identifies PDDNOS: diagnostic agreement between the DSM-5, DSM-IV, and checklist for autism spectrum disorder. Res Autism Spect Disord 7:298–306

Gibbs V, Aldridge F, Chandler F, Witzlsperger E, Smith K (2012) Brief report: an exploratory study comparing diagnostic outcomes for autism spectrum disorders under DSM-IV-TR with the proposed DSM-5 revision. J Autism Dev Disord 42:1750–1756

Kanner L (1943) Autistic disturbances of affective contact. Nervous Child 2:217–250

McPartland JC, Reichow B, Volkmar FR (2012) Sensitivity and specificity of proposed DSM-5 diagnostic criteria for autism spectrum disorder. J Am Acad Child Adolesc Psychiatry 51:368–383

Shattuck PT, Seltzer MM, Greenberg JS, Orsmond GI, Bolt D, Kring S, Lounds J, Lord C (2007) Change in autism symptoms and maladaptive behaviors in adolescents and adults with an autism spectrum disorder. J Autism Dev Disord 37:1735–1747

Volkmar FR, Reichow B (2013) Autism in DSM-5: progress and challenges. Mol Autism 4:13

Volkmar FR, Siegel M, Woodbury-Smith M, King B, McCracken J, State M, American Academy of Child and Adolescent Psychiatry (AACAP), Committee on Quality Issues (CQI) (2014) Practice parameter for the assessment and treatment of children and adolescents with autism spectrum disorder. J Am Acad Child Adolesc Psychiatry 53:237–257

Chapter 3

Epidemiologic Features of Autism Spectrum Disorders

Russell S. Kirby

Abstract Since its discovery, there has been much debate regarding both the etiology of autism as well as its prevalence. While initially thought to be rare, autism has a rising prevalence with a most recent estimate of 14.7 per 1000 in eight year olds. A number of potential risk factors have been investigated including maternal age, prenatal and perinatal factors, socioeconomic status, and ethnicity. However, no direct risk factor for autism has been identified. Additionally, over 100 genes have been identified as putative autism risk genes. Taken together, the number of potential environmental, genetic, developmental, and biological risk factors for autism points to a multifactorial etiology.

Keywords Epidemiology · Prevalence · Risk factors · Autism spectrum disorders · Etiology

While research concerning the causes and risk factors associated with autism spectrum disorders (ASD) continues at a rapid pace, some common themes have emerged in recent years. To date, no direct etiologic links have been identified—there are no known factors which are both necessary and sufficient to cause autism spectrum disorder to occur in any given child. Our purpose in this chapter is to provide a general rather than exhaustive treatment of this subject, to provide the reader with a context and some resources for additional guidance. We begin with definitions of epidemiology and autism spectrum disorder, followed by a discussion of prevalence of ASD, descriptive patterns and reasonably well confirmed associations, and end with some observations that may help guide future research.

3.1 Definitions

Epidemiology is a scientific discipline focusing on the etiology, determinants and treatments of diseases, conditions and health states among humans (Rothman et al. 2012). Taking a population perspective, researchers typically compare groups of

R. S. Kirby (✉)
Department of Community and Family Health, College of Public Health, University of South Florida, 4202 E. Fowler Avenue, Campus Delivery Code MDC56, Tampa, FL 33620, USA
e-mail: rkirby@health.usf.edu

S. Hossein Fatemi (ed.), *The Molecular Basis of Autism,*
Contemporary Clinical Neuroscience, DOI 10.1007/978-1-4939-2190-4_3

study and comparison subjects using cross-sectional, case-control, and cohort study designs. Clear and unambiguous case definitions are critical to epidemiologic research. Differences in case definition have and will continue to frustrate readers of the epidemiologic literature on autism spectrum disorders. While terms such as autism disorder, classical autism, Asperger's syndrome, pervasive developmental disorders and autism spectrum disorders are often seen in the research literature, researchers use varying case definitions depending on age of study subjects, country, and decade when the study was conducted. Studies prior to the 1990s often focus only on autism disorder or classical autism, while recent epidemiologic investigations more commonly examine the broad range of autism spectrum disorders. Generally, ASDs are neurodevelopmental disorders in which the individual is affected by core deficits in the three domains of social interaction, communication, and repetitive or stereotypic behavior. Affected individuals vary markedly in severity of impairment across these domains. The recent implementation of the DSM-5 case definition and clinical criteria will add to the complexity involved in comparing findings of recent epidemiologic studies with those in the coming years. In this chapter, our focus is on the broader range of ASDs, unless otherwise noted.

3.2 Prevalence

Epidemiologists use the term *prevalence* as the frequency of existing cases of a disease or health condition in a population, in contrast to incidence, which refers to newly occurring or *incident* cases in a population. Because timing of diagnosis for ASD can be anywhere from early childhood to school age or in some cases later, prevalence is the more commonly used term in studies of epidemiology of ASD. The onset of symptoms and timing of diagnosis, critical factors for calculating incidence, are complex and variable for ASDs.

When autism and Asperger's syndrome were first characterized in the 1940s (Kanner 1943; Asperger 1944), these conditions were thought to be very rare, and even into the early 1990s there were some reports placing the prevalence in the range of 3–5 cases per 10,000 (Newschaffer et al. 2007). Current estimates place the worldwide prevalence at 0.6–0.7% (Lai et al. 2014; Elsabbagh et al. 2012); however, several recent population-based reports suggest a prevalence of 1–2% (Russell et al. 2014; Kogan et al. 2009; Blumberg et al. 2013). Comparisons of prevalence estimates are fraught with difficulty, as some statistics are based on clinical diagnoses, others on functional assessment for educational purposes, and others on parental self-report. Also, many studies are based on case ascertainment in specific clinics and their findings are difficult to translate to a population basis. In the US, the Autism and Developmental Disabilities Monitoring Network (ADDM) has used a consistent case definition based on laborious review of medical records and diagnostic evaluations to establish the population prevalence of ASD among 8-year-old children in well-defined catchment areas focusing on even numbered calendar years from 2000 on (Rice et al. 2007; van Naarden Braun et al. 2007). In the most recent

ADDM report for 2010 (CDC 2014), ASD prevalence was reported as 14.7 per 1000, with estimates for each site ranging from 5.7 to 21.9 per 1000 8-year old children. A recent British study examining the prevalence of ASD among adults found an overall prevalence of 9.8/1000 (95% C.I. 3.0–16.5) that was not associated with age (Brugha et al. 2011).

The question of whether the prevalence of autism is rising has raised a lively debate (Charman 2011). Matson and Kozlowski (2011) reviewed recent literature, and concluded that, while several factors including expanded diagnostic criteria, greater awareness of ASD as a condition, earlier diagnosis of ASD, and recognition that ASD has lifelong consequences all contribute, the prevalence of ASD has indeed been rising. Isaksen et al. (2013) conducted a similar review, and identified considerable heterogeneity in methods for reported prevalence of ASD, concluding that methods utilized in some studies may be causing the high prevalence reported in some studies. A current controversy involves the recent implementation of the DSM-5 criteria for diagnosis of autism spectrum disorders in most western nations. Maenner et al. (2014) applied the DSM-IV-TR and DSM-5 criteria to the cohort of confirmed cases from the ADDM Network, and concluded that slightly less than one in five current cases would likely not meet the new clinical criteria. However, almost all of these cases had less severe clinical presentations.

3.3 Risk Factors

One of the most striking and persistent features of the descriptive epidemiology of ASD is the extreme preponderance of ASD among males. In most populations, the discordance is on the order of 3–4:1 (Werling and Geschwind 2013). Baron-Cohen et al. (2011) proposed an extreme male brain theory as the basis for gender discordance. Others have hypothesized that ASD-like behaviors are more noticeable in males, or that health care providers are more likely to take parent concerns about sons seriously (Begeer et al. 2013; Giarelli et al. 2010). Among children with ASD, the gender discordance is greater among cases with average or greater intelligence quotient scores (CDC 2014).

That ASD prevalence increases with maternal age is well established (Sandin et al. 2012). Recent studies demonstrate a paternal age effect independent of maternal age (Durkin et al. 2008; Shelton et al. 2010; Hultman et al. 2011; Idring et al. 2014). However, the contribution of live birth order and recurrence within sibships renders these associations somewhat less striking. More studies of autism risk factors among infant siblings of children with ASD may aid in developing our understanding of etiologic mechanisms involved in ASD (Newschaffer et al. 2012). The Early Autism Risk Longitudinal Investigation (EARLI) described by Newschaffer et al. (2012) is an ongoing study designed with this objective in mind.

Prenatal and perinatal factors have been examined in numerous epidemiologic studies of ASD. Several meta-analyses and research syntheses have sought to summarize the common elements of this research. Gardener et al. (2009) examined this

literature through 2007, and found 40 studies that met inclusion criteria. Although more than 50 prenatal factors were examined, consistent evidence for association was found only for parental age, maternal prenatal medication use, maternal bleeding during pregnancy, gestational diabetes, birth order, and maternal nativity status. A companion study (Gardener et al. 2011) examined perinatal and neonatal risk factors for autism. This meta-analysis found that low birth weight, small-for-gestational age, congenital malformations, low 5 min Apgar score, neonatal anemia, ABO or Rh incompatibility, hyperbilirubinemia, and meconium aspiration were associated with autism, as well perinatal factors including season of birth (summer), multiple gestation pregnancy, abnormal presentation, cord complications, fetal distress, and birth injury. Collectively however, most of these risk factors contribute very small increased odds for autism spectrum disorder. For example, Schieve et al. (2014) estimated the population attributable fractions for preterm birth (<37 weeks gestation), small-for-gestational age (<10th percentile birth weight for gestational age), and cesarean delivery among ASD cases compared to birth certificate controls. Among ASD cases born in the year 2000, the population attributable fractions were 2.0, 3.1, and 6.7% for preterm birth, small-for-gestational age, and cesarean delivery, respectively. The summary population attributable fraction was 11.8% (95% C. I.: 7.5–15.9%).

Other perinatal factors have also been investigated. ASD prevalence was found to decrease with increasing parity in a recent Finnish study (Cheslack-Postava et al. 2014), while a study from Norway found higher prevalence of autistic disorder in second-born children of singleton full-sibling pairs with interpregnancy intervals less than 12 months, compared to those with intervals of 36+months (Gunnes et al. 2013). Others have considered the possible role of perinatal ultrasound (Abramowicz 2012), augmentation or induction of labor with oxytocin (Gregory et al. 2013; Vintzileos and Ananth 2013), and a variety of other potential perinatal risk factors.

Maternal lifestyle factors have also been investigated in numerous studies. Lyall et al. (2014) examined this literature, and concluded that among nutrients and supplements, the strongest evidence for a protection against development of ASD is for periconceptional folic acid supplements (Surén et al. 2013; Schmidt et al. 2011). Results of investigations of the role of maternal smoking and alcohol use remain inconclusive. Another emerging area of interest involved the assessment of exposures in the ambient environment during preconception and pregnancy. Several recent studies have implicated specific pollutants, including exposures to traffic-related air pollution (Volk et al. 2013) and to ambient air pollution (Becerra et al. 2013). These observations require confirmation, and a wider array of potential pollutants remains to be studied. Additional studies examining the potential roles of air pollution and pesticides include Windham et al. (2006), Kalkbrenner et al. (2010), Volk et al. (2011), and Roberts et al. (2007).

In the United States, analyses of race/ethnic disparities in the prevalence of ASD have sparked considerable interest. The ADDM Network report for 8-year-old children in 2010 found a higher prevalence of ASD among white non-Hispanic children (15.7 per 1000), compared to 12.1 per 1000 black non-Hispanic children, and 10.8 per 1000 Hispanic children (CDC 2014). A recent report from Los Angeles County,

California focusing on children with a diagnosis of autistic disorder at ages 3–5 found higher risk of severe autism phenotypes among children born to foreign-born black, Hispanic, Vietnamese and Filipino mothers compared to white non-Hispanic mothers born in the U.S. (Becerra et al. 2014). However, these observed patterns, while conflicting, may say more about differential availability of screening and diagnostic services and access to care than reflecting true differences in the prevalence of ASD across race and ethnic groups (Mandell et al. 2009).

Prevalence of ASD has been shown to increase with socioeconomic status (Durkin et al. 2010), but differential patterns were observed in an Australian study of ASD and intellectual deficiency (Leonard et al. 2011). Even in societies with universal access to health services, it is likely that differential access or utilization of screening, diagnosis and treatment occurs.

Issues with timing of diagnosis, availability of programs and services, and access to specialty care fall largely outside this brief review, but there is an extensive and growing literature on these topics.

This review would be incomplete without some discussion of the vaccines and autism hypothesis. Briefly, this hypothesis was brought forward in the 1990s by Wakefield, and generated considerable enthusiasm in the autism advocacy community around the world. There has been no scientific evidence to support this hypothesis, but numerous epidemiologic investigations have all concluded that no association exists. Readers interested in a thorough discussion of this topic should consult (Mnookin 2011).

3.4 Genetics, Genomics, Epigenetics and Gene-Environment Interaction

While not a primary focus of this review of the epidemiology of ASD, identifying etiologic pathways of diseases and health conditions is a primary focus of the discipline of epidemiology. Although estimates of its extent vary widely, most researchers agree that ASD has a genetic component, based on patterns observed in twin pairs, in sibships, and in family histories. Genetic research concerning autism and ASD is published seemingly weekly, and literally thousands of hypotheses have been tested. More than 100 plausible autism candidate genes have been identified and this number continues to grow (Scherer and Dawson 2011; Betancur 2011). However, many of these candidate genes, or candidate regions identified through genome-wide association studies, fail to be replicated when studied with samples drawn from other populations (Newschaffer et al. 2012). Given the large number of candidate genes, one might think we are closing in on a genetic breakthough concerning the etiology of ASD. However, were it possible to apply the methodology for estimating population attributable fractions to all the genes that have been implicated, simultaneously, these likely would account for a very small proportion of a population-based sample of individuals with ASD. Despite the voluminous research already conducted in this area, this field of endeavor is still in the early stages.

Some researchers have hypothesized that gene-gene interaction (Hallmayer et al. 2011), gene-environment interaction, or epigenetic changes (Grafodatskaya et al. 2010) may be on the etiologic pathway to ASD. As suggested by Dietert and Dietert (2008), there are specific windows of vulnerability for the developing central nervous system, both during fetal development, and in infancy and early childhood. Insults occurring at critical points may be preconditions that lead to the potential for environmental, genetic, or developmental factors to contribute to onset of ASD.

3.5 Conclusion

Epidemiologic studies of autism spectrum disorders implicate roles for genetic, biological, environmental, and developmental factors (Elsabbagh et al. 2012; Newschaffer et al. 2007; Lai et al. 2014). It is unlikely that any single condition or event plays a pivotal role in causation of ASD; rather, based on research to date, no identified risk factor is a necessary and sufficient condition for ASD. While theories of causation abound, at present it appears that ASD has a multifactorial etiology to which developmental (in utero and early childhood), environmental, and genetic factors contribute, in as yet unknown but most likely varying ways. Emerging methodologies in genomic, exomic, and exposomic research may hold the key that unlocks the mysteries underlying the epidemiology of autism spectrum disorder, but at present, more is unknown than known about the causes and risk factors for ASD.

References

Abramowicz JS (2012) Ultrasound and autism: association, link, or coincidence? J Ultrasound Med 31:1261–1269

Asperger H (1944) Die "Autistischen Psychopathen" im kindesalter (Autistic psychopaths in childhood) (in German). Archiv Psychiatr Nervenkrankh 117:76–136

Baron-Cohen S, Lombardo MV, Auyeung B, Ashwin E, Chakrabarti B, Knickmeyer R (2011) Why are autism spectrum conditions more prevalent in males? PLoS Biol 9:e1001081

Becerra TA, Wilhelm M, Olsen J, Cockburn M, Ritz B (2013) Ambient air pollution and autism in Los Angeles County, California. Environ Health Perspect 121:380–386

Becerra TA, von Ehrenstein OS, Heck JE, Olsen J, Arah OA, Jeste SS, Rodriguez M, Ritz B (2014) Autism spectrum disorders and race, ethnicity, and nativity: a population-based study. Pediatrics 134:e63–71

Begeer S, Mandell D, Wijnker-Holmes B, Venderbosch S, Rem D, Stekelenberg F, Koot HM (2013) Sex differences in the timing of identification among children and adults with autism spectrum disorders. J Autism Dev Disord 43:1151–1156

Betancur C (2011) Etiological heterogeneity in autism spectrum disorders: more than 100 genetic and genomic disorders and still counting. Brain Res 1380:42–77

Blumberg SJ, Bramlett MD, Kogan MD, Schieve LA, Jones JR, Lu MC (2013) Changes in prevalence of parent-reported autism spectrum disorder in school-aged U.S. children: 2007 to 2011–2012. Natl Health Stat Report 20:1–11

Brugha TS, McManus S, Bankart J, Scott F, Purdon S, Smith J, Bebbington P, Jenkins R, Meltzer H (2011) Epidemiology of autism spectrum disorders in adults in the community in England. Arch Gen Psychiat 68:459–466

Centers for Disease Control and Prevention, Developmental Disabilities Monitoring Network Surveillance Year 2010 Principal Investigators (2014) Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States, 2010. MMWR Surveill Summ 63:1–21

Charman T (2011) The highs and lows of counting autism (editorial). Am J Psychiat 168:873–875

Cheslack-Postava K, Jokiranta E, Suominen A, Lehti V, Sourander A, Brown AS (2014) Variation by diagnostic subtype in risk for autism spectrum disorders associated with maternal parity among finnish births. Paediatr Perinat Epidemiol 28:58–66

Dietert RR, Dietert JM (2008) Potential for early-life immune insult including developmental immunotoxicity in autism and autism spectrum disorders: focus on critical windows of immune vulnerability. J Toxicol Environ Health Part B 11:660–680

Durkin MS, Maenner MJ, Newschaffer CJ, Lee LC, Cunniff CM, Daniels JL, Kirby RS, Leavitt L, Miller L, Zahorodny W, Schieve LA (2008) Advanced paternal age and the risk of the prevalence of autism spectrum disorder. Amer J Epidemiol 168:1268–1276

Durkin MS, Maenner MJ, Meaney FJ, Levy SE, DiGuiseppi C, Nicholas JS, Kirby RS, Pinto-Martin JA, Schieve LA (2010) Socioeconomic status and the prevalence of autism spectrum disorders: evidence of social causation or disparities in access to services? PLoS One 5:e11511

Elsabbagh M, Divan G, Koh YJ, Kim YS, Kauchali S, Marcin S, Montiel-Nava C, Patel V, Paula CS, Wang C, Yasamy MT, Fombonne E (2012) Global prevalence of autism and other pervasive developmental disorders. Autism Res 5:160–179

Gardener H, Spiegelman D, Buka SL (2009) Prenatal risk factors for autism: comprehensive meta-analysis. Br J Psychiat 195:7–14

Gardener H, Spiegelman D, Buka SL (2011) Perinatal and neonatal risk factors for autism: a comprehensive meta-analysis. Pediatrics 128:344–355

Giarelli E, Wiggins LD, Rice CE, Levy SE, Kirby RS, Pinto-Martin J, Mandell D (2010) Sex differences in the evaluation and diagnosis of autism spectrum disorders among children. Disabil Health J 3:107–116

Grafodatskaya D, Chung B, Szatmari P, Weksberg R (2010) Autism spectrum disorders and epigenetics. J Amer Acad Child Adolesc Psychiat 49:794–809

Gregory SG, Anthopolos R, Osgood CE, Grotegut CA, Miranda ML (2013) Association of autism with induced or augmented childbirth in North Carolina Birth Record (1990–1998) and education research (1997–2007) databases. JAMA Pediatr 167:959–966

Gunnes N, Suren P, Bresnahan M, Hornig M, Lie KK, Lipkin WI, Magnus P, Nilsen RM, Reichborn-Kjennerud T, Schjolberg S, Susser ES, Oyen AS, Stoltenberg C (2013) Interpregnancy interval and risk of autistic disorder. Epidemiol 24:906–912

Hallmayer J, Cleveland S, Torres A, Phillips J, Cohen B, Torigoe T, Miller J, Fedele A, Collins J, Smith K, Lotspeich L, Croen LA, Ozonoff S, Lajonchere C, Grether JK, Risch N (2011) Genetic heritability and shared environmental factors among twin pairs with autism. Arch Gen Psychiat 68:1095–1102

Hultman CM, Sandin S, Levine SZ, Lichtenstein P, Reichenberg A (2011) Advancing paternal age and risk of autism: new evidence from a population-based study and a meta-analysis of epidemiological studies. Mol Psychiat 16:1203–1212

Idring S, Magnusson C, Lundberg M, Ek M, Rai D, Svensson AC, Dalman C, Karlsson H, Lee BK (2014) Parental age and the risk of autism spectrum disorders: findings from a Swedish population-based cohort. Int J Epidemiol 43:107–115

Isaksen J, Diseth TH, Schjolberg S, Skjeldal OH (2013) Autism spectrum disorders: are they really epidemic? Eur J Paediatr Neurol 17:327–333

Kalkbrenner AE, Daniels JL, Chen JC, Poole C, Emch M, Morrissey J (2010) Perinatal exposure to hazardous air pollutants and autism spectrum disorders at age 8. Epidemiology 21:631–641

Kanner L (1943) Autistic disturbances of affective contact. Nerv Child 2:217–250

Kogan MD, Blumberg SJ, Schieve LA, Boyle CA, Perrin JM, Ghandour RM, Singh GK, Strickland BB, Trevathan E, van Dyck PC (2009) Prevalence of parent-reported diagnosis of autism spectrum disorder among children in the US, 2007. Pediatrics 124:1395–1403

Lai MC, Lombardo MV, Chakrabarti B, Baron-Cohen S (2014) Autism. Lancet 383:896–910

Leonard L, Glasson E, Nassar N, Whitehouse A, Bebbington A, Bourke J, Jacoby P, Dixon G, Malacova E, Bower C, Stanley F (2011) Autism and intellectual disability are differentially related to sociodemographic background at birth. PLoS One 6:e17875

Lyall K, Schmidt RJ, Hertz-Picciotto I (2014) Maternal lifestyle and environmental risk factors for autism spectrum disorders. Int J Epidemiol 43:443–464

Maenner MJ, Rice CE, Arneson CL, Cunniff C, Schieve LA, Carpenter LA, Van Naarden Braun K, Kirby RS, Bakian AV, Durkin MS (2014) Potential impact of DSM-5 criteria on autism spectrum disorder prevalence estimates. JAMA Psychiat 71:292–300

Mandell DS, Wiggins LD, Carpenter LA, Daniels J, DiGuiseppi C, Durkin MS, Giarelli E, Morrier MJ, Nicholas JS, Pinto-Martin JA, Shattuck PT, Thomas KC, Yeargin-Allsopp M, Kirby RS (2009) Racial and ethnic disparities in the identification of children with autism spectrum disorders. Amer J Public Health 99:493–498

Matson JL, Kozlowski AM (2011) The increasing prevalence of autism spectrum disorders. Res Autism Spectr Disord 5:418–425

Mnookin S. (2011) The panic virus: the true story of the vaccine-autism controversy. Simon & Schuster, New York

Newschaffer CJ, Croen LJ, Daniels J, Giarelli E, Grether JK, Levy SE, Mandell DS, Miller LA, Pinto-Martin J, Reaven J, Reynolds AM, Rice CE, Schendel D, Windham GC (2007) The epidemiology of autism spectrum disorders. Annu Rev Public Health 28:235–258

Newschaffer CJ, Croen LJ, Fallin MD, Hertz-Picciotto I, Nguyen DV, Lee NL, Berry CA, Farzadegan H, Hess HN, Landa RJ, Levy SE, Massolo ML, Meyerer SC, Mohammed SM, Oliver MC, Ozonoff S, Pandey J, Schroeder A, Shedd-Wise KM (2012) Infant siblings and the investigation of autism risk factors. J Neurodev Disord 4:7

Rice C, Baio J, van Naarden Braun K, Doernberg N, Meaney FJ, Kirby RS, for the ADDM Network. (2007) A public health collaboration for the surveillance of autism spectrum disorders. Paediatr Perinat Epidemiol 21:179–190

Roberts EM, English PB, Grether JK, Windham GC, Somberg L, Wolff C (2007) Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California Central Valley. Environ Health Perspect 115:1482–1489

Rothman KJ, Lash TL, Greenland S. (2012) Modern epidemiology, 3rd edn. Lippincott-Raven Publishers, Philadelphia

Russell F, Rodgers LR, Ukoumunne OC, Ford T (2014) Prevalence of parent-reported ASD and ADHD in the UK: findings from the Millenium Cohort Study. J Autism Dev Disord 44:31–40

Sandin S, Hultman CM, Kolevzon A, Gross R, MacCabe JH, Reichenberg A (2012) Advancing maternal age is associated with increasing risk for autism: a review and meta-analysis. J Amer Acad Child Adolesc Psychiat 51:477–486

Scherer SW, Dawson G (2011) Risk factors for autism: translating genomic discoveries into diagnostics. Hum Genet 130:123–148

Schmidt RJ, Hansen RL, Hartiala J, Allayee H, Schmidt LC, Tancredi DJ, Tassone F, Hertz-Picciotto I (2011) Prenatal vitamins, one-carbon metabolism gene variants, and risk for autism. Epidemiol 22:476–485

Shelton JF, Tancredi DJ, Hertz-Picciotto I (2010) Independent and dependent contributions of advanced maternal and paternal ages to autism risk. Autism Res 3:30–39

Schieve LA, Tian L, Baio J, Rankin K, Rosenberg D, Rice C, Wiggins L, Maenner MJ, Yeargin-Allsopp M, Durkin M, King L, Kirby RS, Wingate MS, Devine O (2014) Population attributable fractions for three perinatal risk factors for autism spectrum disorders, 2002 and 2008 autism and developmental disabilities monitoring network. Ann Epidemiol 24:260–266

Surén P, Roth C, Bresnahan M, Haugen M, Hornig M, Hirtz D, Lie KK, Lipkin WI, Magnus P, Reichborn-Kjennerud T, Schjolberg S, Davey Smith G, Oyen AS, Susser E, Stoltenberg C

(2013) Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children. JAMA 309:570–577

van Naarden Braun K, Pettygrove S, Daniels J, Miller L, Nicholas J, Baio J, Schieve L, Kirby RS, Washington A, Brocksen S, Rahbar H, Rice C, Centers for Disease Control and Prevention (2007) Evaluation of a methodology for a collaborative multiple source surveillance network for autism spectrum disorders—autism and developmental disabilities monitoring network, 14 sites, United States, 2002. MMWR Surveill Summ 56:29–40

Vintzileos AM, Ananth CV (2013) Does augmentation or induction of labor with oxytocin increase risk for autism? (editorial). Am J Obstet Gynecol 209:502–504

Volk HE, Hertz-Picciotto I, Delwiche L, Lurmann F, McConnell R (2011) Residential proximity to freeways and autism in the CHARGE study. Environ Health Perspect 119:873–877

Volk HE, Lurmann F, Penfold B, Hertz-Picciotto I, McConnell R (2013) Traffic-related air pollution, particulate matter, and autism. JAMA Psychiat 70:71–77

Werling DM, Geschwind DH (2013) Sex differences in autism spectrum disorders. Curr Opin Neurol 26:146–153

Windham GC, Zhang L, Gunier R, Croen LA, Grether JK (2006) Autism spectrum disorders in relation to distribution of hazardous air pollutants in the San Francisco bay area. Environ Health Perspect 114:1438–1444

Chapter 4

Genetics of Autism Spectrum Disorders: The Opportunity and Challenge in the Genetics Clinic

Yi Wang, Ping Wang, Xiu Xu, Jennifer Goldstein, Allyn McConkie, Sau W. Cheung and Yong-Hui Jiang

Abstract The involvement of genetic etiology in autism spectrum disorders (ASDs) was first suggested from a twin study reported in 1970s. This initial observation has then been validated in many subsequent studies. The identification of single genes in syndromic ASDs in 1990s provided the evidence to support a genetic cause of ASDs. Since 2005, genome-wide copy number variant and sequence analyses have uncovered a list of rare and highly penetrant copy number variants (CNVs) or single nucleotide variants (SNVs) associated with ASDs which strengthen the claim of a genetic etiology for ASDs. However, the cause in majority of ASDs (> 75 %) remains elusive. The increasing prevalence of ASD also has intensified a debate about the role of non-genetic factors, such as epigenetics, environment, and gene and environment interaction, in the ASD etiology. Although it remains a significant challenge to determine the cause in majority cases of ASDs, the findings from ASD genet-

Y.-H. Jiang (✉)
Departments of Pediatrics and Neurobiology, Duke University School of Medicine, Durham, NC 27710, USA
e-mail: yong-hui.jiang@duke.edu

Advisory Professor, The Children's Hospital of Fudan University, Shanghai, China

Y. Wang
Department of Neurology, The Children's Hospital of Fudan University, Shanghai, P. R. China

P. Wang · J. Goldstein · A. McConkie · Y.-H. Jiang
Department of Pediatrics, Duke University School of Medicine, Durham, NC 27710, USA

X. Xu
Department of Child Health Care, The Children's Hospital of Fudan University, Shanghai, P. R. China

S. W. Cheung
Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

S. Hossein Fatemi (ed.), *The Molecular Basis of Autism,*
Contemporary Clinical Neuroscience, DOI 10.1007/978-1-4939-2190-4_4

ics studies now support an important application for molecular diagnostics in the clinical genetics evaluation of ASDs. Various molecular diagnostic assays including single gene tests, targeted multiple gene panels and copy number analysis are all appropriate to be considered in the clinical genetics evaluation of ASDs in clinics. Whole exome sequencing could also be considered in selective clinical cases. However, the challenge remains to determine the causal role of the various types of genetic variants identified through molecular testing. The variable expressivity, pleiotropic effect and incomplete penetrance associated with CNVs and SNVs also present a significant challenge for genetic counseling and prenatal diagnosis.

Keywords Autism spectrum disorders · Comparative genomic hybridization · Chromosomal microarray analysis · Copy number variant · Single nucleotide variant · Next generation sequencing · Whole exome sequencing · Whole genome sequencing

Abbreviations

ASD	Autism spectrum disorder
AOH	Absence of heterozygosity
CGH	Comparative genomic hybridization
CMA	Chromosomal microarray analysis
CNV	Copy number variant
DSM	Diagnostic and Statistical Manual of Mental Disorders
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
LOF	Loss of function
MLPA	Multiplex Ligation-dependent Probe Amplification
NGS	Next generation sequencing
PMS	Phelan-McDermid syndrome
TGP	Targeted gene panel
VCFS	Velocardiofacial syndrome
VUS	Variant of unknown significance
WES	Whole exome sequencing
WGS	Whole genome sequencing
SNP	Single nucleotide polymorphism
SNV	Single nucleotide variant
FISH	Fluorescence *in situ* hybridization

4.1 Brief History of Autism Genetic Studies

It has been 70 years since Kanner and Asperger first coined the term “autism” to describe a group of children with behavioral impairment in social and communication domains. This group of conditions is now defined as autism spectrum disorders (ASDs). The implication of a biological or genetic mechanism in autism spectrum disorders (ASDs) was also first suggested by Leo Kanner (1943). In the landmark paper by Kanner in 1943, he described a group of 11 children with “autism” and con-

cluded with an insightful statement: "We must, then, assume that these children have come into the world with innate inability to form the usually biologically provided affective contact with people, just as other children come into the world with innate physical or intellectual handicaps… For here, we seem to have pure-culture examples of inborn autistic disturbances of affective contact." The use of "inborn" to describe the possible cause of autism is explicit to suggest a biological mechanism. It should be noted that "inborn error" was coined by the British physician Archibald Garrod during the same time period to describe any biochemical defect in metabolism. Kanner also drew a comparison between autism and physical and intellectual disability. For the latter, the etiological role of biological or genetic (Miles 2011) mechanisms was well recognized. Unfortunately, the impact of a biological mechanism in "autism" was not fully investigated until the late 1970s when the first twin study was reported by Susan Folstein and Michael Rutter (1977). Despite the small number of cases in the first twin study, the finding was seminal because for the first time, a genetic factor was suggested to be implicated in the etiology of autism. Subsequently, this finding has been replicated in numerous studies validating the initial observation (Bailey et al. 1995; Hallmayer et al. 2002, 2011; Ronald and Hoekstra 2011; Steffenburg et al. 1989). The identification of genes in many syndromic ASDs such as fragile X and Rett syndrome in the 1990s rendered direct evidence for a genetic etiology or single gene defect in the clinical presentation of autistic disorders (Amir et al. 1999; Pieretti et al. 1991). With the rapid development of new genome technologies, the past decade has seen tremendous advance in the understanding of the genetics of ASDs (Buxbaum et al. 2012; Geschwind 2008; State and Levitt 2011). Findings from these more recent ASD genetic and genomic studies provide the best evidence supporting the role of a genetic etiology in ASDs but also underscore the challenge in fully understanding the molecular basis underlying ASDs. In fact, the cause in the majority of ASD cases (>75%) still remains unknown (Fig. 4.1).

In current clinical practice, the diagnosis of ASDs is purely based on the behavioral assessment using various standardized screening or diagnostic tools. There are no reliable biological biomarkers or brain imaging markers to screen or confirm

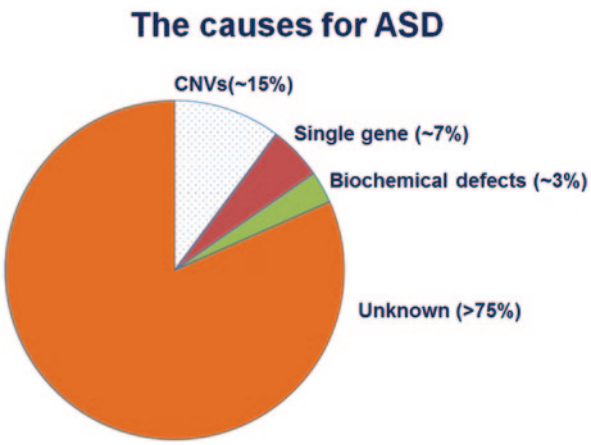


Fig. 4.1 The causes of autism spectrum disorders

the diagnosis for ASDs. The diagnostic criteria of ASDs related to the behavioral domains have been evolving and are frequently a subject of debate in both professional and public forums. In the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) (APA 2000), a diagnosis of autism is made based on recognizing impairments in three core domains of social interaction, communication as well as restricted repetitive and stereotyped patterns of behavior, interests, and activities. Furthermore, patients with autism could be diagnosed with four separate disorders: autistic disorder, Asperger's syndrome, pervasive developmental disorder—not otherwise specified (PDD-NOS) and childhood disintegrative disorder**.** In the recently published DSM-5, an umbrella term of autism spectrum disorders is used to cover all separate diagnoses defined in DSM-IV (APA 2013) such that an individual diagnosis such as Asperger's syndrome will not be used. In DSM-5, the diagnostic criteria of ASDs are based on impairments in two domains (1) persistent deficits in social communication and social interaction across multiple contexts; and (2) restricted, repetitive patterns of behavior, interests, or activities. The severity is based on the level of support that is required to meet the needs of social communication impairments and restricted and repetitive patterns of behaviors. Although this practice has been useful, the limitation is also apparent. Our ability to measure and quantify the impairments of social and communication behaviors in humans is very limited. In many cases with significant comorbidity of intellectual disability, the diagnosis is even more challenging. In DSM-5, the comorbidity of neurological presentations such as intellectual disability and seizures is not considered in the diagnostic criteria of ASDs. Non-behavioral features such as dysmorphic features and congenital anomalies are not taken into account either. However, in the clinical evaluation of ASDs (Schaefer et al. 2013), the probability to uncover a genetic defect is significantly higher in ASD cases with dysmorphic features, congenital anomalies, seizures, and significant intellectual disability (Beaudet 2007; Jacquemont et al. 2006). ASDs are referred to as syndromic if the autistic diagnosis is part of the clinical presentation of a known genetic syndrome or idiopathic if the cause is unknown.

The number of children with the diagnosis of ASDs has been increasing steadily over the last decade. The reported prevalence in children was 1 in 68 in the US in 2014 (DDMNS 2014) compared to 1 in 110 as reported in 2009 (ADDMN 2009) and has a male to female ratio of 4 to 1. The exact underlying reason for the increase in prevalence is not known and is also a subject for debate. The gender difference is also not well understood. There is no clear evidence supporting that mutations in a major gene(s) in the sex chromosomes contribute significantly to the cause in idiopathic ASDs. The alarming increase in the number of new ASD cases has intensified the debate about the role of non-genetic factors, the interaction between genes and the environment, or epigenetics in the etiology of ASDs (Gregory et al. 2013; Hallmayer et al. 2011).

The topic of autism genetics has been reviewed extensively in many recent journal reviews (Devlin and Scherer 2012; Huguet et al. 2013; Miles 2011; Ronemus et al. 2014; Rosti et al. 2014; State and Levitt 2011). In this chapter, we will summarize the key findings from recent ASD genetic and genomic studies using genome-wide copy number variant analysis and next generation sequencing (NGS) techniques, describe the application of these techniques in the clinical genetics

evaluation of ASDs, and discuss the challenges of determining the causal role of copy number and sequence variants and genetic counseling dilemmas associated with ASDs in clinics.

4.2 Genetic Basis of ASDs

The considerable clinical heterogeneity observed in ASDs has created a significant challenge to understanding the genetic inheritance underlying the ASDs. The knowledge learned from syndromic ASDs indicated that autistic disorders can be caused by a defect in a single gene. The inheritance of syndromic ASDs becomes clear once the gene implicated in the genetic syndrome is identified. The classic examples in this category are fragile X syndrome and Rett syndrome (Amir et al. 1999; Pieretti et al. 1991), which are also the most common causes for intellectual disability in males and females respectively. With more reports of autism being a clinical feature of different genetic syndromes, the list of syndromic ASDs is growing (Table 4.1). However, because systematic natural history studies have not been conducted for most of these genetic syndromes, these claims may be overstated in some cases or under-appreciated in others. Regardless, knowledge of syndromic ASDs provides a framework for a differential diagnosis in the clinical evaluation of ASDs. The studies of the genes implicated in these syndromic ASDs will also provide insight for the pathophysiology of idiopathic ASD.

Discussion of the genetic basis in idiopathic ASD cases is more complicated. Mutations in single genes probably account for 5–10 % of ASD cases in all studies in the literature (Buxbaum et al. 2012; Huguet et al. 2013; Schaaf et al. 2011; State and Levitt 2011). Two generation pedigrees with typical ASDs have been rarely reported in the literature. The reduced reproduction fitness in individuals with ASD is often cited as the reason. Although the families with more than one affected child, i.e. multiplex families, are frequently encountered, the clinical presentations among affected siblings usually have more differences than similarities posing an intriguing question as to whether recessive inheritance is a good fit in general. *De novo* genetic defects including copy number variants and single nucleotide variants have been the main focus for autism genetic studies conducted over the last decade. As described below, the results from these studies strongly support an important role of *de novo* and rare genetic mutations in the etiology of ASDs (Devlin and Scherer 2012; Ronemus et al. 2014; State and Levitt 2011).

4.3 Copy Number Variants (CNVs)

Although the term copy number variant is relatively new, the implication of chromosome dosage, i.e. chromosomal deletion and duplication, in genetic diseases have long been recognized for more than two decades. Conceptually, the chromosomal aneuploidy could be considered as the largest CNV in genome that is

Table 4.1 Syndromic ASD

Chr	Gene	Syndromes
2	*MBD5*	2q31 microdeletion sydnrome
2	*SOS1*	Noonan syndrome
5	CDKL5	Rett-like syndrome
5	*NSD1*	Sotos syndrome
7	*CHD7*	CHARGE
7	*CNTNAP2*	Cortical dysplasia-focal epilepsy syndrome
7	*RAF1*	Noonan syndrome
7	*BRAF*	Noonan syndrome
9	*TSC1*	Tuberous sclerosis complex
12	*CACNA1C*	Timothy syndrome
12	*PTPN11*	PTEN associated disorder
12	*KRAS*	Noonan syndrome
14	*PTEN*	PTEN associated disorders
14	*FOXG1*	Angelman-like syndrome
15	*UBE3A*	Angelman syndrome
15	*MAP2K1*	Noonan syndrome
16	*TSC2*	Tuberous sclerosis complex
17	*RAI1*	Smith-Magenis syndrome
18	*TCF4*	Pitt-Hopkins syndrome
22	*SHANK3*	Related to Phelan-McDermid syndrome
X	*MECP2*	Rett syndrome
X	*FMR1*	Fragile X syndrome
X	*SLC6A8*	Creatine transporter
X	*SLC9A6*	X-linked Angelman-like syndrome
X	*HPRT1*	Lesch-Nyhan syndrome
X	*ARX*	ARX related disorders
X	*MED12*	Lujan-Fryns syndrome

always pathogenic. Since the discovery of CNVs in the early 2000, the analyses of CNVs have been conducted in a long list of human diseases. The studies of CNVs associated with ASDs and other neurodevelopmental disorders are probably the most productive (Stankiewicz et al. 2003). Autistic behaviors have been described in several well characterized microdeletion and duplication syndromes including Angelman and Prader-Willi, Smith-Magenis, Potoki-Lupski, Velocadiofacial syndrome (VCFS), and Phelan-McDermid syndromes (PMS) (Laje et al. 2010; Phelan 2008; Potocki et al. 2007; Williams et al. 2001). In the case of idiopathic ASD, one of the first and still best examples of an associated chromosomal rearrangement is the maternally inherited duplication of the chromosomal 15q11-q13 Angelman

and Prader-Willi syndrome region observed using traditional and high resolution chromosome analysis (Cook et al. 1997). Although a variable phenotype has been reported with this particular duplication, autism, developmental delay, seizures and hypotonia are common features (Ingason et al. 2011; Roberts et al. 2002; Thomas et al. 2006). This rearrangement has been validated in numerous subsequent case reports and large scale genome-wide CNV studies using chromosome microarray analysis (CMA) (Boyar et al. 2001; Bucan et al. 2009; Depienne et al. 2009; Gurrieri et al. 1999; Wang et al. 2004).

In an extensive review of 33 studies including 22,698 patients overall, the International Standard Cytogenomic Array Consortium found that CMA offered a diagnostic yield of 15–20 % as compared to 3 % for G-banded chromosome analysis (Miller et al. 2010) in patients with idiopathic ASDs or intellectual disability (Miller et al. 2010). A set of CNVs highly penetrant for ASDs that were identified through research studies using genome-wide CNV analyses in a large set of ASD cases are listed in Table 4.2 (Bucan et al. 2009; Celestino-Soper et al. 2011; Pinto et al. 2010; Sanders et al. 2011). These studies clearly support an important role of CNVs in the genetic etiology of ASDs.

It has been suggested that rare CNVs containing genes involved in neural plasticity or synapse formation and maintenance as well as chromatin modification are associated with ASDs and intellectual disability and that disruption of these shared biological pathways can result in neurodevelopmental disorders (Huguet et al. 2013; Pinto et al. 2014; Toro et al. 2010). In a study comparing 996 ASD individuals of European ancestry to 1287 matched controls, ASD cases were found to carry a higher global burden of rare CNVs, especially for loci previously implicated in either ASD and/or intellectual disability (Pinto et al. 2010). Pinto et al. (2010)

Table 4.2 Clinically relevant ASD associated CNVs

Locus	Candidate gene	Other associated disorders
1q21.1	ND	ID/SCZ/BD
2p16.3	*NRXN1*	ID/SCZ
2q23.1	*MBD5*	ID/SE
3q29	*DLG1/PAK2*	ID/SCZ
7q11.23	*LIMK*	ID
11q13.3	*SHNAK2*	ID/SE
15q11.2	*UBE3A*	ID/SE
15q13.3	*CHRNA7*	ID/SCZ
16p11.2	*KCD13?*	ID/BD/SCZ
22q11.2	ND	ID/SCZ
22q13.3	*SHANK3*	ID/SE/BD
Xp22.1	*PTCHD1*	ID/SE
Xp22.3	*NLGN3*	ID
Xq13.1	*NLGN4X*	ID

ID intellectual disability, *ND* not determined, *SCZ* Schizophrenia, *BD* bipolar disorder, *SE* seizure disorder

analyzed 2446 ASD-affected families and confirmed an excess of gene deletions and duplications in affected versus control groups (Pinto et al. 2014). Genes affected by *de novo* CNVs and/or loss-of-function single-nucleotide variants converged on networks related to neuronal signaling and development, synapse function, and chromatin regulation. Among the CNVs identified, there were numerous *de novo* and inherited events, sometimes in combination in a given family, implicating many novel ASD genes including *CHD2, HDAC4, GDI1, SETD5, MIR137,* and *HDAC9*.

Recently, an exon-targeted oligo array was used to detect intragenic copy number variants in a cohort of 10,362 patients including those with a clinical indication of ASDs (Boone et al. 2010). The more commonly observed CNVs detected in this cohort include those affecting known and candidate genes for ASDs such as *NRXN1*, *CNTNAP2, NLGN4X*, *A2BP1(RBOX1)*, *CNTN4*, *CDH18*, and *TMEM195*. A more detailed clinical and molecular characterization of 24 patients who have intragenic deletions of *NRXN1* revealed that the seventeen patients with deletions involving exons manifested developmental delay/intellectual disability (93%), infantile hypotonia (59%) and ASDs (56%) (Schaaf et al. 2012). These results indicate that a small intragenic deletion is significant enough to contribute to neurodevelopmental disorders, including ASDs, but the detection of these defects could be easily missed by array designs that lack exonic coverage.

Detection of recurring intragenic CNVs in this patient population will support reclassifying candidate genes implicated in neurodevelopmental disorders as disease-causing genes. For example, two patients with developmental delay and ASDs were identified with small duplication CNVs involving single exons that disrupt *AUTS2* (Nagamani et al. 2013). This finding was further verified by another study comparing 17 well-characterized individuals with microdeletions affecting at least one exon of *AUTS2* which enabled the identification of a variable syndromic phenotype including intellectual disability (ID), autism, short stature, microcephaly, cerebral palsy, and facial dysmorphisms (Beunders et al. 2013). These results illustrate the different sensitivities for detecting genetic defects based on the resolution of different arrays. However, comparisons among different array designs to detect disease-causing defects in a clinical setting have not been fully evaluated.

The results from research studies and clinical tests not only have confirmed the previous observations from individual case reports but also consolidated several major findings related to the role of CNVs in ASDs as follows: (1) A number of new CNVs are strongly implicated in ASDs but also show both variable expressivity and pleiotropic effects; (2) between 5–10% of previous idiopathic ASD cases will carry rare CNVs; (3) both *de novo* and inherited CNVs confer a risk in ASD; and (4) there is a 3 fold increase in large and rare *de novo* CNVs in probands compared to their siblings (Buxbaum et al. 2012; Devlin and Scherer 2012). These findings, in general, support the clinical application of copy number analysis in ASDs as the recommended first tier molecular test in the clinical genetics evaluation of ASDs (Miller et al. 2010; Schaefer et al. 2013). However, the translation of these findings into clinical practice remains a challenge because the causal role of these CNVs cannot always be firmly established as discussed below.

4.4 Single Nucleotide Variants (SNVs)

The identification of genes implicated in syndromic ASDs indicated that a sequence change of a single nucleotide in a single gene can confer a significant genetic risk for ASDs. This principle has been validated in idiopathic ASDs using a candidate gene sequencing approach prior to the emergence of next generation sequencing. One of the best examples is the identification of mutations in the *SHANK3* gene (Durand et al. 2007). *SHANK3* was mapped to the critical region of chromosome 22q13.3 in Phelan-McDermid syndrome (PMS) in which autism is a prominent feature (Bonaglia et al. 2006; Durand et al. 2007; Wilson et al. 2003). Subsequently, pathogenic point mutations in *SHANK3* have been identified in idiopathic ASD in many independent reports (Boccuto et al. 2013; Moessner et al. 2007). Similar scenarios have also contributed to the discovery of other genes such as *MBD5*, *CNTNAP2*, Neuroligins and Neurexins, all of which are implicated in ASDs (Arking et al. 2008; Laumonnier et al. 2004; Talkowski et al. 2011; Zweier et al. 2009). The low frequency of these sequence variants posed a challenge in experimental design for discovering new candidate genes until the recent emergence of the new generation sequencing (NGS) technique. Whole exome (WES) and whole genome sequencing (WGS) by NGS have provided an unprecedented opportunity to discover rare variants throughout the genome in ASDs. To date, there are multiple large scale ASD WES and several small scale WGS studies that have been carried out in trios, namely the proband plus the unaffected biological parents (Bi et al. 2012; Chahrour et al. 2012; Iossifov et al. 2012; Jiang et al. 2013; Michaelson et al. 2012; Neale et al. 2012; O'Roak et al. 2012; Sanders et al. 2012; Shi et al. 2013). The majority of these studies were focused on the analysis of *de novo* mutations. Several studies analyzed both rare inherited and *de novo* mutations (Chahrour et al. 2012; Lim et al. 2013; Yu et al. 2013). Among more than 1000 families assessed by these studies, the presence of *de novo* loss-of-function (LOF) mutations was consistently significantly higher in probands compared to controls. These studies have led to confirmation of many known genes and also the discovery of more than a dozen new genes with a high confidence of a causal role in ASDs (Table 4.3). A large number of *de novo* missense variants (10 folder high than LOF allele) have also been identified in ASDs from WES studies. Some of these missense changes are certain to contribute to the risk of ASD susceptibility. However, only 5–10% excess of such mutations was found in ASD cohorts compared to controls, which did not reach statistical significance collectively. It is not possible to assign risk for these missense variants confidently without functional studies or through validation in an even larger number of cases. Only three ASD WGS studies have been reported so far (Arking et al. 2008; Chahrour et al. 2012; Sanders et al. 2012). In contrast to WES, the sample sizes of those evaluated by WGS are relatively small. In one study, WGS of 32 trios detected clinical relevant variants in 16 probands (Jiang et al. 2013). This study supports the feasibility of using WGS as a clinical test and validated the improved sensitivity of WGS compared to WES in detecting pathogenic sequence

Table 4.3 ASD candidate genes from WES/WGS studies

Chr	Gene symbol	Description	MUTATIONS
1	POMGNT1	Protein O-linked mannose beta1,2-N-acetylglucosaminyltransferase	p.R367H
1	NTNG1	Netrin G1	p.T135I (58); p.Y23C
1	POGZ	Pogo transposable element with ZNF domain	Frame shift (57); Frame shift
1	USH2A	Usher syndrome 2A (autosomal recessive, mild)	p.W2075X, p.Y4238X, compound heterozygous
2	DNMT3A	DNA (cytosine-5-)-methyltransferase 3 alpha	p.R635W
2	ARID5A	AT rich interactive domain 5A (MRF1-like)	p.G120V
2	IFIH1	Interferon induced with helicase C domain 1	Splicing site
2	SCN2A	Sodium channel, voltage-gated, type II, alpha subunit	p.G1013X; p.C959X; deletion
2	SCN1A	Sodium channel, voltage-gated, type I, alpha subunit	p.P1894 L (1 missense in additional cases)
3	AMT	Aminomethyltransferase	p.I308F; p.D198G
5	GPR98	G protein-coupled Receptor 98	p.D6252N (3 point mutations in additional cases)
6	PEX7	Peroxisomal biogenesis factor 7	p.W75C
6	SYNE1	Spectrin repeat containing, nuclear envelope 1	p.L3206M
6	VIP	Vasoactive intestinal peptide	p.Y73X (60)
8	VPS13B	Vacuolar protein sorting 13 homolog B (yeast)	Frame shift; p.S824A
8	PKHD1L1	Polycystic kidney and hepatic disease 1 (autosomal recessive)-like 1	Splicing site
9	LAMC3	Laminin, gamma 3	p.D339G (1 missense in additional cases)
10	ANK3	Ankyrin 3, node of Ranvier (ankyrin G)	p.G3690R
10	USP54	Ubiquitin specific peptidase 54	Frame shift
11	MICALCL	MICAL C-terminal like	Frame shift
11	CAPRIN1	Cell cycle associated protein 1	p.Q399X
11	KIRREL3	Kin of IRRE like 3 (Drosophila)	Downstream (3 point mutations in additional cases)
12	GRIN2B	Glutamate receptor, ionotropic, N-methyl D-aspartate 2B	Splicing site (1 non-sense and 1 frame shift in additional cases)
12	NCKAP5L	NCK-associated protein 5-like	p.G11D
12	PAH	Phenylalanine hydroxylase	Deletion; p.Q235X

Table 4.3 (continued)

Chr	Gene symbol	Description	MUTATIONS
12	UBE3B	Ubiquitin protein ligase E3B	p.R40C
14	CHD8	Chromodomain helicase DNA binding protein 8	3 Lof mutations in additional cases; p.Q959X; Frame shift
14	KIAA0284	Centrosomal protein 170B	p.R1122H
16	ABCC12	ATP-binding cassette, sub-family C (CFTR/MRP), member 12	p.W1024X
17	ZNF18	Zinc finger protein 18	p.H377N
18	KATNAL2	Katanin p60 subunit A-like 2	Splicing site; 3 Lof mutations in additional cases
20	KCNQ2	Potassium voltage-gated channel, KQT-like subfamily, member 2	Frame shift
21	DYRK1A	Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A	Splicing site; Frame shift
22	CLTCL1	Clathrin, heavy chain-like 1	p.R125C
X	NLGN4X	Neuroligin 4, X-linked	p.Q329X
X	KAL1	Kallmann syndrome 1 sequence	p.R423X
X	WWC3	WWC family member 3	p.R940Q
X	ZC3H12B	Zinc finger CCCH-type containing 12B	p.R318Q
X	DGAT2L6	Diacylglycerol O-acyltransferase 2-like 6	p.R151X; p.Q214X
X	KIAA2022	KIAA2022	p.Q1471X
X	PCDH11X	Protocadherin 11 X-linked	Splicing site
X	SRPX2	Sushi-repeat containing protein, X-linked 2	Splicing site
X	LUZP4	Leucine zipper protein 4	p.R161X; p.R163X
X	BCORL1	BCL6 corepressor-like 1	p.R1090P
X	AFF2	AF4/FMR2 family, member 2	p.A283X
X	MECP2	Methyl CpG binding protein 2	p.E495X; p.E483X
X	TMLHE	Trimethyllysine hydroxylase, epsilon	Splicing site

Shading recurrent in multiple studies, *WES* whole exome sequencing, *WGS* whole genome sequencing, *Lof* loss-of-function

variants. In addition, the WGS has detected an average of 50–70 *de novo* sequence variants in non-coding regions. It is even more challenging to determine the clinical relevance of these findings to ASDs.

In contrast to the convincing evidence supporting the pathogenicity of rare CNVs and SNVs in ASDs, the implication of common variants remains tenuous (Devlin et al. 2011; Devlin and Scherer 2012). Several large and independent genome-wide association studies have been conducted to identify common variants that exert significant risk for ASDs (Anney et al. 2010; Szatmari et al. 2007; Wang et al. 2009; Weiss 2009). Two earlier large studies (>3000 subjects) reported a significant

Table 4.4 Metabolic diseases with ASD phenotype

1.	Untreated PKU
2.	MTHFR
3.	3β-Hydroxycholesterol-7-reductase deficiency
4.	6-N-trimethyllysine dioxygenase deficiency
5.	Adenylosuccinate lyase deficiency
6.	Cerebral folate deficiency
7.	Disorders of creatine transporter
8.	Mitochondrial Diseases?

association of two different loci at 5p14.1 and 5p15.2, respectively (Wang et al. 2009; Weiss et al. 2009). However, these loci have not been replicated in other studies including WES analysis. Although further investigation may be necessary to clarify the reason underlying the differences among the studies, it is probably fair to conclude that common variants may not have a significant impact on ASDs (Devlin et al. 2011; Zecavati and Spence 2009).

4.5 Biochemical Defects and Mitochondrial Dysfunction

Numerous metabolic abnormalities have been reported in the context of an ASD phenotype but these are usually not specific for a certain diagnosis (Schaefer et al. 2013; Zecavati and Spence 2009). Individual metabolic disorders associated with an ASD phenotype are relatively rare (Table 4.4). However, all together, these disorders may contribute to ~3% of all ASD causes evaluated in clinical genetic clinic. For example, severe ASD is well recognized in individuals with untreated or partially treated phenylketonuria (PKU). Several studies have suggested a link between mitochondrial dysfunction and ASDs (Giulivi et al. 2010; Guevara-Campos et al. 2013; Legido et al. 2013; Rossignol and Frye 2012). However, it remains to be determined whether the mitochondrial dysfunction is primary or secondary to an unidentified defect. Most metabolic disorders in a classical form including mitochondrial diseases are associated with other clinical symptomatology such as seizures and extrapyramidal signs as well as biochemical disturbances. However, it is not known whether the atypical or mild presentations of metabolic disorders may present more commonly as an ASD phenotype and are under-detected.

4.6 Diagnostic Techniques for ASDs in Genetics Clinic

The current practice in the US is that a clinical genetics evaluation is recommended for all children with a confirmed diagnosis of ASD (Johnson and Myers 2007; Schaefer et al. 2013; Zwaigenbaum et al. 2009). It is estimated that a specific genetic

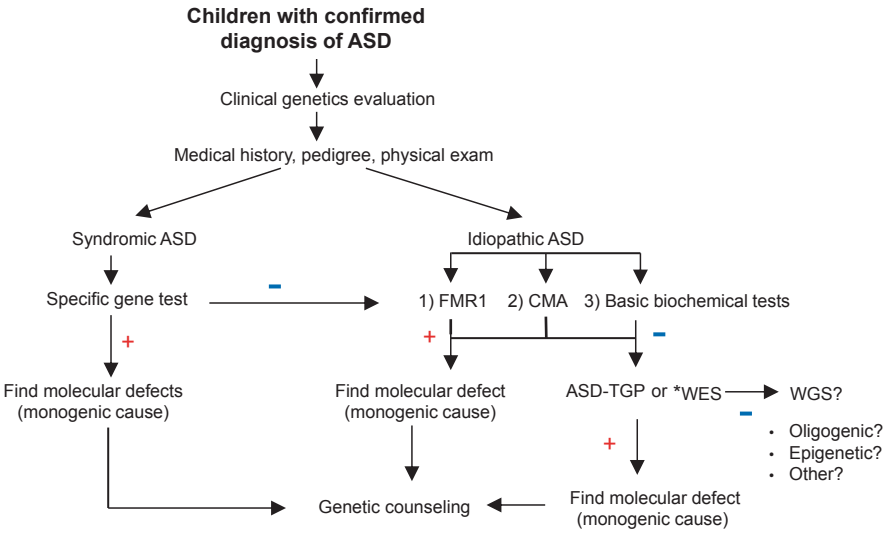


Fig. 4.2 The flowchart for clinical genetics evaluation of ASDs. *TGP* Targeted Gene Panel, *WES* whole exome sequencing, *WGS* whole genome sequencing, *CMA* chromosomal microarray analysis. *Proceeding with WES should be dictated by clinical judgment at this stage

etiology can be determined in about 15–20 % of individuals with an ASD (Schaefer et al. 2013). However, high quality or unbiased clinical data to validate the real yield of a clinical genetics evaluation in ASD is lacking (Herman et al. 2007; Shen et al. 2010). The variable practice models among clinics and different diagnostic platforms used in molecular diagnostic laboratories complicates the assessment of validity. A flowchart for the proposed clinical genetics evaluation of ASDs is presented in Fig. 4.2, which highlights the incorporation of new testing methodologies for determining the molecular defect.

4.7 Copy Number Variant Analysis

Detection of copy number variants is usually achieved through array technology and is now the recommended first tier genetic test for the evaluation of ASDs (Miller et al. 2010; Schaefer et al. 2013). Initially, CMA via array comparative hybridization (aCGH) was used for CNV analysis. Single nucleotide polymorphim (SNP) arrays were then introduced and enabled detection of copy number neutral regions of absence of heterozygosity (AOH) in addition to copy number analysis. The level of genomic resolution achieved by an array analysis depends on the number, size and distance between the interrogating probes. Array CGH utilizes oligonucleotides (60–85 mers) while SNPs (25–50 mers) are the probes for SNP arrays, of which millions of both probe types can be synthesized on one glass slide (Ou et al. 2008).

There are also customized arrays that can detect single exon CNVs involving only a few hundred base pairs (Boone et al. 2010). However, because SNPs predominate in non-coding regions, it is possible that certain regions of the genome such as single exons may not be represented on SNP arrays. In addition, SNP arrays demonstrate a lower signal-to-noise ratio per probe than CMA, which can result in less sensitivity for detecting CNVs. Therefore, to maximize clinical sensitivity, platforms that are a combination of aCGH and SNP genotyping are becoming the predominant type of array used clinically (Papenhausen et al. 2011; Peiffer et al. 2006; Schwartz 2011; Wiszniewska et al. 2014).

An advantage of SNP arrays is that they can reveal regions within the genome that lack heterozygosity, i.e. absence of heterozygosity. Identification of these regions is useful clinically in detecting polyploidy, uniparental disomy, consanguinity and recessive diseases although these are thought to be infrequent causes of ASDs (Alkan et al. 2011). However, a recent study reported that ASD individuals with intellectual disability have more regions of AOH compared to their unaffected siblings (Gamsiz et al. 2013). Furthermore, analysis of the specific ASD AOH regions aided in the discovery of autism candidate genes by either identifying single genes within an AOH interval that are recurrent in ASD or harbor a homozygous, rare deleterious variant upon analysis of exome-sequencing data (Gamsiz et al. 2013).

While the CNVs listed in Table 4.2 represent large-scale, recurrent CNVs that were detected from the first generation of clinical arrays, the increased resolution of arrays currently being used has promise for discovering more ASD-associated CNVs or individual candidate genes. For example, the combined analyses of CNVs in exonic regions and sequencing of coding exons led to a diagnosis of an autosomal recessive disorder by unmasking a recessive allele in a patient with autism. In this case, a deletion of exons 22–25 in the *VPS13B* gene, which is associated with Cohen syndrome, was first detected by CNV analysis using an exon-targeted array (BCM V8) and subsequent sequence analysis of the *VPS13B* gene identified a missense mutation in the other allele (Cohen et al. 2005; Wiszniewska et al. 2014).

These examples highlight that, to discover the precise molecular etiology, it may be necessary to combine different technologies within one test (array CGH + SNP) or use a combination of molecular diagnostic tests (CNV analysis plus medical resequencing of candidate genes or whole exome sequencing).

4.8 Single Nucleotide Variant Analysis

4.8.1 Single Gene Test for Syndromic ASDs

If the patient's clinical presentation strongly suggests a syndromic ASD, the most effective approach is to request the specific gene test for the suspected genetic syndrome. A list of single genes associated with syndromic ASDs is found in Table 4.1.

There are numerous molecular diagnostic tests utilized for single gene testing depending on the type of causative mutation usually observed for that specific gene. Testing methodologies include sequencing analysis (point mutations), FISH (microdeletions/duplications), Southern blotting (large repeat expansions) and multiplex ligation-dependent probe amplification (MLPA, small deletions/duplications). According to the practice guidelines of the American College of Medical Genetics (ACMG) and American Academy of Pediatrics (AAP), DNA testing for fragile X is recommended for all children suspected for an ASD (Myers and Johnson 2007; Schaefer et al. 2013). With the application of CMA, the value of FISH analysis has been phased out gradually in clinical diagnostic practice. However, for a defined microdeletion syndrome with characteristic clinical features, FISH still has its value because of its relative low cost compared to CMA.

4.8.2 Targeted Gene Panels

The development of targeted gene panels (TGP) using NGS technology has been a popular approach in molecular diagnosis practice (Rehm et al. 2013). TGP allows a greater depth of coverage compared to a single gene test and better analytical sensitivity and specificity compared to WES described below. The basic design is to group the genes that are implicated in a disorder with significant genetic heterogeneity or are relevant to particular clinical phenotypes that share the same molecular pathophysiology. TGP is particularly attractive for a disorder with extensive locus heterogeneity that is difficult to differentiate based on clinical presentations. Representative examples include but are not limited to retinitis pigmentosa, Bardet-Bidel syndrome, hearing loss and Noonan syndrome.

In the case of ASDs, the extensive molecular heterogeneity is well recognized despite the fact that the molecular basis for the majority of cases is still not known. Therefore, TGP is an attractive diagnostic strategy for ASDs. Several ASD-related gene panels are offered by clinical molecular diagnostic laboratories and include genes implicated in syndromic ASDs or candidate genes from recent medical re-sequencing studies. The results from WES and WGS have not yet been incorporated in these diagnostic panels. The clinical validity of these panels has not been fully evaluated but it is expected that they will be a useful tool in the clinical setting.

The development of an updated version of an ASD TGP that incorporates the ASD candidate genes from the recent WES and WGS studies is expected to be the next logical step. Although many candidate genes suggested from WES or WGS research studies remain to be validated, it is probably reasonable to include them in the updated version of ASD TGP. This design is a good transition strategy until the use of WES or WGS techniques described below are fully validated for ASDs. The findings from the use of clinical TGP may speed the validation of research findings if the clinical laboratories share results and clinical phenotypes.

4.8.3 *Whole Exome Sequencing (WES)*

The application of WES in clinical genetics practice has gained momentum recently (Gahl et al. 2012; Jacob 2013; Yang et al. 2013). The proof of principle study of using WES in clinical application was first published in 2009 (Ng et al. 2009). In this study, the success of identifying a disease-causing mutation in a patient with a known genetic syndrome was demonstrated by WES in a research protocol. Subsequently, the success of using WES to discover new disease-causing genes was reported in 2010 (Gahl et al. 2012; Gilissen et al. 2011; Hoischen et al. 2011). In 2011, clinical molecular diagnostic laboratories started to offer WES as a clinical test using different array capture platforms and analytic paradigms (Fig. 4.3). Currently, multiple academic and commercial laboratories in US are offering the clinical WES. The indication for WES as a clinical test is quite variable among clinical geneticists or physicians in other specialties. Patients with significant intellectual disability, seizure disorders, multiple congenital anomalies and other unusual clinical presentations are typically good candidates for WES. Because a significant subset of individuals with ASDs, particularly the severe end of ASDs, present with comorbidity of severe intellectual disability, seizure disorders, and other minor congenital anomalies, it is quite reasonable to argue that clinical WES is indicated in these cases if first tier testing is negative (Fig. 4.2). The exact sensitivity of WES in a clinical application cannot be easily obtained. However, accumulated evidence

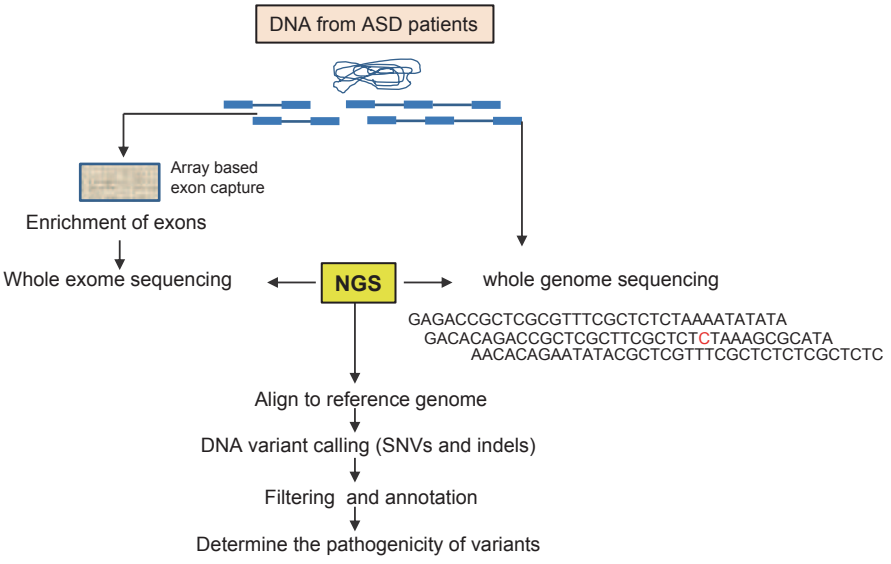


Fig. 4.3 The workflow of whole exome (*WES*) and whole genome sequencing (*WGS*) in clinical diagnostic applications

in one clinical laboratory in the US reported an approximately 25% success rate in determining the cause for ~250 cases with variable clinical presentations referred for WES (Yang et al. 2013). Another group in the Middle East reported the finding of 37 potential variants in 100 probands for known Mendelian diseases (37%) (Rodriguez-Flores et al. 2014). The overall positive detection rate in ASD research WES studies is less than 5%. However, the results from recent ASD WES/WGS research studies should not be used to estimate the positive predictive value for WES in the clinical setting because the study subjects were not carefully selected and the focus was to identify *de novo* mutations.

The American College of Medical Genetics recently published a laboratory standard on the use of WES in clinical testing (Rehm et al. 2013). Several issues with experimental designs are worth discussing regarding the clinical application of WES in ASDs. The sequencing of trios, i.e. proband and both parents, has been the common experimental design in most research WES studies in ASDs. However, clinical laboratories vary regarding sequencing of the parents with some laboratories offering to sequence the trio while others sequence the proband only followed by necessary validation in the parents as indicated by the findings from the proband. The advantage of the proband only model is lower cost. The difference between these two designs is apparent because the sensitivity to detect *de novo* sequence variants is predicted to be higher in the trio design. In addition, the algorithm of variant calling and filtering process varies among different clinical laboratories. All laboratories have an equal opportunity to access public reference genomes such as the NHLBI exome server, 1000 genomes, and dbSNP database. However, each laboratory may have accumulated its own internal references genomes. In addition, different array platforms may be used for the exon enrichment or capture step. Technically, not all coding exons can be captured for sequencing because some fraction of the genome is difficult to capture by design. Therefore, there are inherent differences for each laboratory that may contribute to the difference in sensitivity or success rate. However, it is certain that the technique and sequence analytic ability will improve over time with the diagnostic success rate for WES steadily increasing. One development that is urgently needed in the genetics community is to build a platform and database for information sharing and to curate the clinical phenotypes and exome data world-wide. The complication for this process is that substantial computer storage space is needed and there are issues with human subject protection across different institutes in US or other countries

4.8.4 Whole Genome Sequencing (WGS)

Compared to WES, WGS offers many distinct advantages except for cost and the complexity of data storage and analysis. The DNA sample and library preparations are straightforward because no DNA enrichment step is needed. WGS definitively has better genome coverage for the coding sequences based on the design and also

Table 4.5 Biochemical Screening for ASD phenotype

1.	Plasma amino acid profile
2.	Plasma homocysteine
3.	Urine organic acid
4.	Acylcarnitine profile
5.	Urine carnitine biosynthesis panel
6.	Plasma carnitine biosynthesis panel
7.	Urine creatine/guanidinoacetate analysis
8.	Plasma acylcarnitine profile
9.	Urine purine analysis
10.	Urine pyrimidine analysis
11.	Lactate

has been validated (Jiang et al. 2013). In addition, the non-coding regions that are not covered by WES are included in WGS. WGS will also allow the detection of CNVs in high resolution that current aCGH platforms will not be able to detect or that are currently not optimal for detection. For these reasons, it is reasonable to predict that WGS will eventually become a routine molecular test replacing WES and CMA for all the purposes stated as long as the costs are reduced and the data management and analysis are streamlined.

4.9 Biochemical Testing

There have been no systematic studies examining the diagnostic yield of metabolic testing in an unselected cohort of patients with ASDs. The general opinion expressed about metabolic screening in ASDs is that they are "low incidence yet high impact" because some are treatable. Although no consensus has been reached on what level of testing should be recommended, a baseline biochemical screening (Table 4.5) as a first tier evaluation is probably indicated (Fig. 4.1), particularly in countries or regions where newborn screening for inborn errors of metabolism is not mandatory in clinical practice. For the clinical settings with good clinical expertise for metabolic disorders, a targeted biochemical test after clinical evaluation is probably more cost effective.

4.9.1 Clinical Interpretation

4.9.1.1 Copy Number Variants

The major challenge of using a whole genome analysis approach for clinical application is determining the pathogenic role of the genetic variants identified from these tests. American College of Medical Genetics (ACMG) standards and guidelines

for interpretation and reporting postnatal constitutional copy number variants have been recently updated (South et al. 2013). In general, careful consideration is given to the size of the CNV, genomic position, gene(s) involved and patient's reported phenotype. If the implicated region contains gene(s) with functions compatible with the abnormal clinical findings or previously described patients with a similar imbalance and phenotype, the CNV should be regarded as likely pathogenic. If the clinical significance is still unclear, investigating the parents and additional family members by FISH analysis or CMA (depending on the size of CNV) may often be necessary to interpret and clarify the results. Review of the family history can often provide clues to interpretation of these variants. Although the presence of the CNV in healthy family members suggests it is benign, low penetrance and variable expressivity of the phenotype can complicate the interpretation. A larger, rare CNV that is determined to be *de novo* in origin is more likely to be pathogenic. The *de novo* occurrence of a CNV is, however, not an absolute evidence of its pathogenicity and caution must be exercised for possible non-paternity.

Interpretation of the clinical significance of CNVs remains challenging and requires time-consuming extensive search of the databases as well as literature and collaboration between the laboratory and referring clinician (Stankiewicz et al. 2010). A guide for utilizing public databases can be found in the Diagnostic Interpretation of Array Data Using Public Databases and Internet Sources. Examples of such databases are the Database of Genomic Variants (http://www.projects.tcag.ca/variation/), UCSC Genome Browser (http://www.genome.ucsc.edu), Simons Foundation Autism Research Initiative (SFARI.org) and Autism Chromosome Rearrangement Database (http://projects.tcag.ca/Autism/). As the number of samples screened by copy number analysis has grown significantly over the past several years, the increased experience has revealed many subtleties and complexities of CNV interpretation resulting in a better understanding of the contribution of CNVs to pathogenicity (Hehir-Kwa et al. 2013).

Even in the cases where the role of the CNV is known to confer a significant risk for an ASD, it is still difficult to pinpoint the exact causative gene or genes because there is an average of more than 10 genes usually embedded in a single CNV. In some cases, genes adjacent or outside of the deleted or duplicated intervals may also be affected due to position effect or disruption of a regulatory sequence element. The difficulty is to determine which gene or genes within the CNV are responsible for key features observed in these cases. In some cases, the clue may come from the function of a known gene or genes in the interval that are learned from *in vitro* study or other model organisms. For example, mapping *SHANK3*, a gene known to encode a synaptic scaffolding protein at postsynaptic density (Grabrucker et al. 2011), to the 22q13.3 interval which is deleted in Phelan-McDermid syndrome led to the hypothesis that *SHANK3* is the important gene for ASD in this critical region (Wilson et al. 2003). This conclusion was supported subsequently by the discovery of point mutations in *SHANK3* in idiopathic ASD patients (Durand et al. 2007; Marshall et al. 2008). However, in most cases, genes within the CNV are new and their function is unknown. For example, in the case of the 16p11.2 ASD-associated CNV, there are more than 25 genes within this CNV (Weiss et al. 2008) and the functions

of these genes are not known. Using a zebra fish model, Golzio et al. (2012) conducted a functional screen for the individual genes mapped within the interval and presented evidence suggesting that *KCTD13* is a major driver of mirrored neuroanatomical phenotypes associated with copy number gain or loss in the 16p11.2 region in humans. The increase in the dosage of *KCTD13* resulted in a small brain and loss of the same gene displayed a large brain in fish (Golzio et al. 2012). However, the translation of this finding from zebra fish to humans is not straightforward because neither microcephaly nor macrocephaly are consistent features reported in human ASD patients with CNVs of 16p11.2. Many other phenotypes associated with a gain or loss of 16p11.2 cannot be assessed reliably in a fish model. Despite this caveat, it represents a first and important step to elucidating the contribution of individual genes in the etiology of human ASD.

4.9.2 Single Nucleotide Variants

Interpretation challenges are similar for SNVs identified from targeted gene panels, WES, or WGS. For the protein disrupting mutations in homozygotes or compound heterozygotes, the causal role for these mutations can be made with reasonable confidence although the rarity of the sequence variant(s) itself is still a cause for concern. For missense variants, it is not possible to determine the causality based on the reading of the sequence information alone. Evidence from functional studies in cellular and *in vivo* models is frequently cited to support the causal role of these variants in the research literature.

Another common complication for interpretation of SNVs identified from TGP, WES, or WGS, is that multiple variants of unknown significance (VUS) may be identified in one individual. In most cases, it is difficult to determine the causal role based on the reading of sequencing information. The newly developed genome editing system of CRISPR/Cas9 (clustered regularly interspaced short palindromic repeat) has shown great promise to rapidly manipulate the human genome by introducing SNVs or small inDels (Cong et al. 2013; Mali et al. 2013). The application of CRISPR/Cas9 may then also facilitate the dissection of the role of individual genes within a CNV in a rapid fashion by inactivating the individual genes within the interval. The much needed development of databases to catalog variant annotation for standard mutation nomenclature and a comprehensive reference database of medically important variants that is easily cross referenced to exome and genome sequence data is rapidly in progress.

There is a growing body of evidence suggesting that multiple genetic “hits” ultimately lead to ASDs (Leblond et al. 2012; Schaaf et al. 2011). These results support the suggestion that ASD is a complex genetic disorder resulting from simultaneous genetic variation in a few, several or even multiple genes (El-Fishawy and State 2010). Overall, the term “clan genomics” is introduced to remind us not to focus disproportionately on specific variants but rather to integrate across all classes of

risk-associated variants. In some individuals, risk may be caused by an unusual combination of common variants where in others it will be due to a smaller number of large effect rare variants (Lupski et al. 2011). Furthermore, epigenetic dysregulation of synaptic genes at the transcriptional level could also contribute to ASD susceptibility as observed in several studies (Jiang et al. 2004; Samaco et al. 2005; Zhu et al. 2014) and that further investigations are warranted.

4.9.3 Recurrent Risk

The observed variable expressivity, pleiotropic effect and incomplete penetrance associated with CNVs and SNVs pose a challenge for genetic counseling and determining recurrence risks. For example, the copy number gain and loss of chromosome 16p11.2 was first identified from studies of several large cohorts of ASD patients (Kumar et al. 2008; Marshall et al. 2008; Weiss et al. 2008). Subsequent studies have implicated 16q11.2 in a wide spectrum of neuropsychiatric phenotypes with somatic features like childhood obesity, as well as being present in asymptomatic or unaffected parents or siblings (Bochukova et al. 2010; McCarthy et al. 2009; Miller et al. 2009; Puvabanditsin et al. 2010; Shen et al. 2011; Shinawi et al. 2010; Simons VIP 2012; Walters et al. 2010). The presence of the same CNV in unaffected family members clearly poses a dilemma in counseling families who are interested in using this information for prenatal diagnosis. The extreme variable expressivity and penetrance is not entirely unexpected. However, in many other microdeletion syndromes such as Angelman and Prader-Willi, Williams, 1p36, 22q11.2, and Phelan-McDermid syndromes, these causative CNVs have never been reported in healthy individuals. Even in the case of 22q11.2 deletion syndrome in which the pleiotropic effect has been well documented and 10% are inherited, the penetrance is complete in almost all reported cases. The interesting question is whether ASD-associated CNVs operate under molecular mechanisms that are more amenable to genetic modifiers or environmental influence during evolution. In clinical practice, the identification of novel, *de novo* or inherited CNVs and SNVs has been encountered frequently. They are usually unique and not present in large reference databases or have not been reported by research studies. Therefore, the pathogenicity of these variants related to ASDs cannot be easily determined by genetic analysis.

Given the current complications with interpreting results and providing counseling, genome-wide testing should occur in conjunction with a comprehensive medical genetics evaluation that includes a detailed family history and dysmorphology examination of the patient and relevant family members. In addition, with the quickly changing genomic landscape, it is important for families to return for re-evaluation at recommended time intervals to both review the current interpretation of previously identified variants of unclear clinical significance and determine if any new molecular diagnostic studies are appropriate.

4.10 Prenatal Diagnosis

Prenatal diagnosis for ASDs has been an uncommon request since only rarely was a genetic etiology known for the proband. With the more widespread use of genetic testing for ASDs and discovery of new genetic etiologies, it is expected that prenatal diagnosis will become a more common request. In addition, technologies such as CMA are being increasingly used in prenatal specimens (Wapner et al. 2012) and have the potential to uncover ASD-related variants in fetuses with no prior risk. However, there have only been a few studies in pregnant women documenting the prenatal identification of 16p13.11 and 15q26 deletions, which are predicted to be associated with an ASD phenotype (Law et al. 2009; Poot et al. 2013). Without long term follow-up, there is insufficient data for which to counsel when such findings are unexpectedly identified prenatally.

Prenatal testing for ASD is technically feasible for at-risk pregnancies if there has been prior identification of the causative alteration in the proband. However, it is not always possible to reliably predict the phenotype even if the alteration is present in the fetus. Furthermore, prenatal ultrasonography is of limited use to determine if the fetus is actually affected as there are no pathognomonic signs for the diagnosis of an ASD. If we take 16p11.2 microdeletion as an example, this microdeletion is often *de novo*. Based on current literature reports (Miller et al. 2009), ASD is not diagnosed in most individuals with a 16p11.2 microdeletion but still is much more common than in the general population. Firstly, an important issue that causes difficulties for prenatal diagnosis of ASD is that (i) most pathogenic variants are not found exclusively in individuals with ASD, they also occur in unaffected controls although with a much lower frequency or (ii) they are inherited from a parent with or without a diagnosis of ASD. For example, Sanders et al. (2011) reported *de novo* CNVs in 6% of simplex ASD cases that were also present in 2% of unaffected siblings. Secondly, a current theory regarding the genotype-phenotype correlations in ASDs is determined by the degree of mutational events/burden (CNVs or SNPs) that a given individual carries, which may not be able to be determined in a prenatal setting (Choy et al. 2010).

In summary, whether prenatal diagnosis is appropriate for ASDs is uncertain given the intrinsic difficulty in accurately detecting and predicting the ASD phenotype associated with a given genotype in many cases. The incomplete penetrance and variable expressivity represent the biggest challenges in prenatal testing for ASDs.

4.11 Conclusion and Future Directions

A decade of genetic studies of ASDs has produced evidence to support a major conclusion that rare genetic variants including CNVs and SNVs are strongly implicated in the ASD etiology. The development of new molecular diagnostic technologies such as array CGH/CMA and NGS has provided an unprecedented opportunity to

uncover the rare genetic variants in ASDs. Although these studies have not yet led to a major breakthrough of understanding the molecular basis in the majority of ASD cases, these findings do provide an opportunity for clinical applications.

Many challenges remain to understanding the genetic basis of ASD and developing a robust ASD genetic testing paradigm in clinical practice and for counseling the families about genetic findings. Several important steps may have to be taken toward this direction. Further investigations on potential environmental factor or gene and environment interaction are clearly warranted. The WES/WGS data from ASDs will provide a framework to dissect the gene and environment interaction. Thus, it is urgently needed to develop a reliable functional assay to assist with the interpretation of clinical relevance of genetic variants identified from research studies or clinical tests. To facilitate this process, it would be extremely valuable for different laboratories to share the clinical WES sequence database and clinical phenotype data. To establish an evidence-based practice, it is essential to know the validity or yield of each test modality. This may be obtained from a systematically designed study or accumulated clinical experience. In addition, a cost and benefit analysis should also be conducted to firmly establish a standard of care for the clinical genetics evaluation of ASDs. Despite these many challenges, there are good reasons to believe that the clinical genetics evaluation of ASDs is an important part of clinical care for ASD children and their families. The understanding of molecular basis of ASDs will ultimately lead to the development of effective treatment targeted specially to the genetic defect.

Conflict Interest SWC is faculty member of the Department of Molecular and Human Genetics at Baylor College of Medicine, which derives revenue from the CMA offered in the Medical Genetics Laboratory.

Acknowledgement Y.H.J is supported by National Institute of Health Grant MH-1089441, Autism Speaks, Ruth K. Broad Foundation and Prader-Willi syndrome research foundation. YW is supported by Nature Science Foundation of China (NSFC 81071116), Shanghai science and technology commission (12XD1401100), and China Ministry of Health special scientific research funds (201302002).

References

Alkan C, Coe BP, Eichler EE (2011) Genome structural variation discovery and genotyping. Nat Rev Genet 12:363–376

American Psychiatric Association (2000) Diagnostic and statistical manual of mental disorders: DSM-IV-TR. American Psychiatric Association, Washington, DC

American Psychiatric Association (2013) Diagnostic and statistical manual of mental disorders: DSM-5. American Psychiatric Association, Washington, DC

Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY (1999) Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat Genet 23:185–188

Anney R, Klei L, Pinto D, Regan R, Conroy J, Magalhaes TR, Correia C, Abrahams BS, Sykes N, Pagnamenta AT, Almeida J, Bacchelli E, Bailey AJ, Baird G, Battaglia A, Berney T, Bolshakova

N, Bölte S, Bolton PF, Bourgeron T, Brennan S, Brian J, Carson AR, Casallo G, Casey J, Chu SH, Cochrane L, Corsello C, Crawford EL, Crossett A, Dawson G, de Jonge M, Delorme R, Drmic I, Duketis E, Duque F, Estes A, Farrar P, Fernandez BA, Folstein SE, Fombonne E, Freitag CM, Gilbert J, Gillberg C, Glessner JT, Goldberg J, Green J, Guter SJ, Hakonarson H, Heron EA, Hill M, Holt R, Howe JL, Hughes G, Hus V, Igliozzi R, Kim C, Klauck SM, Kolevzon A, Korvatska O, Kustanovich V, Lajonchere CM, Lamb JA, Laskawiec M, Leboyer M, Le Couteur A, Leventhal BL, Lionel AC, Liu XQ, Lord C, Lotspeich L, Lund SC, Maestrini E, Mahoney W, Mantoulan C, Marshall CR, McConachie H, McDougle CJ, McGrath J, McMahon WM, Melhem NM, Merikangas A, Migita O, Minshew NJ, Mirza GK, Munson J, Nelson SF, Noakes C, Noor A, Nygren G, Oliveira G, Papanikolaou K, Parr JR, Parrini B, Paton T, Pickles A, Piven J, Posey DJ, Poustka A, Poustka F, Prasad A, Ragoussis J, Renshaw K, Rickaby J, Roberts W, Roeder K, Roge B, Rutter ML, Bierut LJ, Rice JP, Salt J, Sansom K, Sato D, Segurado R, Senman L, Shah N, Sheffield VC, Soorya L, Sousa I, Stoppioni V, Strawbridge C, Tancredi R, Tansey K, Thiruvahindrapduram B, Thompson AP, Thomson S, Tryfon A, Tsiantis J, Van Engeland H, Vincent JB, Volkmar F, Wallace S, Wang K, Wang Z, Wassink TH, Wing K, Wittemeyer K, Wood S, Yaspan BL, Zurawiecki D, Zwaigenbaum L, Betancur C, Buxbaum JD, Cantor RM, Cook EH, Coon H, Cuccaro ML, Gallagher L, Geschwind DH, Gill M, Haines JL, Miller J, Monaco AP, Nurnberger JI Jr, Paterson AD, Pericak-Vance MA, Schellenberg GD, Scherer SW, Sutcliffe JS, Szatmari P, Vicente AM, Vieland VJ, Wijsman EM, Devlin B, Ennis S, Hallmayer J (2010) A genome-wide scan for common alleles affecting risk for autism. Hum Mol Genet 19:4072–4082

Anney R, Klei L, Pinto D, Almeida J, Bacchelli E, Baird G, Bolshakova N, Bölte S, Bolton PF, Bourgeron T, Brennan S, Brian J, Casey J, Conroy J, Correia C, Corsello C, Crawford EL, de Jonge M, Delorme R, Duketis E, Duque F, Estes A, Farrar P, Fernandez BA, Folstein SE, Fombonne E, Gilbert J, Gillberg C, Glessner JT, Green A, Green J, Guter SJ, Heron EA, Holt R, Howe JL, Hughes G, Hus V, Igliozzi R, Jacob S, Kenny GP, Kim C, Kolevzon A, Kustanovich V, Lajonchere CM, Lamb JA, Law-Smith M, Leboyer M, Le Couteur A, Leventhal BL, Liu XQ, Lombard F, Lord C, Lotspeich L, Lund SC, Magalhaes TR, Mantoulan C, McDougle CJ, Melhem NM, Merikangas A, Minshew NJ, Mirza GK, Munson J, Noakes C, Nygren G, Papanikolaou K, Pagnamenta AT, Parrini B, Paton T, Pickles A, Posey DJ, Poustka F, Ragoussis J, Regan R, Roberts W, Roeder K, Roge B, Rutter ML, Schlitt S, Shah N, Sheffield VC, Soorya L, Sousa I, Stoppioni V, Sykes N, Tancredi R, Thompson AP, Thomson S, Tryfon A, Tsiantis J, Van Engeland H, Vincent JB, Volkmar F, Vorstman JA, Wallace S, Wing K, Wittemeyer K, Wood S, Zurawiecki D, Zwaigenbaum L, Bailey AJ, Battaglia A, Cantor RM, Coon H, Cuccaro ML, Dawson G, Ennis S, Freitag CM, Geschwind DH, Haines JL, Klauck SM, McMahon WM, Maestrini E, Miller J, Monaco AP, Nelson SF, Nurnberger JI Jr, Oliveira G, Parr JR, Pericak-Vance MA, Piven J, Schellenberg GD, Scherer SW, Vicente AM, Wassink TH, Wijsman EM, Betancur C, Buxbaum JD, Cook EH, Gallagher L, Gill M, Hallmayer J, Paterson AD, Sutcliffe JS, Szatmari P, Vieland VJ, Hakonarson H, Devlin B (2012) Individual common variants exert weak effects on the risk for autism spectrum disorderspi. Hum Mol Genet 21:4781–4792

Arking DE, Cutler DJ, Brune CW, Teslovich TM, West K, Ikeda M, Rea A, Guy M, Lin S, Cook EH, Chakravarti A (2008) A common genetic variant in the neurexin superfamily member CNTNAP2 increases familial risk of autism. Am J Hum Genet 82:160–164

Autism and Developmental Disabilities Monitoring Network, United States, 2006 (2009) Prevalence of autism spectrum disorders.MMWR Surveill Summ 58:1–20

Bailey A, Le Couteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E, Rutter M (1995) Autism as a strongly genetic disorder: evidence from a British twin study. Psychol Med 25:63–77

Beaudet AL (2007) Autism: highly heritable but not inherited. Nat Med 13:534–536

Beunders G, Voorhoeve E, Golzio C, Pardo LM, Rosenfeld JA, Talkowski ME, Simonic I, Lionel AC, Vergult S, Pyatt RE, van de Kamp J, Nieuwint A, Weiss MM, Rizzu P, Verwer LE, van Spaendonk RM, Shen Y, Wu BL, Yu T, Yu Y, Chiang C, Gusella JF, Lindgren AM, Morton CC, van Binsbergen E, Bulk S, van Rossem E, Vanakker O, Armstrong R, Park SM, Greenhalgh L, Maye U, Neill NJ, Abbott KM, Sell S, Ladda R, Farber DM, Bader PI, Cushing T, Drautz JM, Konczal L, Nash P, de Los Reyes E, Carter MT, Hopkins E, Marshall CR, Osborne LR, Gripp

KW, Thrush DL, Hashimoto S, Gastier-Foster JM, Astbury C, Ylstra B, Meijers-Heijboer H, Posthuma D, Menten B, Mortier G, Scherer SW, Eichler EE, Girirajan S, Katsanis N, Groffen AJ, Sistermans EA (2013) Exonic deletions in AUTS2 cause a syndromic form of intellectual disability and suggest a critical role for the C terminus. Am J Hum Genet 92:210–220

Bi C, Wu J, Jiang T, Liu Q, Cai W, Yu P, Cai T, Zhao M, Jiang YH, Sun ZS (2012) Mutations of ANK3 identified by exome sequencing are associated with autism susceptibility. Hum Mutat 33:1635–1638

Boccuto L, Lauri M, Sarasua SM, Skinner CD, Buccella D, Dwivedi A, Orteschi D, Collins JS, Zollino M, Visconti P, Dupont B, Tiziano D, Schroer RJ, Neri G, Stevenson RE, Gurrieri F, Schwartz CE (2013) Prevalence of SHANK3 variants in patients with different subtypes of autism spectrum disorders. Eur J Hum Genet 21:310–316

Bochukova EG, Huang N, Keogh J, Henning E, Purmann C, Blaszczyk K, Saeed S, Hamilton-Shield J, Clayton-Smith J, O'Rahilly S, Hurles ME, Farooqi IS (2010) Large, rare chromosomal deletions associated with severe early-onset obesity. Nature 463:666–670

Bonaglia MC, Giorda R, Mani E, Aceti G, Anderlid BM, Baroncini A, Pramparo T, Zuffardi O (2006) Identification of a recurrent breakpoint within the SHANK3 gene in the 22q13.3 deletion syndrome. J Med Genet 43:822–828

Boone PM, Bacino CA, Shaw CA, Eng PA, Hixson PM, Pursley AN, Kang SH, Yang Y, Wiszniewska J, Nowakowska BA, del Gaudio D, Xia Z, Simpson-Patel G, Immken LL, Gibson JB, Tsai AC, Bowers JA, Reimschisel TE, Schaaf CP, Potocki L, Scaglia F, Gambin T, Sykulski M, Bartnik M, Derwinska K, Wisniowiecka-Kowalnik B, Lalani SR, Probst FJ, Bi W, Beaudet AL, Patel A, Lupski JR, Cheung SW, Stankiewicz P (2010) Detection of clinically relevant exonic copy-number changes by array CGH. Hum Mutat 31:1326–1342

Boyar FZ, Whitney MM, Lossie AC, Gray BA, Keller KL, Stalker HJ, Zori RT, Geffken G, Mutch J, Edge PJ, Voeller KS, Williams CA, Driscoll DJ (2001) A family with a grand-maternally derived interstitial duplication of proximal 15q. Clin Genet 60:421–430

Bucan M, Abrahams BS, Wang K, Glessner JT, Herman EI, Sonnenblick LI, Alvarez Retuerto AI, Imielinski M, Hadley D, Bradfield JP, Kim C, Gidaya NB, Lindquist I, Hutman T, Sigman M, Kustanovich V, Lajonchere CM, Singleton A, Kim J, Wassink TH, McMahon WM, Owley T, Sweeney JA, Coon H, Nurnberger JI, Li M, Cantor RM, Minshew NJ, Sutcliffe JS, Cook EH, Dawson G, Buxbaum JD, Grant SF, Schellenberg GD, Geschwind DH, Hakonarson H (2009) Genome-wide analyses of exonic copy number variants in a family-based study point to novel autism susceptibility genes. PLoS Genet 5:e1000536

Buxbaum JD, Daly MJ, Devlin B, Lehner T, Roeder K, State MW (2012) The autism sequencing consortium: large-scale, high-throughput sequencing in autism spectrum disorders. Neuron 76:1052–1056

Celestino-Soper PB, Shaw CA, Sanders SJ, Li J, Murtha MT, Ercan-Sencicek AG, Davis L, Thomson S, Gambin T, Chinault AC, Ou Z, German JR, Milosavljevic A, Sutcliffe JS, Cook EH Jr, Stankiewicz P, State MW, Beaudet AL (2011) Use of array CGH to detect exonic copy number variants throughout the genome in autism families detects a novel deletion in TMLHE. Hum Mol Genet 20:4360–4370

Chahrour MH, Yu TW, Lim ET, Ataman B, Coulter ME, Hill RS, Stevens CR, Schubert CR, ARRA Autism Sequencing Collaboration, Greenberg ME, Gabriel SB, Walsh CA (2012) Whole-exome sequencing and homozygosity analysis implicate depolarization-regulated neuronal genes in autism. PLoS Genet 8:e1002635

Choy KW, Setlur SR, Lee C, Lau TK (2010) The impact of human copy number variation on a new era of genetic testing. BJOG 117:391–398

Cohen D, Pichard N, Tordjman S, Baumann C, Burglen L, Excoffier E, Lazar G, Mazet P, Pinquier C, Verloes A, Héron D (2005) Specific genetic disorders and autism: clinical contribution towards their identification. J Autism Dev Disord 35:103–116

Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F (2013) Multiplex genome engineering using CRISPR/Cas systems. Science 339:819–823

Cook EH Jr, Lindgren V, Leventhal BL, Courchesne R, Lincoln A, Shulman C, Lord C, Courchesne E (1997) Autism or atypical autism in maternally but not paternally derived proximal 15q duplication. Am J Hum Genet 60:928–934

Depienne C, Moreno-De-Luca D, Heron D, Bouteiller D, Gennetier A, Delorme R, Chaste P, Siffroi JP, Chantot-Bastaraud S, Benyahia B, Trouillard O, Nygren G, Kopp S, Johansson M, Rastam M, Burglen L, Leguern E, Verloes A, Leboyer M, Brice A, Gillberg C, Betancur C (2009) Screening for genomic rearrangements and methylation abnormalities of the 15q11-q13 region in autism spectrum disorders. Biol Psychiatry 66:349–359

Developmental Disabilities Monitoring Network Surveillance Year Principal I, Centers for Disease Control and Prevention (2014) Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States, 2010. MMWR Surveill Summ 63:1–21

Devlin B, Scherer SW (2012) Genetic architecture in autism spectrum disorder. Curr Opin Genet Dev 22:229–237

Devlin B, Melhem N, Roeder K (2011) Do common variants play a role in risk for autism? Evidence and theoretical musings. Brain Res 1380:78–84

Durand CM, Betancur C, Boeckers TM, Bockmann J, Chaste P, Fauchereau F, Nygren G, Rastam M, Gillberg IC, Anckarsäter H, Sponheim E, Goubran-Botros H, Delorme R, Chabane N, Mouren-Simeoni MC, de Mas P, Bieth E, Rogé B, Héron D, Burglen L, Gillberg C, Leboyer M, Bourgeron T (2007) Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. Nat Genet 39:25–27

El-Fishawy P, State MW (2010) The genetics of autism: key issues, recent findings, and clinical implications. Psychiatr Clin North Am 33:83–105

Folstein S, Rutter M (1977) Infantile autism: a genetic study of 21 twin pairs. J Child Psychol Psychiatry 197:297–321

Gahl WA, Markello TC, Toro C, Fajardo KF, Sincan M, Gill F, Carlson-Donohoe H, Gropman A, Pierson TM, Golas G, Wolfe L, Groden C, Godfrey R, Nehrebecky M, Wahl C, Landis DM, Yang S, Madeo A, Mullikin JC, Boerkoel CF, Tifft CJ, Adams D (2012) The national institutes of health undiagnosed diseases program: insights into rare diseases. Genet Med 14:51–59

Gamsiz ED, Viscidi EW, Frederick AM, Nagpal S, Sanders SJ, Murtha MT, Schmidt M, Simons Simplex Collection Genetics Consortium, Triche EW, Geschwind DH, State MW, Istrail S, Cook EH Jr, Devlin B, Morrow EM (2013) Intellectual disability is associated with increased runs of homozygosity in simplex autism. Am J Hum Genet 93:103–109

Geschwind DH (2008) Autism: many genes, common pathways? Cell 135:391–395

Gilissen C, Hoischen A, Brunner HG, Veltman JA (2011) Unlocking Mendelian disease using exome sequencing. Genome Biol 12:228

Giulivi C, Zhang YF, Omanska-Klusek A, Ross-Inta C, Wong S, Hertz-Picciotto I, Tassone F, Pessah IN (2010) Mitochondrial dysfunction in autism. JAMA 304:2389–2396

Golzio C, Willer J, Talkowski ME, Oh EC, Taniguchi Y, Jacquemont S, Reymond A, Sun M, Sawa A, Gusella JF, Kamiya A, Beckmann JS, Katsanis N (2012) KCTD13 is a major driver of mirrored neuroanatomical phenotypes of the 16p11.2 copy number variant. Nature 485:363–367

Grabrucker AM, Knight MJ, Proepper C, Bockmann J, Joubert M, Rowan M, Nienhaus GU, Garner CC, Bowie JU, Kreutz MR, Gundelfinger ED, Boeckers TM (2011) Concerted action of zinc and ProSAP/Shank in synaptogenesis and synapse maturation. EMBO J 30:569–581

Gregory SG, Anthopolos R, Osgood CE, Grotegut CA, Miranda ML (2013) Association of autism with induced or augmented childbirth in North Carolina Birth Record (1990–1998) and Education Research (1997–2007) databases. JAMA Pediatr 167:959–966

Guevara-Campos J, Gonzalez-Guevara L, Puig-Alcaraz C, Cauli O (2013) Autism spectrum disorders associated to a deficiency of the enzymes of the mitochondrial respiratory chain. Metab Brain Dis 28:605–612

Gurrieri F, Battaglia A, Torrisi L, Tancredi R, Cavallaro C, Sangiorgi E, Neri G (1999) Pervasive developmental disorder and epilepsy due to maternally derived duplication of 15q11-q13. Neurology 52:1694–1697

Hallmayer J, Glasson EJ, Bower C, Petterson B, Croen L, Grether J, Risch N (2002) On the twin risk in autism. Am J Hum Genet 71:941–946

Hallmayer J, Cleveland S, Torres A, Phillips J, Cohen B, Torigoe T, Miller J, Fedele A, Collins J, Smith K, Lotspeich L, Croen LA, Ozonoff S, Lajonchere C, Grether JK, Risch N (2011) Genetic heritability and shared environmental factors among twin pairs with autism. Arch Gen Psychiatry 68:1095–1102

Hehir-Kwa JY, Pfundt R, Veltman JA, de Leeuw N (2013) Pathogenic or not? Assessing the clinical relevance of copy number variants. Clin Genet 84:415–421

Herman GE, Henninger N, Ratliff-Schaub K, Pastore M, Fitzgerald S, McBride KL (2007) Genetic testing in autism: how much is enough? Genet Med 9:268–274

Hoischen A, van Bon BW, Rodriguez-Santiago B, Gilissen C, Vissers LE, de Vries P, Janssen I, van Lier B, Hastings R, Smithson SF, Newbury-Ecob R, Kjaergaard S, Goodship J, McGowan R, Bartholdi D, Rauch A, Peippo M, Cobben JM, Wieczorek D, Gillessen-Kaesbach G, Veltman JA, Brunner HG, de Vries BB (2011) De novo nonsense mutations in ASXL1 cause Bohring-Opitz syndrome. Nat Genet 43:729–731

Huguet G, Ey E, Bourgeron T (2013) The genetic landscapes of autism spectrum disorders. Annu Rev Genomics Hum Genet 14:191–213

Ingason A, Kirov G, Giegling I, Hansen T, Isles AR, Jakobsen KD, Kristinsson KT, le Roux L, Gustafsson O, Craddock N, Möller HJ, McQuillin A, Muglia P, Cichon S, Rietschel M, Ophoff RA, Djurovic S, Andreassen OA, Pietiläinen OP, Peltonen L, Dempster E, Collier DA, St Clair D, Rasmussen HB, Glenthøj BY, Kiemeney LA, Franke B, Tosato S, Bonetto C, Saemundsen E, Hreidarsson SJ, GROUP Investigators, Nöthen MM, Gurling H, O'Donovan MC, Owen MJ, Sigurdsson E, Petursson H, Stefansson H, Rujescu D, Stefansson K, Werge T (2011) Maternally derived microduplications at 15q11-q13: implication of imprinted genes in psychotic illness. Am J Psychiatry 168:408–417

Iossifov I, Ronemus M, Levy D, Wang Z, Hakker I, Rosenbaum J, Yamrom B, Lee YH, Narzisi G, Leotta A, Kendall J, Grabowska E, Ma B, Marks S, Rodgers L, Stepansky A, Troge J, Andrews P, Bekritsky M, Pradhan K, Ghiban E, Kramer M, Parla J, Demeter R, Fulton LL, Fulton RS, Magrini VJ, Ye K, Darnell JC, Darnell RB, Mardis ER, Wilson RK, Schatz MC, McCombie WR, Wigler M (2012) De novo gene disruptions in children on the autistic spectrum. Neuron 74:285–299

Jacob HJ (2013) Next-generation sequencing for clinical diagnostics. N Engl J Med 369:1557–1558

Jacquemont ML, Sanlaville D, Redon R, Raoul O, Cormier-Daire V, Lyonnet S, Amiel J, Le Merrer M, Heron D, de Blois MC, Prieur M, Vekemans M, Carter NP, Munnich A, Colleaux L, Philippe A (2006) Array-based comparative genomic hybridisation identifies high frequency of cryptic chromosomal rearrangements in patients with syndromic autism spectrum disorders. J Med Genet 43:843–849

Jiang YH, Sahoo T, Michaelis RC, Bercovich D, Bressler J, Kashork CD, Liu Q, Shaffer LG, Schroer RJ, Stockton DW, Spielman RS, Stevenson RE, Beaudet AL (2004) A mixed epigenetic/genetic model for oligogenic inheritance of autism with a limited role for UBE3A. Am J Med Genet A 131:1–10

Jiang YH, Yuen RK, Jin X, Wang M, Chen N, Wu X, Ju J, Mei J, Shi Y, He M, Wang G, Liang J, Wang Z, Cao D, Carter MT, Chrysler C, Drmic IE, Howe JL, Lau L, Marshall CR, Merico D, Nalpathamkalam T, Thiruvahindrapuram B, Thompson A, Uddin M, Walker S, Luo J, Anagnostou E, Zwaigenbaum L, Ring RH, Wang J, Lajonchere C, Wang J, Shih A, Szatmari P, Yang H, Dawson G, Li Y, Scherer SW (2013) Detection of clinically relevant genetic variants in autism spectrum disorder by whole-genome sequencing. Am J Hum Genet 93:249–263

Johnson CP, Myers SM, American Academy of Pediatrics Council on Children With Disabilities. (2007) Identification and evaluation of children with autism spectrum disorders. Pediatrics 120:1183–1215

Kanner L (1943) Autistic disturbances of affective contact. Nervous Child 2:217–253

Kumar RA, KaraMohamed S, Sudi J, Conrad DF, Brune C, Badner JA, Gilliam TC, Nowak NJ, Cook EH Jr, Dobyns WB, Christian SL (2008) Recurrent 16p11.2 microdeletions in autism. Hum Mol Genet 17:628–638

Laje G, Morse R, Richter W, Ball J, Pao M, Smith AC (2010) Autism spectrum features in Smith-Magenis syndrome. Am J Med Genet C Semin Med Genet 154C:456–462

Laumonnier F, Bonnet-Brilhault F, Gomot M, Blanc R, David A, Moizard MP, Raynaud M, Ronce N, Lemonnier E, Calvas P, Laudier B, Chelly J, Fryns JP, Ropers HH, Hamel BC, Andres C, Barthélémy C, Moraine C, Briault S (2004) X-linked mental retardation and autism are associated with a mutation in the NLGN4 gene, a member of the neuroligin family. Am J Hum Genet 74:552–557

Law LW, Lau TK, Fung TY, Leung TY, Wang CC, Choy KW (2009) De novo 16p13.11 microdeletion identified by high-resolution array CGH in a fetus with increased nuchal translucency. BJOG 116:339–343

Leblond CS, Heinrich J, Delorme R, Proepper C, Betancur C, Huguet G, Konyukh M, Chaste P, Ey E, Rastam M, Anckarsäter H, Nygren G, Gillberg IC, Melke J, Toro R, Regnault B, Fauchereau F, Mercati O, Lemière N, Skuse D, Poot M, Holt R, Monaco AP, Järvelä I, Kantojärvi K, Vanhala R, Curran S, Collier DA, Bolton P, Chiocchetti A, Klauck SM, Poustka F, Freitag CM, Waltes R, Kopp M, Duketis E, Bacchelli E, Minopoli F, Ruta L, Battaglia A, Mazzone L, Maestrini E, Sequeira AF, Oliveira B, Vicente A, Oliveira G, Pinto D, Scherer SW, Zelenika D, Delepine M, Lathrop M, Bonneau D, Guinchat V, Devillard F, Assouline B, Mouren MC, Leboyer M, Gillberg C, Boeckers TM, Bourgeron T (2012) Genetic and functional analyses of SHANK2 mutations suggest a multiple hit model of autism spectrum disorders. PLoS Genet 8:e1002521

Legido A, Jethva R, Goldenthal MJ (2013) Mitochondrial dysfunction in autism. Semin Pediatr Neurol 20:163–175

Lim ET, Raychaudhuri S, Sanders SJ, Stevens C, Sabo A, MacArthur DG, Neale BM, Kirby A, Ruderfer DM, Fromer M, Lek M, Liu L, Flannick J, Ripke S, Nagaswamy U, Muzny D, Reid JG, Hawes A, Newsham I, Wu Y, Lewis L, Dinh H, Gross S, Wang LS, Lin CF, Valladares O, Gabriel SB, dePristo M, Altshuler DM, Purcell SM, NHLBI Exome Sequencing Project, State MW, Boerwinkle E, Buxbaum JD, Cook EH, Gibbs RA, Schellenberg GD, Sutcliffe JS, Devlin B, Roeder K, Daly MJ (2013) Rare complete knockouts in humans: population distribution and significant role in autism spectrum disorders. Neuron 77:235–242

Lupski JR, Belmont JW, Boerwinkle E, Gibbs RA (2011) Clan genomics and the complex architecture of human disease. Cell 147:32–43

Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, Norville JE, Church GM (2013) RNA-guided human genome engineering via Cas9. Science 339:823–826

Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, Skaug J, Shago M, Moessner R, Pinto D, Ren Y, Thiruvahindrapduram B, Fiebig A, Schreiber S, Friedman J, Ketelaars CE, Vos YJ, Ficicioglu C, Kirkpatrick S, Nicolson R, Sloman L, Summers A, Gibbons CA, Teebi A, Chitayat D, Weksberg R, Thompson A, Vardy C, Crosbie V, Luscombe S, Baatjes R, Zwaigenbaum L, Roberts W, Fernandez B, Szatmari P, Scherer SW (2008) Structural variation of chromosomes in autism spectrum disorder. Am J Hum Genet 82:477–488

McCarthy SE, Makarov V, Kirov G, Addington AM, McClellan J, Yoon S, Perkins DO, Dickel DE, Kusenda M, Krastoshevsky O, Krause V, Kumar RA, Grozeva D, Malhotra D, Walsh T, Zackai EH, Kaplan P, Ganesh J, Krantz ID, Spinner NB, Roccanova P, Bhandari A, Pavon K, Lakshmi B, Leotta A, Kendall J, Lee YH, Vacic V, Gary S, Iakoucheva LM, Crow TJ, Christian SL, Lieberman JA, Stroup TS, Lehtimäki T, Puura K, Haldeman-Englert C, Pearl J, Goodell M, Willour VL, Derosse P, Steele J, Kassem L, Wolff J, Chitkara N, McMahon FJ, Malhotra AK, Potash JB, Schulze TG, Nöthen MM, Cichon S, Rietschel M, Leibenluft E, Kustanovich V, Lajonchere CM, Sutcliffe JS, Skuse D, Gill M, Gallagher L, Mendell NR, Wellcome Trust Case Control Consortium, Craddock N, Owen MJ, O'Donovan MC, Shaikh TH, Susser E, Delisi LE, Sullivan PF, Deutsch CK, Rapoport J, Levy DL, King MC, Sebat J (2009) Microduplications of 16p11.2 are associated with schizophrenia. Nat Genet 41:1223–1227

Michaelson JJ, Shi Y, Gujral M, Zheng H, Malhotra D, Jin X, Jian M, Liu G, Greer D, Bhandari A, Wu W, Corominas R, Peoples A, Koren A, Gore A, Kang S, Lin GN, Estabillo J, Gadomski T, Singh B, Zhang K, Akshoomoff N, Corsello C, McCarroll S, Iakoucheva LM, Li Y, Wang J, Sebat J (2012) Whole-genome sequencing in autism identifies hot spots for de novo germline mutation. Cell 151:1431–1442

Miles JH (2011) Autism spectrum disorders–a genetics review. Genet Med 13:278–294

Miller DT NR, Sobeih MM, Shen Y, Wu BL, Hanson E (2009) 16p11.2 microdeletion. Seattle: Gene review internet. www.genetests.org

Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR, Carter NP, Church DM, Crolla JA, Eichler EE, Epstein CJ, Faucett WA, Feuk L, Friedman JM, Hamosh A, Jackson L, Kaminsky EB, Kok K, Krantz ID, Kuhn RM, Lee C, Ostell JM, Rosenberg C, Scherer SW, Spinner NB, Stavropoulos DJ, Tepperberg JH, Thorland EC, Vermeesch JR, Waggoner DJ, Watson MS, Martin CL, Ledbetter DH (2010) Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Am J Hum Genet 86:749–764

Moessner R, Marshall CR, Sutcliffe JS, Skaug J, Pinto D, Vincent J, Zwaigenbaum L, Fernandez B, Roberts W, Szatmari P, Scherer SW (2007) Contribution of SHANK3 mutations to autism spectrum disorder. Am J Hum Genet 81:1289–1297

Myers SM, Johnson CP, American Academy of Pediatrics Council on Children With Disabilities (2007) Management of children with autism spectrum disorders. Pediatrics 120:1162–1182

Nagamani SC, Erez A, Ben-Zeev B, Frydman M, Winter S, Zeller R, El-Khechen D, Escobar L, Stankiewicz P, Patel A, Cheung SW (2013) Detection of copy-number variation in AUTS2 gene by targeted exonic array CGH in patients with developmental delay and autistic spectrum disorders. Eur J Hum Genet 21:343–346

Neale BM, Kou Y, Liu L, Ma'ayan, A, Samocha KE, Sabo A, Lin CF, Stevens C, Wang LS, Makarov V, Polak P, Yoon S, Maguire J, Crawford EL, Campbell NG, Geller ET, Valladares O, Schafer C, Liu H, Zhao T, Cai G, Lihm J, Dannenfelser R, Jabado O, Peralta Z, Nagaswamy U, Muzny D, Reid JG, Newsham I, Wu Y, Lewis L, Han Y, Voight BF, Lim E, Rossin E, Kirby A, Flannick J, Fromer M, Shakir K, Fennell T, Garimella K, Banks E, Poplin R, Gabriel S, DePristo M, Wimbish JR, Boone BE, Levy SE, Betancur C, Sunyaev S, Boerwinkle E, Buxbaum JD, Cook EH Jr, Devlin B, Gibbs RA, Roeder K, Schellenberg GD, Sutcliffe JS, Daly MJ (2012) Patterns and rates of exonic de novo mutations in autism spectrum disorders. Nature 485:242–245

Ng SB, Turner EH, Robertson PD, Flygare SD, Bigham AW, Lee C, Shaffer T, Wong M, Bhattacharjee A, Eichler EE, Bamshad M, Nickerson DA, Shendure J (2009) Targeted capture and massively parallel sequencing of 12 human exomes. Nature 461:272–276

O'Roak BJ, Vives L, Girirajan S, Karakoc E, Krumm N, Coe BP, Levy R, Ko A, Lee C, Smith JD, Turner EH, Stanaway IB, Vernot B, Malig M, Baker C, Reilly B, Akey JM, Borenstein E, Rieder MJ, Nickerson DA, Bernier R, Shendure J, Eichler EE (2012) Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. Nature 485:246–250

Ou Z, Kang SH, Shaw CA, Carmack CE, White LD, Patel A, Beaudet AL, Cheung SW, Chinault AC (2008) Bacterial artificial chromosome-emulation oligonucleotide arrays for targeted clinical array-comparative genomic hybridization analyses. Genet Med 10:278–289

Papenhausen P, Schwartz S, Risheg H, Keitges E, Gadi I, Burnside RD, Jaswaney V, Pappas J, Pasion R, Friedman K, Tepperberg J (2011) UPD detection using homozygosity profiling with a SNP genotyping microarray. Am J Med Genet A 155A:757–768

Peiffer DA, Le JM, Steemers FJ, Chang W, Jenniges T, Garcia F, Haden K, Li J, Shaw CA, Belmont J, Cheung SW, Shen RM, Barker DL, Gunderson KL (2006) High-resolution genomic profiling of chromosomal aberrations using Infinium whole-genome genotyping. Genome Res 16:1136–1148

Phelan MC (2008) Deletion 22q13.3 syndrome. Orphanet J Rare Dis 3:14

Pieretti M, Zhang FP, Fu YH, Warren ST, Oostra BA, Caskey CT, Nelson DL (1991) Absence of expression of the FMR-1 gene in fragile X syndrome. Cell 66:817–822

Pinto D, Pagnamenta AT, Klei L, Anney R, Merico D, Regan R, Conroy J, Magalhaes TR, Correia C, Abrahams BS, Almeida J, Bacchelli E, Bader GD, Bailey AJ, Baird G, Battaglia A, Berney T, Bolshakova N, Bölte S, Bolton PF, Bourgeron T, Brennan S, Brian J, Bryson SE, Carson AR, Casallo G, Casey J, Chung BH, Cochrane L, Corsello C, Crawford EL, Crossett A, Cytrynbaum C, Dawson G, de Jonge M, Delorme R, Drmic I, Duketis E, Duque F, Estes A, Farrar P, Fernandez BA, Folstein SE, Fombonne E, Freitag CM, Gilbert J, Gillberg C, Glessner JT, Goldberg J, Green A, Green J, Guter SJ, Hakonarson H, Heron EA, Hill M, Holt R, Howe JL, Hughes G, Hus V, Igliozzi R, Kim C, Klauck SM, Kolevzon A, Korvatska O, Kustanovich V, Lajonchere CM, Lamb JA, Laskawiec M, Leboyer M, Le Couteur A, Leventhal BL, Lionel AC, Liu XQ, Lord C, Lotspeich L, Lund SC, Maestrini E, Mahoney W, Mantoulan C, Marshall CR, McConachie H, McDougle CJ, McGrath J, McMahon WM, Merikangas A, Migita O, Minshew NJ, Mirza GK, Munson J, Nelson SF, Noakes C, Noor A, Nygren G, Oliveira G, Papanikolaou K, Parr JR, Parrini B, Paton T, Pickles A, Pilorge M, Piven J, Ponting CP, Posey DJ, Poustka A, Poustka F, Prasad A, Ragoussis J, Renshaw K, Rickaby J, Roberts W, Roeder K, Roge B, Rutter ML, Bierut LJ, Rice JP, Salt J, Sansom K, Sato D, Segurado R, Sequeira AF, Senman L, Shah N, Sheffield VC, Soorya L, Sousa I, Stein O, Sykes N, Stoppioni V, Strawbridge C, Tancredi R, Tansey K, Thiruvahindrapduram B, Thompson AP, Thomson S, Tryfon A, Tsiantis J, Van Engeland H, Vincent JB, Volkmar F, Wallace S, Wang K, Wang Z, Wassink TH, Webber C, Weksberg R, Wing K, Wittemeyer K, Wood S, Wu J, Yaspan BL, Zurawiecki D, Zwaigenbaum L, Buxbaum JD, Cantor RM, Cook EH, Coon H, Cuccaro ML, Devlin B, Ennis S, Gallagher L, Geschwind DH, Gill M, Haines JL, Hallmayer J, Miller J, Monaco AP, Nurnberger JI Jr, Paterson AD, Pericak-Vance MA, Schellenberg GD, Szatmari P, Vicente AM, Vieland VJ, Wijsman EM, Scherer SW, Sutcliffe JS, Betancur C (2010) Functional impact of global rare copy number variation in autism spectrum disorders. Nature 466:368–372

Pinto D, Delaby E, Merico D, Barbosa M, Merikangas A, Klei L, Thiruvahindrapuram B, Xu X, Ziman R, Wang Z, Vorstman JA, Thompson A, Regan R, Pilorge M, Pellecchia G, Pagnamenta AT, Oliveira B, Marshall CR, Magalhaes TR, Lowe JK, Howe JL, Griswold AJ, Gilbert J, Duketis E, Dombroski BA, De Jonge MV, Cuccaro M, Crawford EL, Correia CT, Conroy J, Conceição IC, Chiocchetti AG, Casey JP, Cai G, Cabrol C, Bolshakova N, Bacchelli E, Anney R, Gallinger S, Cotterchio M, Casey G, Zwaigenbaum L, Wittemeyer K, Wing K, Wallace S, van Engeland H, Tryfon A, Thomson S, Soorya L, Rogé B, Roberts W, Poustka F, Mouga S, Minshew N, McInnes LA, McGrew SG, Lord C, Leboyer M, Le Couteur AS, Kolevzon A, Jiménez González P, Jacob S, Holt R, Guter S, Green J, Green A, Gillberg C, Fernandez BA, Duque F, Delorme R, Dawson G, Chaste P, Café C, Brennan S, Bourgeron T, Bolton PF, Bölte S, Bernier R, Baird G, Bailey AJ, Anagnostou E, Almeida J, Wijsman EM, Vieland VJ, Vicente AM, Schellenberg GD, Pericak-Vance M, Paterson AD, Parr JR, Oliveira G, Nurnberger JI, Monaco AP, Maestrini E, Klauck SM, Hakonarson H, Haines JL, Geschwind DH, Freitag CM, Folstein SE, Ennis S, Coon H, Battaglia A, Szatmari P, Sutcliffe JS, Hallmayer J[65], Gill M, Cook EH, Buxbaum JD, Devlin B, Gallagher L, Betancur C, Scherer SW (2014) Convergence of genes and cellular pathways dysregulated in autism spectrum disorders. Am J Hum Genet 94:677–694

Poot M, Verrijn Stuart AA, van Daalen E, van Iperen A, van Binsbergen E, Hochstenbach R (2013) Variable behavioural phenotypes of patients with monosomies of 15q26 and a review of 16 cases. Eur J Med Genet 56:346–350

Potocki L, Bi W, Treadwell-Deering D, Carvalho CM, Eifert A, Friedman EM, Glaze D, Krull K, Lee JA, Lewis RA, Mendoza-Londono R, Robbins-Furman P, Shaw C, Shi X, Weissenberger G, Withers M, Yatsenko SA, Zackai EH, Stankiewicz P, Lupski JR (2007) Characterization of Potocki-Lupski syndrome (dup(17)(p11.2p11.2)) and delineation of a dosage-sensitive critical interval that can convey an autism phenotype. Am J Hum Genet 80:633–649

Puvabanditsin S, Nagar MS, Joshi M, Lambert G, Garrow E, Brandsma E (2010) Microdeletion of 16p11.2 associated with endocardial fibroelastosis. Am J Med Genet A 152A:2383–2386

Rehm HL, Bale SJ, Bayrak-Toydemir P, Berg JS, Brown KK, Deignan JL, Friez MJ, Funke BH, Hegde MR, Lyon E, Working Group of the American College of Medical Genetics and Genomics Laboratory Quality Assurance Committee (2013) ACMG clinical laboratory standards for next-generation sequencing. Genet Med 15:733–747

Roberts SE, Dennis NR, Browne CE, Willatt L, Woods G, Cross I, Jacobs PA, Thomas S (2002) Characterisation of interstitial duplications and triplications of chromosome 15q11-q13. Hum Genet 110:227–234

Rodriguez-Flores JL, Fakhro K, Hackett NR, Salit J, Fuller J, Agosto-Perez F, Gharbiah M, Malek JA, Zirie M, Jayyousi A, Badii R, Al-Nabet Al-Marri A, Chouchane L, Stadler DJ, Mezey JG, Crystal RG (2014) Exome sequencing identifies potential risk variants for Mendelian disorders at high prevalence in Qatar. Hum Mutat 35:105–116

Ronald A, Hoekstra RA (2011) Autism spectrum disorders and autistic traits: a decade of new twin studies. Am J Med Genet B Neuropsychiatr Genet 156B:255–274

Ronemus M, Iossifov I, Levy D, Wigler M (2014) The role of de novo mutations in the genetics of autism spectrum disorders. Nat Rev Genet 15:133–141

Rossignol DA, Frye RE (2012) Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis. Mol Psychiatry 17:290–314

Rosti RO, Sadek AA, Vaux KK, Gleeson JG (2014) The genetic landscape of autism spectrum disorders. Dev Med Child Neurol 56:12–18

Samaco RC, Hogart A, LaSalle JM (2005) Epigenetic overlap in autism-spectrum neurodevelopmental disorders: MECP2 deficiency causes reduced expression of UBE3A and GABRB3. Hum Mol Genet 14:483–492

Sanders SJ, Ercan-Sencicek AG, Hus V, Luo R, Murtha MT, Moreno-De-Luca D, Chu SH, Moreau MP, Gupta AR, Thomson SA, Mason CE, Bilguvar K, Celestino-Soper PB, Choi M, Crawford EL, Davis L, Wright NR, Dhodapkar RM, DiCola M, DiLullo NM, Fernandez TV, Fielding-Singh V, Fishman DO, Frahm S, Garagaloyan R, Goh GS, Kammela S, Klei L, Lowe JK, Lund SC, McGrew AD, Meyer KA, Moffat WJ, Murdoch JD, O'Roak BJ, Ober GT, Pottenger RS, Raubeson MJ, Song Y, Wang Q, Yaspan BL, Yu TW, Yurkiewicz IR, Beaudet AL, Cantor RM, Curland M, Grice DE, Günel M, Lifton RP, Mane SM, Martin DM, Shaw CA, Sheldon M, Tischfield JA, Walsh CA, Morrow EM, Ledbetter DH, Fombonne E, Lord C, Martin CL, Brooks AI, Sutcliffe JS, Cook EH Jr, Geschwind D, Roeder K, Devlin B, State MW (2011) Multiple recurrent de novo CNVs, including duplications of the 7q11.23 Williams syndrome region, are strongly associated with autism. Neuron 70:863–885

Sanders SJ, Murtha MT, Gupta AR, Murdoch JD, Raubeson MJ, Willsey AJ, Ercan-Sencicek AG, DiLullo NM, Parikshak NN, Stein JL, Walker MF, Ober GT, Teran NA, Song Y, El-Fishawy P, Murtha RC, Choi M, Overton JD, Bjornson RD, Carriero NJ, Meyer KA, Bilguvar K, Mane SM, Sestan N, Lifton RP, Günel M, Roeder K, Geschwind DH, Devlin B, State MW (2012) De novo mutations revealed by whole-exome sequencing are strongly associated with autism. Nature 485:237–241

Schaaf CP, Goin-Kochel RP, Nowell KP, Hunter JV, Aleck KA, Cox S, Patel A, Bacino CA, Shinawi M (2011) Expanding the clinical spectrum of the 16p11.2 chromosomal rearrangements: three patients with syringomyelia. Eur J Hum Genet 19:152–156

Schaaf CP, Boone PM, Sampath S, Williams C, Bader PI, Mueller JM, Shchelochkov OA, Brown CW, Crawford HP, Phalen JA, Tartaglia NR, Evans P, Campbell WM, Tsai AC, Parsley L, Grayson SW, Scheuerle A, Luzzi CD, Thomas SK, Eng PA, Kang SH, Patel A, Stankiewicz P, Cheung SW (2012) Phenotypic spectrum and genotype-phenotype correlations of NRXN1 exon deletions. Eur J Hum Genet 20:1240–1247

Schaefer GB, Mendelsohn NJ, Professional Practice and Guidelines Committee (2013) Clinical genetics evaluation in identifying the etiology of autism spectrum disorders: 2013 guideline revisions. Genet Med 15:399–407

Schwartz S (2011) Clinical utility of single nucleotide polymorphism arrays. Clin Lab Med 31:581–594, viii

Shen Y, Dies KA, Holm IA, Bridgemohan C, Sobeih MM, Caronna EB, Miller KJ, Frazier JA, Silverstein I, Picker J, Weissman L, Raffalli P, Jeste S, Demmer LA, Peters HK, Brewster SJ, Kowalczyk SJ, Rosen-Sheidley B, McGowan C, Duda AW 3rd, Lincoln SA, Lowe KR, Schonwald A, Robbins M, Hisama F, Wolff R, Becker R, Nasir R, Urion DK, Milunsky JM, Rappaport L, Gusella JF, Walsh CA, Wu BL, Miller DT, Autism Consortium Clinical Genetics/DNA Diagnostics Collaboration (2010) Clinical genetic testing for patients with autism spectrum disorders. Pediatrics 125:e727–e735

Shen Y, Chen X, Wang L, Guo J, Shen J, An Y, Zhu H, Zhu Y, Xin R, Bao Y, Gusella JF, Zhang T, Wu BL (2011) Intra-family phenotypic heterogeneity of 16p11.2 deletion carriers in a three-generation Chinese family. Am J Med Genet B Neuropsychiatr Genet 156:225–232

Shi L, Zhang X, Golhar R, Otieno FG, He M, Hou C, Kim C, Keating B, Lyon GJ, Wang K, Hakonarson H (2013) Whole-genome sequencing in an autism multiplex family. Mol Autism 4:8

Shinawi M, Liu P, Kang SH, Shen J, Belmont JW, Scott DA, Probst FJ, Craigen WJ, Graham BH, Pursley A, Clark G, Lee J, Proud M, Stocco A, Rodriguez DL, Kozel BA, Sparagana S, Roeder ER, McGrew SG, Kurczynski TW, Allison LJ, Amato S, Savage S, Patel A, Stankiewicz P, Beaudet AL, Cheung SW, Lupski JR (2010) Recurrent reciprocal 16p11.2 rearrangements associated with global developmental delay, behavioural problems, dysmorphism, epilepsy, and abnormal head size. J Med Genet 47:332–341

Simons VIP Consortium (2012) Simons Variation in Individuals Project (Simons VIP) a genetics-first approach to studying autism spectrum and related neurodevelopmental disorders. Neuron 73:1063–1067

South ST, Lee C, Lamb AN, Higgins AW, Kearney HM, Working Group for the American College of Medical Genetics and Genomics Laboratory Quality Assurance Committee (2013) ACMG Standards and Guidelines for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications: revision 2013. Genet Med 15:901–909

Stankiewicz P, Inoue K, Bi W, Walz K, Park SS, Kurotaki N, Shaw CJ, Fonseca P, Yan J, Lee JA, Khajavi M, Lupski JR (2003) Genomic disorders: genome architecture results in susceptibility to DNA rearrangements causing common human traits. Cold Spring Harb Symp Quant Biol 68:445–454

Stankiewicz P, Pursley AN, Cheung SW (2010) Challenges in clinical interpretation of microduplications detected by array CGH analysis. Am J Med Genet A 152A:1089–1100

State MW, Levitt P (2011) The conundrums of understanding genetic risks for autism spectrum disorders. Nat Neurosci 14:1499–1506

Steffenburg S, Gillberg C, Hellgren L, Andersson L, Gillberg IC, Jakobsson G, Bohman M (1989) A twin study of autism in Denmark, Finland, Iceland, Norway and Sweden. J Child Psychol Psychiatry 30:405–416

Szatmari P, Paterson AD, Zwaigenbaum L, Roberts W, Brian J, Liu XQ, Vincent JB, Skaug JL, Thompson AP, Senman L, Feuk L, Qian C, Bryson SE, Jones MB, Marshall CR, Scherer SW, Vieland VJ, Bartlett C, Mangin LV, Goedken R, Segre A, Pericak-Vance MA, Cuccaro ML, Gilbert JR, Wright HH, Abramson RK, Betancur C, Bourgeron T, Gillberg C, Leboyer M, Buxbaum JD, Davis KL, Hollander E, Silverman JM, Hallmayer J, Lotspeich L, Sutcliffe JS, Haines JL, Folstein SE, Piven J, Wassink TH, Sheffield V, Geschwind DH, Bucan M, Brown WT, Cantor RM, Constantino JN, Gilliam TC, Herbert M, Lajonchere C, Ledbetter DH, Lese-Martin C, Miller J, Nelson S, Samango-Sprouse CA, Spence S, State M, Tanzi RE, Coon H, Dawson G, Devlin B, Estes A, Flodman P, Klei L, McMahon WM, Minshew N, Munson J, Korvatska E, Rodier PM, Schellenberg GD, Smith M, Spence MA, Stodgell C, Tepper PG, Wijsman EM, Yu CE, Rogé B, Mantoulan C, Wittemeyer K, Poustka A, Felder B, Klauck SM, Schuster C, Poustka F, Bölte S, Feineis-Matthews S, Herbrecht E, Schmötzer G, Tsiantis J, Papanikolaou K, Maestrini E, Bacchelli E, Blasi F, Carone S, Toma C, Van Engeland H, de Jonge M, Kemner C, Koop F, Langemeijer M, Hijmans C, Staal WG, Baird G, Bolton PF, Rutter ML, Weisblatt E, Green J, Aldred C, Wilkinson JA, Pickles A, Le Couteur A, Berney T, McConachie H, Bailey AJ, Francis K, Honeyman G, Hutchinson A, Parr JR, Wallace S, Monaco AP, Barnby G, Kobayashi K, Lamb JA, Sousa I, Sykes N, Cook EH, Guter SJ, Leventhal BL, Salt J, Lord C, Corsello C, Hus V, Weeks DE, Volkmar F, Tauber M, Fombonne E, Shih A, Meyer KJ (2007) Mapping autism risk loci using genetic linkage and chromosomal rearrangements. Nat Genet 39:319–328

Talkowski ME, Mullegama SV, Rosenfeld JA, van Bon BW, Shen Y, Repnikova EA, Gastier-Foster J, Thrush DL, Kathiresan S, Ruderfer DM, Chiang C, Hanscom C, Ernst C, Lindgren AM, Morton CC, An Y, Astbury C, Brueton LA, Lichtenbelt KD, Ades LC, Fichera M, Romano C, Innis JW, Williams CA, Bartholomew D, Van Allen MI, Parikh A, Zhang L, Wu BL, Pyatt RE, Schwartz S, Shaffer LG, de Vries BB, Gusella JF, Elsea SH (2011) Assessment of 2q23.1

microdeletion syndrome implicates MBD5 as a single causal locus of intellectual disability, epilepsy, and autism spectrum disorder. Am J Hum Genet 89:551–563

Thomas NS, Durkie M, Potts G, Sandford R, Van Zyl B, Youings S, Dennis NR, Jacobs PA (2006) Parental and chromosomal origins of microdeletion and duplication syndromes involving 7q11. 23, 15q11-q13 and 22q11. Eur J Hum Genet 14:831–837

Toro R, Konyukh M, Delorme R, Leblond C, Chaste P, Fauchereau F, Coleman M, Leboyer M, Gillberg C, Bourgeron T (2010) Key role for gene dosage and synaptic homeostasis in autism spectrum disorders. Trends Genet 26:363–372

Walters RG, Jacquemont S, Valsesia A, de Smith AJ, Martinet D, Andersson J, Falchi M, Chen F, Andrieux J, Lobbens S, Delobel B, Stutzmann F, El-Sayed Moustafa JS, Chèvre JC, Lecoeur C, Vatin V, Bouquillon S, Buxton JL, Boute O, Holder-Espinasse M, Cuisset JM, Lemaitre MP, Ambresin AE, Brioschi A, Gaillard M, Giusti V, Fellmann F, Ferrarini A, Hadjikhani N, Campion D, Guilmatre A, Goldenberg A, Calmels N, Mandel JL, Le Caignec C, David A, Isidor B, Cordier MP, Dupuis-Girod S, Labalme A, Sanlaville D, Béri-Dexheimer M, Jonveaux P, Leheup B, Ounap K, Bochukova EG, Henning E, Keogh J, Ellis RJ, Macdermot KD, van Haelst MM, Vincent-Delorme C, Plessis G, Touraine R, Philippe A, Malan V, Mathieu-Dramard M, Chiesa J, Blaumeiser B, Kooy RF, Caiazzo R, Pigeyre M, Balkau B, Sladek R, Bergmann S, Mooser V, Waterworth D, Reymond A, Vollenweider P, Waeber G, Kurg A, Palta P, Esko T, Metspalu A, Nelis M, Elliott P, Hartikainen AL, McCarthy MI, Peltonen L, Carlsson L, Jacobson P, Sjöström L, Huang N, Hurles ME, O'Rahilly S, Farooqi IS, Männik K, Jarvelin MR, Pattou F, Meyre D, Walley AJ, Coin LJ, Blakemore AI, Froguel P, Beckmann JS (2010) A new highly penetrant form of obesity due to deletions on chromosome 16p11.2. Nature 463:671–675

Wang NJ, Liu D, Parokonny AS, Schanen NC (2004) High-resolution molecular characterization of 15q11-q13 rearrangements by array comparative genomic hybridization (array CGH) with detection of gene dosage. Am J Hum Genet 75:267–281

Wang K, Zhang H, Ma D, Bucan M, Glessner JT, Abrahams BS, Salyakina D, Imielinski M, Bradfield JP, Sleiman PM, Kim CE, Hou C, Frackelton E, Chiavacci R, Takahashi N, Sakurai T, Rappaport E, Lajonchere CM, Munson J, Estes A, Korvatska O, Piven J, Sonnenblick LI, Alvarez Retuerto AI, Herman EI, Dong H, Hutman T, Sigman M, Ozonoff S, Klin A, Owley T, Sweeney JA, Brune CW, Cantor RM, Bernier R, Gilbert JR, Cuccaro ML, McMahon WM, Miller J, State MW, Wassink TH, Coon H, Levy SE, Schultz RT, Nurnberger JI, Haines JL, Sutcliffe JS, Cook EH, Minshew NJ, Buxbaum JD, Dawson G, Grant SF, Geschwind DH, Pericak-Vance MA, Schellenberg GD, Hakonarson H (2009) Common genetic variants on 5p14.1 associate with autism spectrum disorders. Nature 459:528–533

Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM, Savage M, Platt LD, Saltzman D, Grobman WA, Klugman S, Scholl T, Simpson JL, McCall K, Aggarwal VS, Bunke B, Nahum O, Patel A, Lamb AN, Thom EA, Beaudet AL, Ledbetter DH, Shaffer LG, Jackson L (2012) Chromosomal microarray versus karyotyping for prenatal diagnosis. N Engl J Med 367:2175–2184

Weiss LA (2009) Autism genetics: emerging data from genome-wide copy-number and single nucleotide polymorphism scans. Expert Rev Mol Diagn 9:795–803

Weiss LA, Shen Y, Korn JM, Arking DE, Miller DT, Fossdal R, Saemundsen E, Stefansson H, Ferreira MA, Green T, Platt OS, Ruderfer DM, Walsh CA, Altshuler D, Chakravarti A, Tanzi RE, Stefansson K, Santangelo SL, Gusella JF, Sklar P, Wu BL, Daly MJ, Autism Consortium (2008) Association between microdeletion and microduplication at 16p11.2 and autism. N Engl J Med 358:667–675

Weiss LA, Arking DE, Gene Discovery Project of John Hopkins & Autism Consortiumm, Daly MJ, Chakravarti A (2009) A genome-wide linkage and association scan reveals novel loci for autism. Nature 461:802–808

Williams CA, Lossie A, Driscoll D (2001) Angelman syndrome: mimicking conditions and phenotypes. Am J Med Genet 101:59–64

Wilson HL, Wong AC, Shaw SR, Tse WY, Stapleton GA, Phelan MC, Hu S, Marshall J, McDermid HE (2003) Molecular characterisation of the 22q13 deletion syndrome supports the role of

haploinsufficiency of SHANK3/PROSAP2 in the major neurological symptoms. J Med Genet 40:575–584

Wiszniewska J, Bi W, Shaw C, Stankiewicz P, Kang SH, Pursley AN, Lalani S, Hixson P, Gambin T, Tsai CH, Bock HG, Descartes M, Probst FJ, Scaglia F, Beaudet AL, Lupski JR, Eng C, Wai Cheung S, Bacino C, Patel A (2014) Combined array CGH plus SNP genome analyses in a single assay for optimized clinical testing. Eur J Hum Genet 22:79–87

Yang Y, Muzny DM, Reid JG, Bainbridge MN, Willis A, Ward PA, Braxton A, Beuten J, Xia F, Niu Z, Hardison M, Person R, Bekheirnia MR, Leduc MS, Kirby A, Pham P, Scull J, Wang M, Ding Y, Plon SE, Lupski JR, Beaudet AL, Gibbs RA, Eng CM (2013) Clinical whole-exome sequencing for the diagnosis of mendelian disorders. N Engl J Med 369:1502–1511

Yu TW, Chahrour MH, Coulter ME, Jiralerspong S, Okamura-Ikeda K, Ataman B, Schmitz-Abe K, Harmin DA, Adli M, Malik AN, D'Gama AM, Lim ET, Sanders SJ, Mochida GH, Partlow JN, Sunu CM, Felie JM, Rodriguez J, Nasir RH, Ware J, Joseph RM, Hill RS, Kwan BY, Al-Saffar M, Mukaddes NM, Hashmi A, Balkhy S, Gascon GG, Hisama FM, LeClair E, Poduri A, Oner O, Al-Saad S, Al-Awadi SA, Bastaki L, Ben-Omran T, Teebi AS, Al-Gazali L, Eapen V, Stevens CR, Rappaport L, Gabriel SB, Markianos K, State MW, Greenberg ME, Taniguchi H, Braverman NE, Morrow EM, Walsh CA (2013) Using whole-exome sequencing to identify inherited causes of autism. Neuron 77:259–273

Zecavati N, Spence SJ (2009) Neurometabolic disorders and dysfunction in autism spectrum disorders. Curr Neurol Neurosci Rep 9:129–136

Zhu L, Wang X, Li XL, Towers A, Cao X, Wang P, Bowman R, Yang H, Goldstein J, Li YJ, Jiang YH (2014) Epigenetic dysregulation of SHANK3 in brain tissues from individuals with autism spectrum disorders. Hum Mol Genet 23:1563–1578

Zwaigenbaum L, Bryson S, Lord C, Rogers S, Carter A, Carver L, Chawarska K, Constantino J, Dawson G, Dobkins K, Fein D, Iverson J, Klin A, Landa R, Messinger D, Ozonoff S, Sigman M, Stone W, Tager-Flusberg H, Yirmiya N (2009) Clinical assessment and management of toddlers with suspected autism spectrum disorder: insights from studies of high-risk infants. Pediatrics 123:1383–1391

Zweier C, de Jong EK, Zweier M, Orrico A, Ousager LB, Collins AL, Bijlsma EK, Oortveld MA, Ekici AB, Reis A, Schenck A, Rauch A (2009) CNTNAP2 and NRXN1 are mutated in autosomal-recessive Pitt-Hopkins-like mental retardation and determine the level of a common synaptic protein in Drosophila. Am J Hum Genet 85:655–666

Chapter 5
Epigenetic Regulation in Autism

Cyril J. Peter, Abraham Reichenberg and Schahram Akbarian

Abstract The exploration of brain epigenomes, including DNA methylation and covalent histone modifications, has provided novel insights into the mechanisms of normal and diseased brain development, and furthermore, deleterious mutations and rare structural variants in more than 50 genes encoding various types of chromatin regulators have been linked to autism spectrum disorders. In this book chapter, we will provide a general introduction on the basic principles of epigenetic regulation, and then discuss matters of epigenetic heritability as it pertains to autism spectrum disorders, highlight monogenic forms of the disorder associated with disordered chromatin structure and function, summarize the current knowledge base as it pertains to epigenetic regulation during normal aging and development, including the alterations that were reported in postmortem brain studies in autism spectrum disorders. We conclude the chapter with a brief discussion on novel epigenetic therapies for neurodevelopmental disease.

Keywords Nucleosome · Chromatin fiber · Neuronal epigenome · Histone · Post-translational histone modification · DNA methylation · Chromatin remodeling

Autism spectrum disorder (ASD) is a summary term for neurodevelopmental conditions bound together by broad syndromic overlap in three key behavioral domains including deficits in social interaction, communication, and restricted, stereotypical or repetitive behavior. ASDs also present with intellectual disability, neurological manifestations including seizure disorder, and other movement disorders. The age of onset of ASD almost always falls within early childhood, mostly within the first 36 months after birth. It is currently estimated that one of every 88 children born in the US will be diagnosed with ASD.

Behavioral genetic studies using twin and family study designs provide compelling evidence for a strong genetic contribution (Lichtenstein et al. 2010), yet environmental influences may also be etiologically important (Lichtenstein et al. 2010; Hallmayer et al. 2011). Indeed, over the course of the last 10 years, advances in molecular genetic methods have led significant developments in exploring the

S. Akbarian (✉) · C. J. Peter · A. Reichenberg
Department of Psychiatry, Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, Hess Center for Science and Medicine, Room 9-105, 1470 Madison Avenue, 10029 New York, NY, USA
e-mail: Schahram.akbarian@mssm.edu

S. Hossein Fatemi (ed.), *The Molecular Basis of Autism,*
Contemporary Clinical Neuroscience, DOI 10.1007/978-1-4939-2190-4_5

genetic risk architecture of ASD. Thus, approximately 10% of so-called sporadic cases of ASD are thought to be the direct result of deletions, duplications and other copy number variants affecting several kilo- to megabases of a select set of chromosomal loci which, when mutated, carry high penetrance/high disease risk. Single nucleotide polymorphisms and allelic variants, many of which are fairly common (defined as minor allele frequency above 5%) in the general population, also make a significant contribution to the ASD genetic risk architecture, which shows significant overlap in a range of psychiatric disorders such as depression, schizophrenia and attention-deficit hyperactivity disorder.

However, despite these advances in ASD genetics, which undoubtedly continue to occur at rapid pace, the genetic risk architecture remains poorly understood for a large majority, or at least 70–85% of cases. Therefore, additional efforts will be required to obtain better insights into the underlying neurobiology of ASD, requiring—like for any complex medical condition—a multipronged approach that will reach far beyond clinical genetics. Here, we discuss the potential contribution of epi-(*greek* for "over," "above") genetics, a term which we apply broadly to describe the regulation and organization of chromatin structures and, more generally, three-dimensional genome architectures and functions. After providing

i. a general introduction on the basic principles of epigenetic regulation, we will discuss
ii. epigenetic heritability,
iii. monogenic forms of ASD due to mutations in genes encoding chromatin regulatory proteins,
iv. epigenetic regulation in the human brain during normal aging and development,
v. epigenetic dysregulation in the autistic brain and
vi. novel epigenetic therapies for neurodevelopmental disease and then conclude with
vii. a synopsis and outlook.

5.1 Chromatin and Epigenetic Regulation—General Principles

The elementary unit of chromatin is the nucleosome, or 146 bp of genomic DNA wrapped around an octamer of core histones, connected by linker DNA and linker histones. The collective set of covalent DNA & histone modifications and variant histones provide the major building blocks for the 'epigenome', or the epigenetic landscapes that define the functional architecture of the genome, including its organization into many tens of thousands of transcriptional units, clusters of condensed chromatin and other features that are differentially regulated in different cell types and developmental stages of the organism (Rodriguez-Paredes and Esteller 2011; Li and Reinberg 2011). For an in depth description for some of the epigenetic markings, see (Zhou et al. 2011; Ederveen et al. 2011; Kinney et al. 2011).

Common terminology used in chromatin studies includes (i) *nucleosomes*, comprised of a protein octamer of 4 small proteins, the *nucleosome core histones,* around which 146 bp of DNA is wrapped around. Transcription start sites are often defined by a nucleosome-free interval, probably for increased access of the transcriptional initiation complex and other regulators of gene expression. Arrays of nucleosomes, connected by linker DNA and linker histones, comprise the 10 nm 'beads-on-a-string' chromatin fiber; (ii) *Euchromatin* defines loose chromatin typically at sites of actively transcribed genes and units poised for transcription; (iii) *Heterochromatin* defines tightly packed nucleosomal arrays. The terms *Euchromatin* and *Heterochromatin* were initially described by their differential microscopic appearance (Heitz 1928). Constitutive heterochromatin remains highly condensed in most interphase nuclei. Examples include pericentric and telomeric repeat DNA, the inactivated X-chromosome ('Barr body') of female somatic cells, and other chromosomal structures often found in close proximity to the nuclear envelope and also around the nucleolus (see Fig. 5.1). Facultative heterochromatin includes silenced genes that upon differentiation or other stimuli could switch to a state of active transcription.

DNA (Hydroxy)-Methylation: Two related but functionally very different types of DNA modifications, methylation (m) and hydroxymethylation (hm) of cytosines in CpG dinucleotides, provide the bulk of the epigenetic modifications in vertebrate DNA (Kriaucionis and Heintz 2009). There are additional types of DNA modifications, which are mostly chemical intermediates in the context of mC5 and hmC5 (cytosines methylated at the carbon 5 position) synthesis and breakdown (Ito et al. 2011). While the majority of DNA (hydroxy)-methylation is found at sites of CpG dinucleotides and, more generally, in the CpG enriched sequences of the genome, a recent study in rat cerebral cortex reported that up to 25% of mC5 in brain is found at nonCpG sites, a fraction that is far higher than previously assumed (Xie et al. 2012). The mC5 and hmC5 markings show a differential (but not mutually exclusive) pattern of genomic occupancy. The hmC5 mark is concentrated towards the 5' end of genes and the proximal most portion of transcriptional units, and broadly correlates with local gene expression levels (Jin et al. 2011; Song et al. 2011). There is increasing evidence that the genome-wide distribution of hmC5 is regulated in tissue-specific manner. For example, in brain, one of the tissues with highest levels of hmC5, the mark is enriched in many active genes (Mellen et al. 2012) and could play a role in the regulation of intron/exon boundaries and splicing events of neuron-specific gene transcripts (Khare et al. 2012). Some of these findings in brain resonate with observations on the hmC5 distribution in embryonic stem cells. For example, (Wu et al. 2011) performed genome-wide profile of 5hmC in both wild-type and Tet1-depleted mouse embryonic stem (ES) cells, and their data suggest that 5hmC is enriched at both gene bodies of actively transcribed genes and extended promoter regions of Polycomb-repressed developmental regulators. Furthermore, there is evidence that hmC5 is also enriched in enhancer region marked by H3K4me1 and H3K27ac in human embryonic stem cells (Stroud et al. 2011; Szulwach et al. 2011).

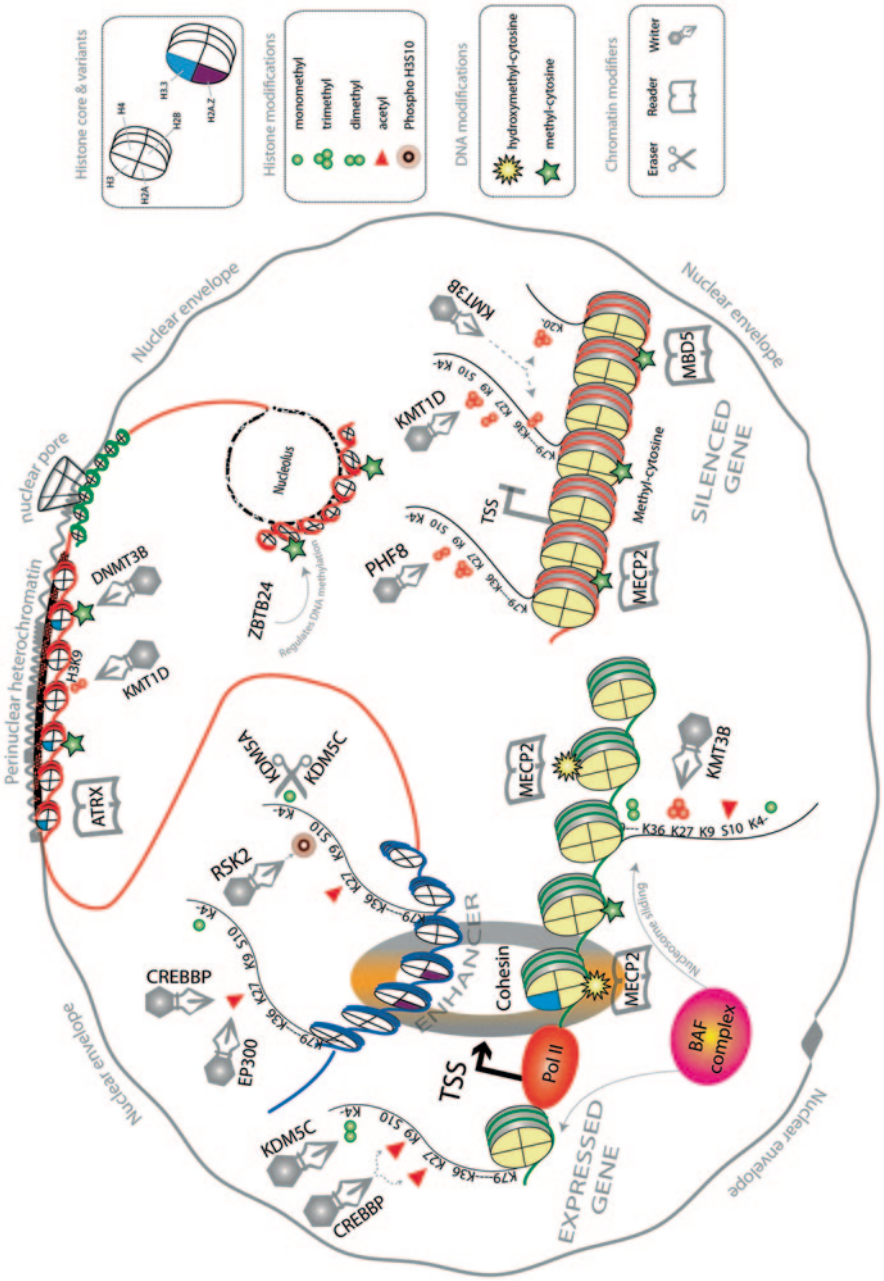


Fig. 5.1 The Epigenome, from nucleus to nucleosome. Schematic illustration of gene (*green*) poised for transcription by polymerase II (Pol II) initiation complex, with nucleosome free interval at transcription start site (*TSS*). distal enhancer sequence (*blue*) which in loop-like structure moves in close proximity to active gene. Subset of heterochromatic portions of the genome (*red*), including silenced genes, border the nuclear envelope and pore complex and the nucleolar periphery. A

On the other hand, less than <3% of methyl-cytosine (mC5) markings are positioned around the 5' end of genes (Maunakea et al. 2010). The classical concept on the transcriptional regulatory role of DNA methylation, which also has guided many brain related studies, is that promoter-bound repressive chromatin remodeling complexes negatively regulate transcription (Sharma et al. 2005). There are many studies that report changes in promoter DNA methylation (mostly in conjunction with decreased gene expression) in preclinical models of psychosis, depression and addiction, as well as in brain tissue in subjects diagnosed with one of these conditions. Interestingly, however, while the largest amount, or 97%, of mC5s are found in intra- and intergenic sequences and within DNA repeats (Maunakea et al. 2010), only few of these studies have explored brain DNA methylation changes at repeat DNA and other sequences outside of promoters.

Histone Modifications: The epigenetic regulation of chromatin by virtue of chemical histone modifications is even more complex than DNA methylation discussed above, and it is now thought that there are far more than 100 amino acid residue-specific post-translational modifications (PTMs) in a typical vertebrate cell (Tan et al. 2011), including mono (me1), di (me2)- and tri (me3) methylation, acetylation and crotonylation, polyADP-ribosylation and small protein (ubiquitin, SUMO) modification of specific lysine residues, as well as arginine (R) methylation and 'citrullination', serine (S) phosphorylation, tyrosine (T) hydroxylation, and several others (Kouzarides 2007; Taverna et al. 2007; Tan et al. 2011). These site- and residue-specific PTMs are typically explored in the context of chromatin structure and function, with an epigenetic histone code (a combinatorial set of histone PTMs that differentiates between promoters, gene bodies, enhancer and other regulatory sequences, condensed heterochromatin, and so on) (Zhou et al. 2011). It is important to emphasize that histone PTMs rarely occur in isolation, and instead multiple histone PTMs appear to be co-regulated and, as a group, define the aforementioned chromatin states (Berger 2007). Many active promoters, for example, are defined by high levels of histone H3 lysine 4 methylation in combination with various histone lysine acetylation markings (Zhou et al. 2011). Repressive histone PTMs, including the trimethylated forms of H3K9, H3K27 and H4K20, potentially co-localize to some of the same loci in the genome, and so forth. Furthermore, there is also

small subset of representative histone variants and histone H3 site-specific lysine (K) residues at N-terminal tail (K4, K9, K27, K36, K79) and H4K20 residue are shown as indicated, together with panel of mono- and trimethyl, or acetyl modifications that differentiate between active promoters, transcribed gene bodies, and repressive chromatin, as indicated. DNA cytosines that are hydroxymethylated at the C5 position are in the nervous system most prominent at active promoters and gene bodies, while methylated cytosines are positioned around repressed promoters and in constitutive heterochromatin, and within the body of some of the actively transcribed genes. Specific examples of chromatin regulatory proteins associated with monogenic forms of neurodevelopmental disorders, including autism are provided. For an updated listing of the >50 genes encoding chromatin regulators and neurodevelopmental risk genes, which includes not only (i) various regulators of DNA methylation and histone PTM and variants, but also (ii) multiple components of the cohesin complex which tethers together promoter-enhancer and other types of chromosomal loopings, and (iii) multiple members of the BAF nucleosome sliding/chromatin remodeling complex, see (Ronan et al. 2013)

evidence for a coordinated and sequential regulation; phosphorylation of histone H3 at the serine (S)10 position often serves as a trigger for subsequent acetylation of neighboring lysine residues histone H3 lysine 9 (H3K9) and lysine 14 (H3K14) in the context of transcriptional activation, while at the same time blocking repression-associated methylation of H3K9 (Nowak and Corces 2004). Of note, proteins associated with the regulation of histone PTMs are sometimes referred to as 'writers', or 'erasers' or 'readers', essentially differentiating between the process of establishing or removing a mark as opposed to its docking functions for chromatin remodeling complexes that regulate transcription, or induce and maintain chromatin condensation (Taverna et al. 2007; Mosammaparast and Shi 2010; Justin et al. 2010). Obviously, the concept of chromatin 'reader', 'writer' and 'eraser' proteins could easily be expanded to proteins associated with DNA methylation (Fig. 5.1).

Histone Variants: In addition to the core histones H2A/H2B/H3/H4, histone variants such as H3.3, H2A.Z and H2A.X exist. The role of these variant histones, which differ from the canonical histones only at very few amino acid positions, is often discussed in the context of replication-independent expression and assembly (Woodcock 2006), and several histone variants robustly affect nucleosome stability and compaction (Jin and Felsenfeld 2007). One popular model postulates that during the process of gene expression, RNA polymerase and the transcriptional activation and elongator complexes destabilize nucleosomes, which in turn promotes nucleosome remodeling and variant histone incorporation which then further potentiate or stabilize gene expression (Sutcliffe et al. 2009; Bintu et al. 2011).

Chromatin-Bound RNAs (CBRs): While the process of gene expression is obviously defined by nascent RNA emerging from genomic DNA packaged into chromatin, the term CBR could be reserved to RNA species as part of a chromatin structure, thereby regulating its functions. None of these definitions are mutually exclusive, however. According to some estimates, up to 2–3 % of the nucleic acid content in chromatin is contributed by polyadenylated RNAs (Rodriguez-Campos and Azorin 2007). One of the best known examples of a CBR is provided by the *X-chromosome Inactive Transcript (XIST)* (Brockdorff 2013; Zhao et al. 2008).

Perhaps one of the most illustrative and complex examples as it pertains to ASD-associated CBR involves chromosome 15q11-13, a highly regulated locus, subject to genomic imprinting (parent-of-origin-specific gene expression) and responsible for a range of neurodevelopmental syndromes, including Prader-Willi and Angelman, as well as for a subset of cases diagnosed with ASD (Leung et al. 2011). Furthermore, DNA structural variants within this locus could contribute to genetic risk to schizophrenia and bipolar disorder, further emphasizing that this locus is broadly relevant for a range of neuropsychiatric disease (Leung et al. 2011). Of note, a very large ncRNA arises from *15q11-13*, covering 1 Mb in the mouse and 600 kb in humans and 148 exons and introns (Le Meur et al. 2005). This long *SNPRN-UBE3A* ncRNA, which normally is highly expressed on the paternal but not on the maternal chromosome, includes clusters of smaller non-coding RNAs that are thought to modulate nucleolar functions in neurons, and an antisense transcript, *UBE3A-AS* which suppresses UBE3A sense transcription of the same gene on the paternal chro-

mosome (Leung et al. 2011). Evidence has been that the *SNPRN-UBE3A* ncRNA, and the smaller RNAs derived from it, produce a 'RNA cloud' in *cis*, which contributes to lasting decondensation of this locus on the paternal chromosome, including epigenetic decoration with open chromatin-bound histone modifications and loss of repressive chromatin-associated histone and DNA methylation (Leung et al. 2011; Xin et al. 2001). Interestingly, UBE3A (also known as E6-AP) encodes a ubiquitin ligase that targets RING-1B, a component of Polycomb repressive complex PRC1, for its subsequent degradation (Zaaroor-Regev et al. 2010). Because PRC1 is a key regulator for genome-wide repressive histone (H3K27) methylation, dysregulated expression of long *SNPRN-UBE3A* ncRNA in the context of 15q11-13 imprinting disorders and/or genetic mutations and polymorphisms, may affect orderly activity of the PRC1 complex in developing brain (Vogel et al. 2006; Tarabykin et al. 2000; Golden and Dasen 2012), perhaps resulting in chromatin defects across widespread portions of neuronal or glial genomes, with serious implications for brain function and behavior.

Chromatin Remodeling and Nucleosome Positioning: Chromatin remodeling complexes are comprised of multiple subunits, that according to their classical definition regulate sliding and mobility of nucleosomes, powered by ATP hydrolysis, thereby regulating gene expression and RNA polymerase II access at transcription start sites (Ronan et al. 2013). Examples of well known chromatin remodelers with a critical role in brain development include the BAF (SWI/SNF) complex and CHD family of proteins (Ronan et al. 2013). Interestingly, mutations in numerous members of the BAF complex and multiple CHD proteins have now been linked to psychiatric disease and developmental brain disorders (Ronan et al. 2013).

Higher Order Chromatin Structures: Epigenetic decoration of nucleosomes, including the DNA and histone modifications, and histone variants described above, in itself, would fall short to adequately describe the epigenome, or even the localized chromatin architecture at any given (genomic) locus. This is because nucleosomal organization leads to only a 7-fold increase in packaging density of the genetic material, as compared to naked DNA; however, the actual level of compaction in the vertebrate nucleus in interphase (which defines the nucleus during the time period a cell is not dividing, including postmitotic cells such as neurons) is about three orders of magnitude higher (Belmont 2006). The chromosomal arrangements in the interphase nucleus are not random, however. Specifically, loci at sites of active gene expression are more likely to be clustered together and positioned towards a central position within the nucleus, while heterochromatin and silenced loci move more towards the nuclear periphery (Cremer and Cremer 2001; Duan et al. 2010). Chromosomal loopings, in particular, are among the most highly regulated 'supranucleosomal' structures and are associated with transcriptional regulation, by, for example, positioning distal regulatory enhancer or silencer elements that—in the linear genome—are positioned potentially many hundred of kilobases apart from a gene, to interact directly with that specific promoter (Wood et al. 2010; Gaszner and Felsenfeld 2006).

The regulation of higher order chromatin is certainly of critical importance for human health, including orderly brain development and function. For example, Cornelia de Lange Syndrome (CdLS) with an estimated incidence of 1:10–30,000 live births among the more frequent genetic disorders (source http://ghr.nlm.nih.gov) is associated with severe developmental delay and a range of neuropsychiatric symptoms, including ASD and psychosis (Moss et al. 2008). CdLS (including *Online Mendelian Inheritance of Man (OMIM)* 122470 and 300590) involves causative mutations in the cohesin complex, a multisubunit protein that includes, among others, nipped B-like protein (NIPBL), structural maintenance of chromosomal proteins SMC1A and SMC3, and histone deacetylase HDAC8 (Deardorff et al. 2012; Gervasini et al. 2013). Cohesin is thought to form ring-like structures bringing together DNA segments from different locations, and by interaction with transcriptional co-activators such as the Mediator multi-protein complex, these protein networks could provide the foundation for chromosomal loop formations, including promoter-enhancer loopings that define cell-type specific gene expression programs (Kagey et al. 2010).

Thus, there can be little doubt that epigenetic regulation is of critical importance for orderly brain development. The list of chromatin regulators (Ronan et al. 2013) includes not only methyl-CpG-binding proteins (incl. *MECP2*, the Rett Syndrome gene) but also multiple members of the BAF and also CHD complexes regulating nucleosome mobility (Ronan et al. 2013). Another pathway with multiple genes found to have mutations in larger ASD cohorts includes H3K4 methyltransferases and demethylases *MLL1* (Jones et al. 2012), *MLL2* (Hannibal et al. 2011), *MLL3* (O'Roak et al. 2012; Kleefstra et al. 2012; Neale et al. 2012), and *KDM5A*, *KDM5C/JARID1C/SMCX* (Adegbola et al. 2008; Najmabadi et al. 2011; Jensen et al. 2005). As we pointed out above, these detailed findings from clinical genetics will require further workup of the cell type(s) and developmental stage(s) at risk, because the neurobiology of disease driven by these genes and their mutations remains essentially unresolved.

5.2 'Epigenetic Heritability' in ASD?

Significant strides have been achieved in unraveling the genetic risk architecture of ASD. Thus, it is now estimated that up to 30 % of cases harbor either (i) identifiable chromosomal microdeletions and duplications (copy number variants) that often encompass many hundreds of kilobases of sequence on the linear genome (7–20 % of cases), or (ii) single gene disorders such as Fragile X, Rett Syndrome and others that in toto account for 5–7 % of all cases, and another (iii) 5 % of cases are due to metabolic diseases including mitochrondrial disease, phenylketonuria, adenylosuccinate lyase deficiency etc (Schaaf and Zoghbi 2011). This still leaves 70 % of cases with no straightforward genetic explanation, albeit genetic approaches such as whole exome and whole genome sequencing are beginning to make additional inroads in the complex field of ASD genetics, mostly by identifying additional rare variants

of potentially high disease risk (Gratten et al. 2013; Shi et al. 2013). There are also ongoing refinements in mapping some of the common (mostly single nucleotide) polymorphisms that contribute to the genetic risk of ASD and other psychiatric and neurodevelopmental disorders (Smoller et al. 2013).

In addition to the above described genetic risk structure of ASD—there is both indirect and direct evidence for epigenetic component(s) in the etiology of the disorder. At the population level, twin studies have almost exclusively been used to demonstrate substantial *genetic* contribution to ASD etiology. However, it is important to underscore that the concordance rates are not exceeding 50 % in monozygotic twin studies in ASD and related disease (Steffenburg et al. 1989; Bailey et al. 1995; Chang et al. 2013)—providing ample evidence for the role of environmental factors, and for epigenetic processes in the etiology of ASD. The framework for the epigenetics of ASD includes an increasing list of deleterious mutations and DNA structural variants directly resulting in dysregulated chromatin, including several portions of chromosomes 15q and 7q that are classical sites for genomic imprinting, as defined by allele-specific DNA methylation signatures differentially regulated on the maternally vs. paternally derived chromosomes (Schanen 2006). Therefore, it is entirely possible that disruption of gene expression via epigenetic mechanisms not reflected in the primary nucleotide sequence, or so called 'epialleles', either due to *de novo* dysregulation in very early development, or by inheritance and carry-over of epigenetic information through the parental germline, plays an important role in some causes of ASD and other psychiatric disorders.

Here, we define epigenetic heritability simply as a phenotype or predisposition that is transmitted from one generation to the next, by some sort of molecular information in the germ cell, that is not encoded by the base pair DNA sequence of the (transmitted) genome. It is often speculated that 'abnormal' epigenetic decoration of the haploid germ cell genome (including DNA and histone modifications, see below) contribute to heritable risk of common (including psychiatric) disorders but convincing evidence, or even proof, of 'true epigenetic heritability' in disorders such as ASD is extremely difficult to accomplish with present day technologies, because it would, among others, require demonstration that DNA structural variants either in cis (at the site of the epigenetic defect) or trans (other locations in the genome) were not driving the chromatin alterations. Nonetheless, there is an increasing amount of correlative evidence in support of epigenetic heritability in humans, including the role of the ancestral nutritional environment in metabolic disease risk in children and grandchildren (Rando 2012). In the following sections, we summarize some of the most interesting findings as it pertains to the possibility for disease-relevant epigenetic heritability:

1. Nucleosomal organization and DNA:histone octamer packaging is retained in 4 % of the haploid genome in sperm, and these retained nucleosomes are enriched significantly at loci of developmental importance, including imprinted gene clusters, microRNA clusters, homeobox (HOX) gene clusters, and the promoters of stand-alone developmental transcription and signaling factors (Hammoud et al. 2009). Furthermore, DNA methylation patterns and histone

modifications are also preserved in the portion of the genome that maintains nucleosomal organization in sperm cells (Hammoud et al. 2009; Okada et al. 2010). The limitations of present day genome technology, including the lack of genome-wide high quality sequence information when only few cells are available as input has so far prevented our ability to map the epigenome of human oocytes (McGraw et al. 2013); however, high quality DNA methylation maps have also been established for mouse oocytes (Shirane et al. 2013; Smallwood et al. 2011). Therefore, in principle, chromatin templates, including their epigenetic markings, could be passed on to the next generation for perhaps as much as 4 % of the human genome (Hammoud et al. 2009), and therefore the portion of the genome subject to transgenerational epigenetic inheritance would indeed be larger than the entire protein coding sequence ('exome') which amounts to 'only' 1–1.5 % of the genome.

2. Paternal age, (and specifically advanced parental age), has been linked to an increased risk for neurodevelopmental disorders, including ASD, and much of this effect could be attributed to an age-associated risk of rare de novo mutation, with a doubling of the mutational burden through the paternal age every 16 years, according to some of the more widely publicized findings (Kong et al. 2012). Twin studies have demonstrated that advancing paternal age modifies twin concordance for autism (Lundstrom et al. 2012). There is evidence that advanced grandpaternal age contributes independently to increase the risk for ASD by approximately 1.7-fold both for the maternal and paternal lines (Frans et al. 2013). Preclinical work in mice and rat would suggest that, at least for the paternal age effect, epigenetic mechanism could play a role too. Thus, brain of offspring from older fathers showed DNA methylation abnormalities at multiple imprinted gene loci (imprinted loci show parent-of-origin specific epigenetic regulation and are by some authors considered to be more sensitive to epigenetic pertubations) (Smith et al. 2013). In addition, aberrant DNA methylation in sperm of older animals has been observed at DNA repeats encoding ribosomal genes (Oakes et al. 2003).
3. In the rodent, there is ample evidence for transgenerational effects of environmental influences, including nutrition, stress and drugs, impacting brain and behavior for at least one, or few generations. These involve DNA methylation and histone modification changes in a number of molecules involved in serotonergic signaling and neurotrophins (including brain-derived neurotrophic factor (Bdnf)) (Bohacek and Mansuy 2013; Morgan and Bale 2011; Vassoler et al. 2013).
4. In humans, adverse environmental conditions, including famine around the time of conception, have been linked to lasting DNA methylation changes at the site of some of the best studied imprinted gene loci, including IGF2R/H19 (Heijmans et al. 2008) and could contribute to the increased risk for neurodevelopmental and also endocrine disorders that are associated with these conditions, including diabetes and schizophrenia (Lumey et al. 2011).

5. Maternal prenatal use of prescribed medications has been recently associated with risk for ASD. Risks for ASD have been reported for selective serotonin reuptake inhibitors (SSRI) and other monoamine reuptake inhibitors (Rai et al. 2013), and mood stabilizers such as valproate (Meador and Loring 2013; Christensen et al. 2013). Psychiatric and neurologic medications are used in >3% of women of child-bearing potential, all cross the placenta and animal models of their exposure result in aberrant neurodevelopment (Walsh et al. 2008; Pardo and Eberhart 2007). Adverse effects of these drugs include interference in GABAergic, dopaminergic, serotoninergic, and glutamatergic pathways. Importantly, they have been shown to effect DNA methylation (Mill et al. 2008; Tsankova et al. 2007; Melas et al. 2012) and histone modifications (Peter and Akbarian 2011; Li et al. 2004).

5.3 Monogenic Forms of ASD Due to Mutations in Genes Encoding Chromatin Regulatory Proteins

The list of mutations carrying a high risk for ASD, even as singular genetic factors, includes an increasing number of genes encoding a chromatin regulatory protein. This is not too surprising, because chromatin remodeling and proper assignment of epigenetic marks is of fundamental importance for brain ontogenesis and a key control point in the stepwise transition from pluripotency to neural precursor to terminally differentiated neurons and glia (Ho and Crabtree 2010), and involved in developmental events such as neuronal migration and connectivity formation (Fuentes et al. 2011). On the other hand, ASD-associated chromatin disorders were initially known only in the context of embryonic defects and multi-organ syndromes, with some of the more recently discovered gene defects affecting selectively the brain without significant involvement of peripheral organs. Well known examples of the former type of gene defects include Rubinstein-Taybi syndrome (RSTS) 1 (*Online Mendelian Inheritance of Man*, OMIM 180849) and 2 (OMIM 613684) (Roelfsema and Peters 2007), as well as the Immunodeficiency, centromere instability, facial anomalies (ICF1) mental retardation syndrome (OMIM 242860) (de Greef et al. 2011; Ehrlich et al. 2008), among others. The most well known example of the latter type of gene defects includes Rett Syndrome, an X-linked neurological disorder of early childhood (OMIM 312750). To date, deleterious mutations in over 50 different chromatin regulators have been associated with psychiatric disorders including ASD and schizophrenia (Ronan et al. 2013).

5.4 Epigenetic Regulation in the Human Brain During Normal Development and Aging—Implications for ASD

Chromatin remodeling and epigenetic mechanisms are involved in developmental events such as neuronal migration and connectivity formation (Fuentes et al. 2011). Furthermore, there is increasing evidence that the *normal* course of maturation and aging is associated with changes in the brain's epigenome. On the one hand, this is an attractive hypothesis given that there are widespread age-related changes in gene expression in the cerebral cortex, including downregulation of many neuronal genes (Erraji-Benchekroun et al. 2005; Tang et al. 2009). However, in contrast to the accumulation of somatic mutations and other structural brain DNA changes that affect promoter function during aging (which are likely to be irreversible) (Lu et al. 2004), most or perhaps all epigenetic markings studied to date are now thought to be reversible, and there is no *a priori* reason for unidirectional accumulation of a specific epigenetic mark in aging brain chromatin. Nonetheless, an increasing body of literature indicates that a substantial reorganization of the epigenome occurs during postnatal development and aging. Human cerebral cortex, for example, shows complex and gene-specific changes in levels of methyl-cytosine (mC5; cytosines are methylated at the carbon 5 position), with a steady rise at many promoters that continues into old age in conjunction with subtle changes (mostly a decline) in gene expression (Siegmund et al. 2007; Hernandez et al. 2011).

The increasing list of monogenic forms of neurodevelopmental disease due to mutations in genes encoding specific chromatin remodelers speaks to the importance of these mechanisms for brain ontogenesis and early maturation. It is interesting to note that DNA and histone methylation signatures show, on a genome-wide scale, the most dramatic changes during prenatal development, and the transition phases from perinatal period to early infancy and the perinatal period; in striking contrast, epigenetic changes during subsequent periods of maturation and aging, including puberty and various phases of adulthood, are comparatively minor (Siegmund et al. 2007; Numata et al. 2012; Shulha et al. 2013; Cheung et al. 2010). Cell-type specific epigenome profilings confirmed that these developmental changes are not explained by the various shifts in neuron-to glia ratios and other changes in cell type composition and instead, point to large scale chromatin remodeling in cortical neurons during prenatal and early postnatal development (Cheung et al. 2010; Shulha et al. 2013; Siegmund et al. 2007).

Thus, one could conclude that immature neurons are, both in utero and for the first few months or years after birth, particularly vulnerable to epigenetic pertubations, and some of these could have played a role in the etiology of some cases on the autism spectrum. This hypothesis is further supported by the observation that prenatal exposure to certain types of drugs, including the short chain fatty acid derivative and histone deacetylase inhibitor, sodium valproate, is associated with a 2–3 fold increase in the risk of the offspring to develop ASD (Christensen et al. 2013). Because epigenetic mechanisms literally serve as a molecular bridge linking

the genome to the environment, it is worth noting in this context that a recent large twin cohort attributed the estimated risk of shared in utero environment at 30–80 % for ASD, exceeding the estimated genetic risk of 14–67 % (Hallmayer et al. 2011).

5.5 Gene Expression and Chromatin Alterations in ASD Postmortem Brain

ASD, like many other psychiatric disorders, lacks a unifying neuropathology and is probably comprised of a diverse set of syndromes of considerable genetic and etiologic heterogeneity. Interestingly, however, studies even in fairly small postmortem brain cohorts with typically less than 20 cases and equal number of controls have shown significant, disease-associated changes in RNA and protein levels. For example, there is literature reporting downregulated $GABA_A$ and $GABA_B$ receptor expression and ligand binding across multiple areas of the cerebral cortex and cerebellum, in conjunction with altered expression of GABA synthesis enzymes and GABA neuron-specific peptides in cerebral cortex, hippocampus and cerebellum, reviewed in (Blatt and Fatemi 2011). As in other conditions, including schizophrenia (Akbarian and Huang 2006), changes in the GABAergic transcriptome appear to affect a significant portion of cases on the autism spectrum, albeit the reported RNA alterations are not sufficiently consistent to reach the level of significance in all of the postmortem studies (Huang et al. 2010). Nonetheless, this work in the clinical tissue samples provided one of the cornerstones for the popular hypothesis that alterations in the balance of excitatory and inhibitory (E/I) activity play a critical role in the pathophysiology of a substantial number of cases on the autism spectrum, affecting cortical inhibitory (GABAergic) circuitry and synchronization of electrical activity across widespread brain regions in the autistic brain (Uhlhaas and Singer 2012; Rubenstein 2010).

The complete set of gene expression changes in the autistic brain are likely to go far beyond these GABAergic RNAs. For example, recent microarray studies identified a diverse set of neuronal genes and noncoding RNAs, many of which positioned in genomic loci conferring genetic ASD susceptibility and that showed altered expression in prefrontal and temporal cortex or cerebellar cortices (Voineagu et al. 2011; Ziats and Rennert 2013), with additional gene expression changes indicative of a dysfunction of immune regulation in at least some of the disease cases (Voineagu et al. 2011). Given that transcriptional regulation is closely associated with dynamic changes in chromatin structure and function (Lee and Young 2013), it would not be too surprising that these gene expression alterations in the diseased brain are associated with epigenetic changes in cis-regulatory sequences such as transcription start sites.

However, one challenge for epigenetic studies in brain is the enormous cellular heterogeneity of the postmortem tissue, with its mixed population of glutamatergic and GABAergic neurons, mature oligodendrocytes and astrocytes and their precursors as well as endothelial cells. Furthermore, some brains from subjects on the

autism spectrum show evidence for inflammation, including activation and proliferation of microglia as the brain's immune surveillance cells (Theoharides et al. 2013; Suzuki et al. 2013; Tetreault et al. 2012). This is an important problem given that cell-type specific differences in DNA and histone methylation landscapes are, on a genome-wide scale, far greater than, for example, developmentally regulated changes within a specific cell type, or species-specific regulation in the primate brain (Shulha et al. 2013, 2012b; Cheung et al. 2010). Thus, conventional chromatin assays, which are designed to detect and quantify DNA methylation and histone modifications requiring an input material between 10^3–10^8 nuclei (Huang et al. 2006; Adli and Bernstein 2011), could be severly confounded by changes in glia-to-neuron ratios that could show considerable fluctuations across different developmental or disease states. To date, many studies exploring epigenetic dysregulation of gene expression in major psychiatric disorders examined DNA methylation and histone modifications in tissue homogenates, thus ignoring the fact that the gene(s)-of-interest often are expressed only in a select subpopulation of neurons or other cells.

To bypass these limitations, a recent postmortem brain study, conducted on 16 subjects on the autism spectrum and 16 controls of child and adult age, profiled the transcriptional mark, histone H3-trimethylated at lysine 4, on a genome-wide scale in prefrontal cortical neurons and separately, in non-neuronal chromatin from the same cases (Shulha et al. 2012a). The study identified 711 "epigenetic risk" loci were affected in variable subsets of autistic individuals, including the synaptic vesicle gene *RIMS3*, the retinoic acid signaling regulated gene *RAI1* (Nakamine et al. 2008), the histone demethylase *JMJD1C* (Castermans et al. 2007), the astrotactin *ASTN2* (Glessner et al. 2009), the adhesion molecules *NRCAM* (Sakurai et al. 2006; Bonora et al. 2005; Petek et al. 2001) and *SEMA5* (Weiss et al. 2009; Melin et al. 2006), the ubiquitin ligase *PARKIN2* (*PARK2*) (Scheuerle and Wilson 2011; Glessner et al. 2009) and many other genes for which rare structural DNA variations could carry high disease penetrance. Intriguingly, for most of these epigenetic changes, the H3K4me3 changes occurred selectively in prefrontal neurons, but not in their surrounding non-neuronal cells (Shulha et al. 2012a). Therefore, the disease-associated epigenetic signatures in ASD are cell-type specific and, at least in neurons, show significant overlap with the genetic risk architecture of neurodevelopmental disorders (Shulha et al. 2012a).

Another study, using peripheral blood cells from monozygotic twins discordant for ASD, identified multiple disease-associated differentially DNA methylated regions, which would suggest that at least some of the epigenetic alterations in subjects with ASD may be unrelated to the genetic risk architectures in the affected cases (Wong et al. 2013).

5.6 Epigenetic Drug Targets to Treat ASD

To date, no medication has been approved for the treatment of core symptoms of ASD, including social and other cognitive defects and problems associated with language and communication and interaction (Payakachat et al. 2012). Preliminary data suggest that intranasal application of the nonapeptide, oxytocin, when administered in research and laboratory settings, elicits a modest improvement in repetitive behaviors and some of the test measures for social cognition (Bakermans-Kranenburg and van Ijzendoorn 2013). In addition, antipsychotic medications (originally designed to treat schizophrenia and other types of psychosis) such as risperidone and aripiprazole demonstrated improvement in parent-reported measures of challenging behaviors such as repetitive or aggressive behaviors, but carry a significant side-effect burden (Payakachat et al. 2012). Other types of drugs, including anticonvulsants and anxiolytics may be very beneficial in selected cases to treat seizures and anxiety but obviously do not address the core symptoms. Thus, there is a pressing need to pursue novel and innovative treatment options for these types of conditions. It is not unreasonable to explore promising epigenetic drug targets in ASD.

One recent, impressive example for epigenetic drug targets is provided by topoisomerases (topos), DNA cleaving enzymes that are important in the processes for replication and recombination, transcription and chromatin remodeling (Salerno et al. 2010). A recent study using an unbiased high-content screening approach, using mouse primary cortical neurons, discovered that a diverse group of molecules, that share an inhibitory activity against DNA topoisomerase type I or II enzymes can unlock the expression of the normally epigenetically silenced paternal allele of the gene encoding ubiquitin protein ligase E3A (*Ube3a*). These topo inhibitors mediate their effect by reducing the expression of the imprinted *Ube3a* antisense RNA (*Ube3a-ATS*) (Huang et al. 2011). The expression of this antisense RNA is normally repressed in the maternal chromosome in conjunction with the allele-specific DNA methylation of an imprinting center (a DNA or chromatin structure that carries epigenetic information about parental origin) (Bressler et al. 2001). Similar to hundreds of other loci defined by parent-of-origin-specific gene expression, *Ube3a* was considered epigenetically stable throughout life (Reik 2007). This hypothesis now needs to be revised, however, given that even a single intrathecal infusion of the FDA-approved topoisomerase inhibitor topotecan was sufficient to relieve silencing of the paternal *Ube3a* (sense) transcript in lumbar spinal neurons for an extended period of at least 3 months (Huang et al. 2011). The most obvious explanation for topotecan's mechanism of action—altered DNA methylation of the *Ube3a* imprinting center—has been ruled out, thus the underlying mechanism(s) remain a mystery. Thus, the reactivation of paternal *UBE3A* expression via topoisomerase inhibition could provide a starting point to investigate potential therapies for Angelman syndrome, which is caused by loss of function mutations and deletions at the maternal *UBE3A* locus (Kishino et al. 1997; Matsuura et al. 1997; Sutcliffe et al. 1997), and for which there are currently no effective treatments. Currently, it remains unknown whether topoisomerase-mediated reversal of imprinting-related

gene expression is specific to the *UBE3A* locus. However, this issue could be addressed by, for example, fusing topoisomerase enzymes to customized motifs for sequence-specific binding at *UBE3A*. For example, zinc finger nucleases or transcription activator-like (TAL) effectors of plant pathogenic bacteria have been fused to the FokI restriction enzyme, allowing the induction of 'custom-made' DNA strand breaks at specific and even at unique loci in the genome (Porteus and Baltimore 2003; Bogdanove and Voytas 2011). An even more promising toolkit was recently introduced to the field, utilizing a genome defense mechanism in bacteria, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas (CRISPR-associated) (Bhaya et al. 2011). The Type II CRISPR/Cas system requires a single protein, Cas9, to catalyze DNA cleavage (Sapranauskas et al. 2011) and is now widely used to induce targeted mutations in eukaryotic systems, including human and mouse cells (Cong et al. 2013).

Of note, drugs that inhibit histone deacetylases (these enzymes typically target a wide range of histone and non-histone proteins for deacetylation) might have therapeutic potential in depression and other psychiatric illnesses (Morris et al. 2010; Covington et al. 2009; Schroeder et al. 2007) but caution is warranted given that the already mentioned histone deacetylase inhibitor (HDACi), sodium valproate, confers a 2–3 fold increase in causation of ASD following prenatal exposure (Christensen et al. 2013). Curiously, in a rat model for prenatal valproate exposure, the ensuing deficits in social cognition could be significantly ameliorated with HDACi (Foley et al. 2012), thereby drawing a distinction between the adverse effects of HDACi (or at least of one HDACi drug, valproate) during prenatal development on the one hand and potential therapeutic benefits later in life.

Interestingly, data from 85,000 participants in the prospective Norwegian Mother and Child Cohort study showed that prenatal exposure to folic acid (also known as Vitamin B9) around the time of conception was associated with a significant decrease in the risk for ASD associated with severe language delay in the offspring (Suren et al. 2013). Of note, folic acid is an essential substrate for the generation of methyl-Vitamin B12. Moreover, the folate and vitamin B_{12}-dependent enzyme, methionine synthase, affects molecular and cellular mechanisms, including DNA and histone methylation modifications, and is expressed at very high levels in human cerebral cortex at 28 weeks of gestation, then shows an exponential decline, resulting in 400-fold lower levels in aged (80+ years) control subjects; this protein shows abnormally low levels in brain of some cases diagnosed with ASD (Muratore et al. 2013). These extremely interesting observations justify more detailed follow-up work, because when viewed together, they definitively point to the importance of general regulators of cellular methylation metabolism for normal and diseased neurodevelopment. Furthermore, DNA methylation inhibitors, including the cytidine analogues 5-azacytidine (5-Aza-CR), zebularine and nucleoside analogs that sequester DNMT enzymes after being incorporated into DNA (Kelly et al. 2010), when administered directly into brain tissue, disrupt synaptic plasticity and hippocampal learning and memory, and thereby act as powerful modulators of reward and addiction behaviors (Levenson et al. 2006; Han et al. 2010; Miller and Sweatt 2007; LaPlant et al. 2010). In addition, inhibitors for the histone H3K9-specific

methyltransferases G9a/Glp (Kubicek et al. 2007) strongly enhance the development of reward behaviors in mice exposed to the stimulant drug cocaine (Maze et al. 2010). It will be interesting to explore, in the preclinical model, the role of these specific DNA and histone methylation inhibitors on social cognition and other behavioral domains specifically important for ASD.

5.7 Synopsis and Outlook

In the living cell, the functional definition of the human genome goes far beyond its linear sequence of 6 (or when haploid, 3) billion basepairs. It is the 'epi-(*greek for* '*over*', '*above*')genome', with a rich cache of highly regulated structural modifications of DNA cytosine and histone residues and variants, which defines the moldings and three-dimensional structure of the genomic material inside the cell nucleus, thereby providing a molecular bridge between genes and 'the environment', and orchestrating the myriads of transcriptional units, condensed chromatin clusters and many of the other features that distinguish between various cell types and development- or disease-states sharing the same genome within the same subject. Rapid advances in our knowledge about the basic principles of epigenetic regulation, and more specifically, epigenetic mechanisms in the developing nervous system, are dramatically reshaping current thinking of neurological and psychiatric disease. A number of postmortem studies of autism and control brains provided convincing evidence that dysregulation of chromatin structure and function is part of the pathophysiology of disease, at least in some of the affected cases. Furthermore, based on the current knowledge base from clinical genetics, deleterious mutations and structural variants in at least 50 genes, each encoding a different chromatin-associated protein, are associated with intellectual disability and ASD, further emphasizing that the fine-tuning of epigenetic regulation is broadly relevant for the developing human brain. Preclinical work, complemented by prospective and epidemiological studies, are beginning to identify promising epigenetic drug targets for the treatment of neurodevelopmental disorders. It is the authors expectation that in the near future, the blossoming field of neuroepigenetics will significantly contribute to a better understanding of the neurobiology of ASD, and perhaps pave the way for novel and effective treatments and prevention.

Acknowledgements Work in the author's laboratory is supported by the National Institutes of Health and the Brain & Behavior Research Foundation.

References

Adegbola A, Gao H, Sommer S, Browning M (2008) A novel mutation in JARID1C/SMCX in a patient with autism spectrum disorder (ASD). Am J Med Genet Part A 146A:505–511

Adli M, Bernstein BE (2011) Whole-genome chromatin profiling from limited numbers of cells using nano-ChIP-seq. Nat Protoc 6:1656–1668

Akbarian S, Huang HS (2006) Molecular and cellular mechanisms of altered GAD1/GAD67 expression in schizophrenia and related disorders. Brain Res Rev 52:293–304
Bailey A, Le Couteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E, Rutter M (1995) Autism as a strongly genetic disorder: evidence from a British twin study. Psychol Med 25:63–77
Bakermans-Kranenburg MJ, van Ijzendoorn MH (2013) Sniffing around oxytocin: review and meta-analyses of trials in healthy and clinical groups with implications for pharmacotherapy. Transl Psychiatry 3:e258
Belmont AS (2006) Mitotic chromosome structure and condensation. Curr Opin Cell Biol 18:632–638
Berger SL (2007) The complex language of chromatin regulation during transcription. Nature 447:407–412
Bhaya D, Davison M, Barrangou R (2011) CRISPR-Cas systems in bacteria and archaea: versatile small RNAs for adaptive defense and regulation. Annu Rev Genet 45:273–297
Bintu L, Kopaczynska M, Hodges C, Lubkowska L, Kashlev M, Bustamante C (2011) The elongation rate of RNA polymerase determines the fate of transcribed nucleosomes. Nat Struct Mol Biol 18:1394–1399
Blatt GJ, Fatemi SH (2011) Alterations in GABAergic biomarkers in the autism brain: research findings and clinical implications. Anat Rec (Hoboken) 294:1646–1652
Bogdanove AJ, Voytas DF (2011) TAL effectors: customizable proteins for DNA targeting. Science 333:1843–1846
Bohacek J, Mansuy IM (2013) Epigenetic inheritance of disease and disease risk. Neuropsychopharmacology 38:220–236
Bonora E, Lamb JA, Barnby G, Sykes N, Moberly T, Beyer KS, Klauck SM, Poustka F, Bacchelli E, Blasi F, Maestrini E, Battaglia A, Haracopos D, Pedersen L, Isager T, Eriksen G, Viskum B, Sorensen EU, Brondum-Nielsen K, Cotterill R, Engeland H, Jonge M, Kemner C, Steggehuis K, Scherpenisse M, Rutter M, Bolton PF, Parr JR, Poustka A, Bailey AJ, Monaco AP (2005) Mutation screening and association analysis of six candidate genes for autism on chromosome 7q. Eur J Hum Genet 13:198–207
Bressler J, Tsai TF, Wu MY, Tsai SF, Ramirez MA, Armstrong D, Beaudet AL (2001) The SNRPN promoter is not required for genomic imprinting of the Prader-Willi/Angelman domain in mice. Nat Genet 28:232–240
Brockdorff N (2013) Noncoding RNA and Polycomb recruitment. RNA 19:429–442
Castermans D, Vermeesch JR, Fryns JP, Steyaert JG, Van de Ven WJ, Creemers JW, Devriendt K (2007) Identification and characterization of the TRIP8 and REEP3 genes on chromosome 10q21.3 as novel candidate genes for autism. Eur J Hum Genet 15:422–431
Chang Z, Lichtenstein P, Asherson PJ, Larsson H (2013) Developmental twin study of attention problems: high heritabilities throughout development. JAMA Psychiatry 70:311–318
Cheung I, Shulha HP, Jiang Y, Matevossian A, Wang J, Weng Z, Akbarian S (2010) Developmental regulation and individual differences of neuronal H3K4me3 epigenomes in the prefrontal cortex. Proc Natl Acad Sci U S A 107:8824–8829
Christensen J, Gronborg TK, Sorensen MJ, Schendel D, Parner ET, Pedersen LH, Vestergaard M (2013) Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. JAMA 309:1696–1703
Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F (2013) Multiplex genome engineering using CRISPR/Cas systems. Science 339:819–823
Covington HE, 3rd, Maze I, LaPlant QC, Vialou VF, Ohnishi YN, Berton O, Fass DM, Renthal W, Rush AJ, 3rd, Wu EY, Ghose S, Krishnan V, Russo SJ, Tamminga C, Haggarty SJ, Nestler EJ (2009) Antidepressant actions of histone deacetylase inhibitors. J Neurosci 29:11451–11460
Cremer T, Cremer C (2001) Chromosome territories, nuclear architecture and gene regulation in mammalian cells. Nat Rev Genet 2:292–301
de Greef JC, Wang J, Balog J, den Dunnen JT, Frants RR, Straasheijm KR, Aytekin C, van der Burg M, Duprez L, Ferster A, Gennery AR, Gimelli G, Reisli I, Schuetz C, Schulz A, Smeets DF, Sznajer Y, Wijmenga C, van Eggermond MC, van Ostaijen-Ten Dam MM, Lankester AC,

van Tol MJ, van den Elsen PJ, Weemaes CM, van der Maarel SM (2011) Mutations in ZBTB24 are associated with immunodeficiency, centromeric instability, and facial anomalies syndrome type 2. Am J Hum Genet 88:796–804

Deardorff MA, Bando M, Nakato R, Watrin E, Itoh T, Minamino M, Saitoh K, Komata M, Katou Y, Clark D, Cole KE, De Baere E, Decroos C, Di Donato N, Ernst S, Francey LJ, Gyftodimou Y, Hirashima K, Hullings M, Ishikawa Y, Jaulin C, Kaur M, Kiyono T, Lombardi PM, Magnaghi-Jaulin L, Mortier GR, Nozaki N, Petersen MB, Seimiya H, Siu VM, Suzuki Y, Takagaki K, Wilde JJ, Willems PJ, Prigent C, Gillessen-Kaesbach G, Christianson DW, Kaiser FJ, Jackson LG, Hirota T, Krantz ID, Shirahige K (2012) HDAC8 mutations in Cornelia de Lange syndrome affect the cohesin acetylation cycle. Nature 489:313–317

Duan Z, Andronescu M, Schutz K, McIlwain S, Kim YJ, Lee C, Shendure J, Fields S, Blau CA, Noble WS (2010) A three-dimensional model of the yeast genome. Nature 465:363–367

Ederveen TH, Mandemaker IK, Logie C (2011) The human histone H3 complement anno 2011. Biochim Biophys Acta 1809:577–586

Ehrlich M, Sanchez C, Shao C, Nishiyama R, Kehrl J, Kuick R, Kubota T, Hanash SM (2008) ICF, an immunodeficiency syndrome: DNA methyltransferase 3B involvement, chromosome anomalies, and gene dysregulation. Autoimmunity 41:253–271

Erraji-Benchekroun L, Underwood MD, Arango V, Galfalvy H, Pavlidis P, Smyrniotopoulos P, Mann JJ, Sibille E (2005) Molecular aging in human prefrontal cortex is selective and continuous throughout adult life. Biol Psychiatry 57:549–558

Foley AG, Gannon S, Rombach-Mullan N, Prendergast A, Barry C, Cassidy AW, Regan CM (2012) Class I histone deacetylase inhibition ameliorates social cognition and cell adhesion molecule plasticity deficits in a rodent model of autism spectrum disorder. Neuropharmacol 63:750–760

Frans EM, Sandin S, Reichenberg A, Langstrom N, Lichtenstein P, McGrath JJ, Hultman CM (2013) Autism risk across generations: a population-based study of advancing grandpaternal and paternal age. JAMA Psychiatry 70:516–521

Fuentes P, Canovas J, Berndt FA, Noctor SC, Kukuljan M (2011) CoREST/LSD1 control the development of pyramidal cortical neurons. Cereb Cortex 22:1431–1441

Gaszner M, Felsenfeld G (2006) Insulators: exploiting transcriptional and epigenetic mechanisms. Nat Rev Genet 7:703–713

Gervasini C, Parenti I, Picinelli C, Azzollini J, Masciadri M, Cereda A, Selicorni A, Russo S, Finelli P, Larizza L (2013) Molecular characterization of a mosaic NIPBL deletion in a Cornelia de Lange patient with severe phenotype. Eur J Med Genet 56:138–143

Glessner JT, Wang K, Cai G, Korvatska O, Kim CE, Wood S, Zhang H, Estes A, Brune CW, Bradfield JP, Imielinski M, Frackelton EC, Reichert J, Crawford EL, Munson J, Sleiman PM, Chiavacci R, Annaiah K, Thomas K, Hou C, Glaberson W, Flory J, Otieno F, Garris M, Soorya L, Klei L, Piven J, Meyer KJ, Anagnostou E, Sakurai T, Game RM, Rudd DS, Zurawiecki D, McDougle CJ, Davis LK, Miller J, Posey DJ, Michaels S, Kolevzon A, Silverman JM, Bernier R, Levy SE, Schultz RT, Dawson G, Owley T, McMahon WM, Wassink TH, Sweeney JA, Nurnberger JI, Coon H, Sutcliffe JS, Minshew NJ, Grant SF, Bucan M, Cook EH, Buxbaum JD, Devlin B, Schellenberg GD, Hakonarson H (2009) Autism genome-wide copy number variation reveals ubiquitin and neuronal genes. Nature 459:569–573

Golden MG, Dasen JS (2012) Polycomb repressive complex 1 activities determine the columnar organization of motor neurons. Genes Dev 26:2236–2250

Gratten J, Visscher PM, Mowry BJ, Wray NR (2013) Interpreting the role of de novo protein-coding mutations in neuropsychiatric disease. Nature Genet 45:234–238

Hallmayer J, Cleveland S, Torres A, Phillips J, Cohen B, Torigoe T, Miller J, Fedele A, Collins J, Smith K, Lotspeich L, Croen LA, Ozonoff S, Lajonchere C, Grether JK, Risch N (2011) Genetic heritability and shared environmental factors among twin pairs with autism. Arch Gen Psychiatry 68:1095–1102

Hammoud SS, Nix DA, Zhang H, Purwar J, Carrell DT, Cairns BR (2009) Distinctive chromatin in human sperm packages genes for embryo development. Nature 460:473–478

Han J, Li Y, Wang D, Wei C, Yang X, Sui N (2010) Effect of 5-aza-2-deoxycytidine microinjecting into hippocampus and prelimbic cortex on acquisition and retrieval of cocaine-induced place preference in C57BL/6 mice. Eur J Pharmacol 642:93–98

Hannibal MC, Buckingham KJ, Ng SB, Ming JE, Beck AE, McMillin MJ, Gildersleeve HI, Bigham AW, Tabor HK, Mefford HC, Cook J, Yoshiura K, Matsumoto T, Matsumoto N, Miyake N, Tonoki H, Naritomi K, Kaname T, Nagai T, Ohashi H, Kurosawa K, Hou JW, Ohta T, Liang D, Sudo A, Morris CA, Banka S, Black GC, Clayton-Smith J, Nickerson DA, Zackai EH, Shaikh TH, Donnai D, Niikawa N, Shendure J, Bamshad MJ (2011) Spectrum of MLL2 (ALR) mutations in 110 cases of Kabuki syndrome. Am J M Genet Part A 155A:1511–1516

Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, Slagboom PE, Lumey LH (2008) Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci U S A 105:17046–17049

Heitz E (1928) Das Heterochromatin der Moose. Jahrbuch Wiss Bot 69:762–780

Hernandez DG, Nalls MA, Gibbs JR, Arepalli S, van der Brug M, Chong S, Moore M, Longo DL, Cookson MR, Traynor BJ, Singleton AB (2011) Distinct DNA methylation changes highly correlated with chronological age in the human brain. Hum Mol Genet 20:1164–1172

Ho L, Crabtree GR (2010) Chromatin remodelling during development. Nature 463:474–484

Huang HS, Matevossian A, Jiang Y, Akbarian S (2006) Chromatin immunoprecipitation in postmortem brain. J Neurosci Methods 156:284–292

Huang HS, Cheung I, Akbarian S (2010) RPP25 is developmentally regulated in prefrontal cortex and expressed at decreased levels in autism spectrum disorder. Autism Res 3:153–161

Huang HS, Allen JA, Mabb AM, King IF, Miriyala J, Taylor-Blake B, Sciaky N, Dutton JW, Lee HM, Chen X, Jin J, Bridges AS, Zylka MJ, Roth BL, Philpot BD (2011) Topoisomerase inhibitors unsilence the dormant allele of Ube3a in neurons. Nature 481:185–189

Ito S, Shen L, Dai Q, Wu SC, Collins LB, Swenberg JA, He C, Zhang Y (2011) Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. Science 333:1300–1303

Jensen LR, Amende M, Gurok U, Moser B, Gimmel V, Tzschach A, Janecke AR, Tariverdian G, Chelly J, Fryns JP, Van Esch H, Kleefstra T, Hamel B, Moraine C, Gecz J, Turner G, Reinhardt R, Kalscheuer VM, Ropers HH, Lenzner S (2005) Mutations in the JARID1C gene, which is involved in transcriptional regulation and chromatin remodeling, cause X-linked mental retardation. Am J Hum Genet 76:227–236

Jin C, Felsenfeld G (2007) Nucleosome stability mediated by histone variants H3.3 and H2A.Z. Genes Dev 21:1519–1529

Jin SG, Wu X, Li AX, Pfeifer GP (2011) Genomic mapping of 5-hydroxymethylcytosine in the human brain. Nucleic Acids Res 39:5015–5024

Jones WD, Dafou D, McEntagart M, Woollard WJ, Elmslie FV, Holder-Espinasse M, Irving M, Saggar AK, Smithson S, Trembath RC, Deshpande C, Simpson MA (2012) De novo mutations in MLL cause Wiedemann-Steiner syndrome. Am J Hum Genet 91:358–364

Justin N, De Marco V, Aasland R, Gamblin SJ (2010) Reading, writing and editing methylated lysines on histone tails: new insights from recent structural studies. Curr Opin Struct Biol 20:730–738

Kagey MH, Newman JJ, Bilodeau S, Zhan Y, Orlando DA, van Berkum NL, Ebmeier CC, Goossens J, Rahl PB, Levine SS, Taatjes DJ, Dekker J, Young RA (2010) Mediator and cohesin connect gene expression and chromatin architecture. Nature 467:430–435

Kelly TK, De Carvalho DD, Jones PA (2010) Epigenetic modifications as therapeutic targets. Nat Biotechnol 28:1069–1078

Khare T, Pai S, Koncevicius K, Pal M, Kriukiene E, Liutkeviciute Z, Irimia M, Jia P, Ptak C, Xia M, Tice R, Tochigi M, Morera S, Nazarians A, Belsham D, Wong AH, Blencowe BJ, Wang SC, Kapranov P, Kustra R, Labrie V, Klimasauskas S, Petronis A (2012) 5-hmC in the brain is abundant in synaptic genes and shows differences at the exon–intron boundary. Nat Struct Mol Biol 19:1037–1043

Kinney SM, Chin HG, Vaisvila R, Bitinaite J, Zheng Y, Esteve PO, Feng S, Stroud H, Jacobsen SE, Pradhan S (2011) Tissue-specific distribution and dynamic changes of 5-hydroxymethylcytosine in mammalian genomes. J Biol Chem 286:24685–24693

Kishino T, Lalande M, Wagstaff J (1997) UBE3A/E6-AP mutations cause Angelman syndrome. Nat Genet 15:70–73

Kleefstra T, Kramer JM, Neveling K, Willemsen MH, Koemans TS, Vissers LE, Wissink-Lindhout W, Fenckova M, van den Akker WM, Kasri NN, Nillesen WM, Prescott T, Clark RD, Devriendt K, van Reeuwijk J, de Brouwer AP, Gilissen C, Zhou H, Brunner HG, Veltman JA, Schenck A, van Bokhoven H (2012) Disruption of an EHMT1-associated chromatin-modification module causes intellectual disability. Am J Hum Genet 91:73–82

Kong A, Frigge ML, Masson G, Besenbacher S, Sulem P, Magnusson G, Gudjonsson SA, Sigurdsson A, Jonasdottir A, Jonasdottir A, Wong WS, Sigurdsson G, Walters GB, Steinberg S, Helgason H, Thorleifsson G, Gudbjartsson DF, Helgason A, Magnusson OT, Thorsteinsdottir U, Stefansson K (2012) Rate of de novo mutations and the importance of father's age to disease risk. Nature 488:471–475

Kouzarides T (2007) Chromatin modifications and their function. Cell 128:693–705

Kriaucionis S, Heintz N (2009) The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. Science 324:929–930

Kubicek S, O'Sullivan RJ, August EM, Hickey ER, Zhang Q, Teodoro ML, Rea S, Mechtler K, Kowalski JA, Homon CA, Kelly TA, Jenuwein T (2007) Reversal of H3K9me2 by a small-molecule inhibitor for the G9a histone methyltransferase. Mol Cell 25:473–481

LaPlant Q, Vialou V, Covington HE, 3rd, Dumitriu D, Feng J, Warren BL, Maze I, Dietz DM, Watts EL, Iniguez SD, Koo JW, Mouzon E, Renthal W, Hollis F, Wang H, Noonan MA, Ren Y, Eisch AJ, Bolanos CA, Kabbaj M, Xiao G, Neve RL, Hurd YL, Oosting RS, Fan G, Morrison JH, Nestler EJ (2010) Dnmt3a regulates emotional behavior and spine plasticity in the nucleus accumbens. Nat Neurosci 13:1137–1143

Le Meur E Watrin F Landers M Sturny R Lalande M Muscatelli F (2005) Dynamic developmental regulation of the large non-coding RNA associated with the mouse 7C imprinted chromosomal region. Dev Biol 286:587–600

Lee TI, Young RA (2013) Transcriptional regulation and its misregulation in disease. Cell 152:1237–1251

Leung KN, Chamberlain SJ, Lalande M, LaSalle JM (2011) Neuronal chromatin dynamics of imprinting in development and disease. J Cell Biochem 112:365–373

Levenson JM, Roth TL, Lubin FD, Miller CA, Huang IC, Desai P, Malone LM, Sweatt JD (2006) Evidence that DNA (cytosine-5) methyltransferase regulates synaptic plasticity in the hippocampus. J Biol Chem 281:15763–15773

Li G, Reinberg D (2011) Chromatin higher-order structures and gene regulation. Curr Opin Genet Dev 21:175–186

Li J, Guo Y, Schroeder FA, Youngs RM, Schmidt TW, Ferris C, Konradi C, Akbarian S (2004) Dopamine D2-like antagonists induce chromatin remodeling in striatal neurons through cyclic AMP-protein kinase A and NMDA receptor signaling. J Neurochem 90:1117–1131

Lichtenstein P, Carlstrom E, Rastam M, Gillberg C, Anckarsater H (2010) The genetics of autism spectrum disorders and related neuropsychiatric disorders in childhood. Am J Psychiatry 167:1357–1363

Lu T, Pan Y, Kao SY, Li C, Kohane I, Chan J, Yankner BA (2004) Gene regulation and DNA damage in the ageing human brain. Nature 429:883–891

Lumey LH, Stein AD, Susser E (2011) Prenatal famine and adult health. Annu Rev Public Health 32:237–262

Lundstrom S, Chang Z, Rastam M, Gillberg C, Larsson H, Anckarsater H, Lichtenstein P (2012) Autism spectrum disorders and autistic like traits: similar etiology in the extreme end and the normal variation. Archives of general psychiatry 69:46–52

Matsuura T, Sutcliffe JS, Fang P, Galjaard RJ, Jiang YH, Benton CS, Rommens JM, Beaudet AL (1997) De novo truncating mutations in E6-AP ubiquitin-protein ligase gene (UBE3A) in Angelman syndrome. Nat Genet 15:74–77

Maunakea AK, Nagarajan RP, Bilenky M, Ballinger TJ, D'Souza C, Fouse SD, Johnson BE, Hong C, Nielsen C, Zhao Y, Turecki G, Delaney A, Varhol R, Thiessen N, Shchors K, Heine VM, Rowitch DH, Xing X, Fiore C, Schillebeeckx M, Jones SJ, Haussler D, Marra MA, Hirst M,

Wang T, Costello JF (2010) Conserved role of intragenic DNA methylation in regulating alternative promoters. Nature 466:253–257

Maze I, Covington HE, 3rd, Dietz DM, LaPlant Q, Renthal W, Russo SJ, Mechanic M, Mouzon E, Neve RL, Haggarty SJ, Ren Y, Sampath SC, Hurd YL, Greengard P, Tarakhovsky A, Schaefer A, Nestler EJ (2010) Essential role of the histone methyltransferase G9a in cocaine-induced plasticity. Science 327:213–216

McGraw S, Shojaei Saadi HA, Robert C (2013) Meeting the methodological challenges in the molecular mapping of the embryonic epigenome. Mol Hum Reprod 19:809–827

Meador KJ, Loring DW (2013) Risks of in utero exposure to valproate. JAMA 309:1730–1731

Melas PA, Rogdaki M, Lennartsson A, Bjork K, Qi H, Witasp A, Werme M, Wegener G, Mathe AA, Svenningsson P, Lavebratt C (2012) Antidepressant treatment is associated with epigenetic alterations in the promoter of P11 in a genetic model of depression. Int J Neuropsychopharmacol 15:669–679

Melin M, Carlsson B, Anckarsater H, Rastam M, Betancur C, Isaksson A, Gillberg C, Dahl N (2006) Constitutional downregulation of SEMA5A expression in autism. Neuropsychobiology 54:64–69

Mellen M, Ayata P, Dewell S, Kriaucionis S, Heintz N (2012) MeCP2 binds to 5hmC enriched within active genes and accessible chromatin in the nervous system. Cell 151:1417–1430

Mill J, Tang T, Kaminsky Z, Khare T, Yazdanpanah S, Bouchard L, Jia P, Assadzadeh A, Flanagan J, Schumacher A, Wang SC, Petronis A (2008) Epigenomic profiling reveals DNA-methylation changes associated with major psychosis. Am J Hum Genet 82:696–711

Miller CA, Sweatt JD (2007) Covalent modification of DNA regulates memory formation. Neuron 53:857–869

Morgan CP, Bale TL (2011) Early prenatal stress epigenetically programs dysmasculinization in second-generation offspring via the paternal lineage. J Neuroscience 31:11748–11755

Morris MJ, Karra AS, Monteggia LM (2010) Histone deacetylases govern cellular mechanisms underlying behavioral and synaptic plasticity in the developing and adult brain. Behav Pharmacol 21:409–419

Mosammaparast N, Shi Y (2010) Reversal of histone methylation: biochemical and molecular mechanisms of histone demethylases. Annu Rev Biochem 79:155–179

Moss JF, Oliver C, Berg K, Kaur G, Jephcott L, Cornish K (2008) Prevalence of autism spectrum phenomenology in Cornelia de Lange and Cri du Chat syndromes. Am J Ment Retard 113:278–291

Muratore CR, Hodgson NW, Trivedi MS, Abdolmaleky HM, Persico AM, Lintas C, De la Monte S, Deth RC (2013) Age-dependent decrease and alternative splicing of methionine synthase mRNA in human cerebral cortex and an accelerated decrease in autism. PloS One 8:e56927

Najmabadi H, Hu H, Garshasbi M, Zemojtel T, Abedini SS, Chen W, Hosseini M, Behjati F, Haas S, Jamali P, Zecha A, Mohseni M, Puttmann L, Vahid LN, Jensen C, Moheb LA, Bienek M, Larti F, Mueller I, Weissmann R, Darvish H, Wrogemann K, Hadavi V, Lipkowitz B, Esmaeeli-Nieh S, Wieczorek D, Kariminejad R, Firouzabadi SG, Cohen M, Fattahi Z, Rost I, Mojahedi F, Hertzberg C, Dehghan A, Rajab A, Banavandi MJ, Hoffer J, Falah M, Musante L, Kalscheuer V, Ullmann R, Kuss AW, Tzschach A, Kahrizi K, Ropers HH (2011) Deep sequencing reveals 50 novel genes for recessive cognitive disorders. Nature 478:57–63

Nakamine A, Ouchanov L, Jimenez P, Manghi ER, Esquivel M, Monge S, Fallas M, Burton BK, Szomju B, Elsea SH, Marshall CR, Scherer SW, McInnes LA (2008) Duplication of 17(p11.2p11.2) in a male child with autism and severe language delay. Am J Med Genet A 146A:636–643

Neale BM, Kou Y, Liu L, Ma'ayan A, Samocha KE, Sabo A, Lin CF, Stevens C, Wang LS, Makarov V, Polak P, Yoon S, Maguire J, Crawford EL, Campbell NG, Geller ET, Valladares O, Schafer C, Liu H, Zhao T, Cai G, Lihm J, Dannenfelser R, Jabado O, Peralta Z, Nagaswamy U, Muzny D, Reid JG, Newsham I, Wu Y, Lewis L, Han Y, Voight BF, Lim E, Rossin E, Kirby A, Flannick J, Fromer M, Shakir K, Fennell T, Garimella K, Banks E, Poplin R, Gabriel S, DePristo M, Wimbish JR, Boone BE, Levy SE, Betancur C, Sunyaev S, Boerwinkle E, Buxbaum JD, Cook EH Jr, Devlin B, Gibbs RA, Roeder K, Schellenberg GD, Sutcliffe JS, Daly

MJ (2012) Patterns and rates of exonic de novo mutations in autism spectrum disorders. Nature 485:242–245
Nowak SJ, Corces VG (2004) Phosphorylation of histone H3: a balancing act between chromosome condensation and transcriptional activation. Trends Genet 20:214–220
Numata S, Ye T, Hyde TM, Guitart-Navarro X, Tao R, Wininger M, Colantuoni C, Weinberger DR, Kleinman JE, Lipska BK (2012) DNA methylation signatures in development and aging of the human prefrontal cortex. Am J Hum Genet 90:260–272
O'Roak BJ, Vives L, Girirajan S, Karakoc E, Krumm N, Coe BP, Levy R, Ko A, Lee C, Smith JD, Turner EH, Stanaway IB, Vernot B, Malig M, Baker C, Reilly B, Akey JM, Borenstein E, Rieder MJ, Nickerson DA, Bernier R, Shendure J, Eichler EE (2012) Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. Nature 485:246–250
Oakes CC, Smiraglia DJ, Plass C, Trasler JM, Robaire B (2003) Aging results in hypermethylation of ribosomal DNA in sperm and liver of male rats. Proc Natl Acad Sci U S A 100:1775–1780
Okada Y, Yamagata K, Hong K, Wakayama T, Zhang Y (2010) A role for the elongator complex in zygotic paternal genome demethylation. Nature 463:554–558
Pardo CA, Eberhart CG (2007) The neurobiology of autism. Brain Pathol 17:434–447
Payakachat N, Tilford JM, Kovacs E, Kuhlthau K (2012) Autism spectrum disorders: a review of measures for clinical, health services and cost-effectiveness applications. Expert Rev Pharmacoecon Outcomes Res 12:485–503
Petek E, Windpassinger C, Vincent JB, Cheung J, Boright AP, Scherer SW, Kroisel PM, Wagner K (2001) Disruption of a novel gene (IMMP2L) by a breakpoint in 7q31 associated with Tourette syndrome. Am J Hum Genet 68:848–858
Peter CJ, Akbarian S (2011) Balancing histone methylation activities in psychiatric disorders. Trends Mol Med 17:372–379
Porteus MH, Baltimore D (2003) Chimeric nucleases stimulate gene targeting in human cells. Science 300:763
Rai D, Lee BK, Dalman C, Golding J, Lewis G, Magnusson C (2013) Parental depression, maternal antidepressant use during pregnancy, and risk of autism spectrum disorders: population based case-control study. BMJ 346:f2059
Rando OJ (2012) Daddy issues: paternal effects on phenotype. Cell 151:702–708
Reik W (2007) Stability and flexibility of epigenetic gene regulation in mammalian development. Nature 447:425–432
Rodriguez-Campos A, Azorin F (2007) RNA is an integral component of chromatin that contributes to its structural organization. PloS One 2:e1182
Rodriguez-Paredes M, Esteller M (2011) Cancer epigenetics reaches mainstream oncology. Nat Med 17:330–339
Roelfsema JH, Peters DJ (2007) Rubinstein-Taybi syndrome: clinical and molecular overview. Expert Rev Mol Med 9:1–16
Ronan JL, Wu W, Crabtree GR (2013) From neural development to cognition: unexpected roles for chromatin. Nat Rev Genet 14:347–359
Rubenstein JL (2010) Three hypotheses for developmental defects that may underlie some forms of autism spectrum disorder. Curr Opin Neurol 23:118–123
Sakurai T, Ramoz N, Reichert JG, Corwin TE, Kryzak L, Smith CJ, Silverman JM, Hollander E, Buxbaum JD (2006) Association analysis of the NrCAM gene in autism and in subsets of families with severe obsessive-compulsive or self-stimulatory behaviors. Psychiatr Genet 16:251–257
Salerno S, Da Settimo F, Taliani S, Simorini F, La Motta C, Fornaciari G, Marini AM (2010) Recent advances in the development of dual topoisomerase I and II inhibitors as anticancer drugs. Curr Med Chem 17:4270–4290
Sapranauskas R, Gasiunas G, Fremaux C, Barrangou R, Horvath P, Siksnys V (2011) The Streptococcus thermophilus CRISPR/Cas system provides immunity in Escherichia coli. Nucleic Acids Res 39:9275–9282
Schaaf CP, Zoghbi HY (2011) Solving the autism puzzle a few pieces at a time. Neuron 70:806–808

Schanen NC (2006) Epigenetics of autism spectrum disorders. Hum Mol Genet 15(Spec No 2):R138–R150

Scheuerle A, Wilson K (2011) PARK2 copy number aberrations in two children presenting with autism spectrum disorder: further support of an association and possible evidence for a new microdeletion/microduplication syndrome. Am J Med Genet Part B Neuropsychiatr Genet 156B:413–420

Schroeder FA, Lin CL, Crusio WE, Akbarian S (2007) Antidepressant-like effects of the histone deacetylase inhibitor, sodium butyrate, in the mouse. Biol Psychiatry 62:55–64

Sharma RP, Grayson DR, Guidotti A, Costa E (2005) Chromatin, DNA methylation and neuron gene regulation—the purpose of the package. J Psychiatry Neurosci 30:257–263

Shi L, Zhang X, Golhar R, Otieno FG, He M, Hou C, Kim C, Keating B, Lyon GJ, Wang K, Hakonarson H (2013) Whole-genome sequencing in an autism multiplex family. Mol Autism 4:8

Shirane K, Toh H, Kobayashi H, Miura F, Chiba H, Ito T, Kono T, Sasaki H (2013) Mouse oocyte methylomes at base resolution reveal genome-wide accumulation of non-CpG methylation and role of DNA methyltransferases. PLoS Genetics 9:e1003439

Shulha HP, Cheung I, Guo Y, Akbarian S, Weng Z (2013) Coordinated cell type-specific epigenetic remodeling in prefrontal cortex begins before birth and continues into early adulthood. PLoS Genet 9:e1003433

Shulha HP, Cheung I, Whittle C, Wang J, Virgil D, Lin CL, Guo Y, Lessard A, Akbarian S, Weng Z (2012a) Epigenetic signatures of autism: trimethylated H3K4 landscapes in prefrontal neurons. Arch Gen Psychiatry 69:314–324

Shulha HP, Crisci JL, Reshetov D, Tushir JS, Cheung I, Bharadwaj R, Chou HJ, Houston IB, Peter CJ, Mitchell AC, Yao WD, Myers RH, Chen JF, Preuss TM, Rogaev EI, Jensen JD, Weng Z, Akbarian S (2012b) Human-specific histone methylation signatures at transcription start sites in prefrontal neurons. PLoS Biol 10:e1001427

Siegmund KD, Connor CM, Campan M, Long TI, Weisenberger DJ, Biniszkiewicz D, Jaenisch R, Laird PW, Akbarian S (2007) DNA methylation in the human cerebral cortex is dynamically regulated throughout the life span and involves differentiated neurons. PLoS One 2:e895

Smallwood SA, Tomizawa S, Krueger F, Ruf N, Carli N, Segonds-Pichon A, Sato S, Hata K, Andrews SR, Kelsey G (2011) Dynamic CpG island methylation landscape in oocytes and preimplantation embryos. Nat Genet 43:811–814

Smith RG, Reichenberg A, Kember RL, Buxbaum JD, Schalkwyk LC, Fernandes C, Mill J (2013) Advanced paternal age is associated with altered DNA methylation at brain-expressed imprinted loci in inbred mice: implications for neuropsychiatric disease. Mol Psychiatry 18:635–636

Smoller JW, Craddock N, Kendler K, Lee PH, Neale BM, Nurnberger JI, Ripke S, Santangelo S, Sullivan PF (2013) Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. Lancet 381:1371–1379

Song CX, Szulwach KE, Fu Y, Dai Q, Yi C, Li X, Li Y, Chen CH, Zhang W, Jian X, Wang J, Zhang L, Looney TJ, Zhang B, Godley LA, Hicks LM, Lahn BT, Jin P, He C (2011) Selective chemical labeling reveals the genome-wide distribution of 5-hydroxymethylcytosine. Nat Biotechnol 29:68–72

Steffenburg S, Gillberg C, Hellgren L, Andersson L, Gillberg IC, Jakobsson G, Bohman M (1989) A twin study of autism in Denmark, Finland, Iceland, Norway and Sweden. J Child Psychol Psychiatry 30:405–416

Stroud H, Feng S, Morey Kinney S, Pradhan S, Jacobsen SE (2011) 5-Hydroxymethylcytosine is associated with enhancers and gene bodies in human embryonic stem cells. Genome Biol 12:R54

Suren P, Roth C, Bresnahan M, Haugen M, Hornig M, Hirtz D, Lie KK, Lipkin WI, Magnus P, Reichborn-Kjennerud T, Schjolberg S, Davey Smith G, Oyen AS, Susser E, Stoltenberg C (2013) Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children. JAMA 309:570–577

Sutcliffe JS, Jiang YH, Galijaard RJ, Matsuura T, Fang P, Kubota T, Christian SL, Bressler J, Cattanach B, Ledbetter DH, Beaudet AL (1997) The E6-Ap ubiquitin-protein ligase (UBE3A) gene is localized within a narrowed Angelman syndrome critical region. Genome Res 7:368–377

Sutcliffe EL, Parish IA, He YQ, Juelich T, Tierney ML, Rangasamy D, Milburn PJ, Parish CR, Tremethick DJ, Rao S (2009) Dynamic histone variant exchange accompanies gene induction in T cells. Mol Cell Biol 29:1972–1986

Suzuki K, Sugihara G, Ouchi Y, Nakamura K, Futatsubashi M, Takebayashi K, Yoshihara Y, Omata K, Matsumoto K, Tsuchiya KJ, Iwata Y, Tsujii M, Sugiyama T, Mori N (2013) Microglial activation in young adults with autism spectrum disorder. JAMA Psychiatry 70:49–58

Szulwach KE, Li X, Li Y, Song CX, Han JW, Kim S, Namburi S, Hermetz K, Kim JJ, Rudd MK, Yoon YS, Ren B, He C, Jin P (2011) Integrating 5-hydroxymethylcytosine into the epigenomic landscape of human embryonic stem cells. PLoS Genet 7:e1002154

Tan M, Luo H, Lee S, Jin F, Yang JS, Montellier E, Buchou T, Cheng Z, Rousseaux S, Rajagopal N, Lu Z, Ye Z, Zhu Q, Wysocka J, Ye Y, Khochbin S, Ren B, Zhao Y (2011) Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification. Cell 146:1016–1028

Tang B, Chang WL, Lanigan CM, Dean B, Sutcliffe JG, Thomas EA (2009) Normal human aging and early-stage schizophrenia share common molecular profiles. Aging Cell 8:339–342

Tarabykin V, Britanova O, Fradkov A, Voss A, Katz LS, Lukyanov S, Gruss P (2000) Expression of PTTG and prc1 genes during telencephalic neurogenesis. Mech Dev 92:301–304

Taverna SD, Li H, Ruthenburg AJ, Allis CD, Patel DJ (2007) How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. Nat Struct Mol Biol 14:1025–1040

Tetreault NA, Hakeem AY, Jiang S, Williams BA, Allman E, Wold BJ, Allman JM (2012) Microglia in the cerebral cortex in autism. J Autism Dev Disord 42:2569–2584

Theoharides TC, Asadi S, Patel AB (2013) Focal brain inflammation and autism. J Neuroinflammation 10:46

Tsankova N, Renthal W, Kumar A, Nestler EJ (2007) Epigenetic regulation in psychiatric disorders. Nat Rev Neurosci 8:355–367

Uhlhaas PJ, Singer W (2012) Neuronal dynamics and neuropsychiatric disorders: toward a translational paradigm for dysfunctional large-scale networks. Neuron 75:963–980

Vassoler FM, White SL, Schmidt HD, Sadri-Vakili G, Pierce RC (2013) Epigenetic inheritance of a cocaine-resistance phenotype. Nat Neurosci 16:42–47

Vogel T, Stoykova A, Gruss P (2006) Differential expression of polycomb repression complex 1 (PRC1) members in the developing mouse brain reveals multiple complexes. Dev Dyn 235:2574–2585

Voineagu I, Wang X, Johnston P, Lowe JK, Tian Y, Horvath S, Mill J, Cantor RM, Blencowe BJ, Geschwind DH (2011) Transcriptomic analysis of autistic brain reveals convergent molecular pathology. Nature 474:380–384

Walsh CA, Morrow EM, Rubenstein JL (2008) Autism and brain development. Cell 135:396–400

Weiss LA, Arking DE, Daly MJ, Chakravarti A (2009) A genome-wide linkage and association scan reveals novel loci for autism. Nature 461:802–808

Wong CC, Meaburn EL, Ronald A, Price TS, Jeffries AR, Schalkwyk LC, Plomin R, Mill J (2013) Methylomic analysis of monozygotic twins discordant for autism spectrum disorder and related behavioural traits. Mol Psychiatry 19:495–503

Wood AJ, Severson AF, Meyer BJ (2010) Condensin and cohesin complexity: the expanding repertoire of functions. Nat Rev Genet 11:391–404

Woodcock CL (2006) Chromatin architecture. Curr Opin Struct Biol 16:213–220

Wu H, D'Alessio AC, Ito S, Wang Z, Cui K, Zhao K, Sun YE, Zhang Y (2011) Genome-wide analysis of 5-hydroxymethylcytosine distribution reveals its dual function in transcriptional regulation in mouse embryonic stem cells. Genes Dev 25:679–684

Xie W, Barr CL, Kim A, Yue F, Lee AY, Eubanks J, Dempster EL, Ren B (2012) Base-resolution analyses of sequence and parent-of-origin dependent DNA methylation in the mouse genome. Cell 148:816–831

Xin Z, Allis CD, Wagstaff J (2001) Parent-specific complementary patterns of histone H3 lysine 9 and H3 lysine 4 methylation at the Prader-Willi syndrome imprinting center. Am J Hum Genet 69:1389–1394

Zaaroor-Regev D, de Bie P, Scheffner M, Noy T, Shemer R, Heled M, Stein I, Pikarsky E, Ciechanover A (2010) Regulation of the polycomb protein Ring1B by self-ubiquitination or by E6-AP may have implications to the pathogenesis of Angelman syndrome. Proc Natl Acad Sci U S A 107:6788–6793

Zhao J, Sun BK, Erwin JA, Song JJ, Lee JT (2008) Polycomb proteins targeted by a short repeat RNA to the mouse X chromosome. Science 322:750–756

Zhou VW, Goren A, Bernstein BE (2011) Charting histone modifications and the functional organization of mammalian genomes. Nat Rev Genet 12:7–18

Ziats MN, Rennert OM (2013) Aberrant expression of long noncoding RNAs in autistic brain. J Mol Neurosci 49:589–593Xie W, Barr CL, Kim A, Yue F, Lee AY, Eubanks J, Dempster EL, Ren B (2012) Base-resolution analyses of sequence and parent-of-origin dependent DNA methylation in the mouse genome. Cell 148:816–831

Xin Z, Allis CD, Wagstaff J (2001) Parent-specific complementary patterns of histone H3 lysine 9 and H3 lysine 4 methylation at the Prader-Willi syndrome imprinting center. Am J Hum Genet 69:1389–1394

Zaaroor-Regev D, de Bie P, Scheffner M, Noy T, Shemer R, Heled M, Stein I, Pikarsky E, Ciechanover A (2010) Regulation of the polycomb protein Ring1B by self-ubiquitination or by E6-AP may have implications to the pathogenesis of Angelman syndrome. Proc Natl Acad Sci U S A 107:6788–6793

Zhao J, Sun BK, Erwin JA, Song JJ, Lee JT (2008) Polycomb proteins targeted by a short repeat RNA to the mouse X chromosome. Science 322:750–756

Zhou VW, Goren A, Bernstein BE (2011) Charting histone modifications and the functional organization of mammalian genomes. Nat Rev Genet 12:7–18

Ziats MN, Rennert OM (2013) Aberrant expression of long noncoding RNAs in autistic brain. J Mol Neurosci 49:589–593

Chapter 6
Immunology of Autism

Destanie R. Rose and Paul Ashwood

Abstract Autism spectrum disorders (ASD) are developmental disorders characterized by behavioral deficits in verbal and nonverbal communication as well as social interactions, and are accompanied by repetitive or stereotyped behaviors and interests. Numerous studies over the last forty years have recognized altered immune responses in individuals with ASD; concurrently basic research has highlighted the myriad of neuroimmune interactions and the cross talk that occurs between nervous and immune systems. Neuroinflammation, particularly in the cerebellum, has been found in post mortem brain tissues from individuals with ASD and is characterized by the presence of profound glia activation processes. This and altered gene expression profiles indicating perturbed immune suggest a contributing role for immunological systems in the pathology of ASD. Peripheral immune abnormalities have also been found; shifts in both direction of TH1 and TH2 skewing have been reported as well as autoantibody production, increased NK cell activation, T cell responses and monocyte cell function overwhelmingly suggesting the presence of immune dysfunction in individuals with ASD. Many of these findings are associated with worsening behavioral scores, suggesting treatment of immune function could be useful in alleviating symptoms associated with ASD. Immune activation *in utero* is also associated with an increased risk of the child for having a diagnosis of ASD, where increased cytokine production in the offspring is directly linked to changes in offspring behavior. In addition to peripheral changes, brain and CSF immune variations in ASD are reported as well as an increase in gastrointestinal/mucosal dysfunction which has led to an increased interest in exploring the gut-brain- immune connections and its role in ASD. Further research in neuroimmune interactions may bring further insight and elicit new therapeutic tools for ASD.

Keywords Adaptive immune system · Autism spectrum disorders · Behavior · Cerebellum · Cytokine · Innate immune system · Immunity · Maternal immune activation · Neuroimmunology · Social interactions · T cells · Gastrointestinal

P. Ashwood (✉) · D. R. Rose
Department of Medical Microbiology and Immunology and M.I.N.D. Institute,
University of California, Davis, 2805 Wet Lab Building,
Sacramento, CA, 95817, USA
email: pashwood@ucdavis.edu

S. Hossein Fatemi (ed.), *The Molecular Basis of Autism,*
Contemporary Clinical Neuroscience, DOI 10.1007/978-1-4939-2190-4_6

6.1 Immune-Brain Interactions

6.1.1 Cytokines and Neurodevelopment

The immune system is involved in three main areas—surveillance of foreign antigen, fighting infections and tissue remodeling and participates in these functions in all types of tissue during development, health, disease and wound healing. The brain is no exception; many immune responses take part in shaping and maintaining normal central nervous system (CNS) function (Fig. 6.1). During the course of neurodevelopment and throughout adulthood, normal immunological processes take part in influencing appropriate neurological features and functions. Cytokines, the immune system's signaling molecules have a significant role in both of these areas. Cytokines act as both chemoattractants, guiding direction of growth and migration as well as acting as neurotrophic factors that promote survival to developing neurons in the brain and spinal cord (Deverman and Patterson 2009). Interleukin (IL)-1β a prominent inflammatory cytokine of the immune system has been found to be involved in many different functions of the CNS. During development, IL-1β expression is observed in the embryonic spinal cord of both chickens (stage 17-HH) and rats (embryonic day 12) (de la Mano et al. 2007). Delivery of IL-1β on microbeads implanted near the spinal cord of chick embryos increased the number of proliferating ($BrdU^+$) neuroepithelial cells in the dorsal spinal cord and led to a reduction in the ventral spinal cord. Blocking IL-1β through anti- IL-1β antibodies reduced BrdU incorporation in the dorsal spinal cord. (de la Mano et al. 2007). In the mammalian adult brain, cytokines including IL-1β can act on the neural and progenitor stem cells in response to injury, disease and stress influencing proliferation and neurogenesis. (Carpentier and Palmer 2009). IL-1β is also believed to be required for normal learning and memory processes in the hippocampus (Goshen et al. 2007). Gene expression of IL-1β has been found to be increased in the hippocampus 24 h after contextual learning and blocking IL-1β results in impairments in spatial memory and fear conditioning tests (Goshen et al. 2007). IL-1 receptor knockout mice were found to have a reduction in neuronal dendritic spine size which may contribute to the defects seen in memory in these mice (Goshen et al. 2009).

Other pro-inflammatory cytokines that are involved in spatial learning and memory include both IL-6 and Tumor necrosis factor (TNF)-α; however, these cytokines also have complex roles in shaping memory and learning and have reported beneficial and detrimental effects. A recent study showed that TNF-α signaling through the NFκB pathway lead to increased neural stem cell (NSC) proliferation. This proliferation was attributed to the activation of the IKK-β and NFκB pathway leading to up regulation of cyclin D1 (Widera et al. 2006). How cytokines affect the CNS is context dependent and environments surrounding the release of these cytokines play an equally important role in determining the ultimate effect than any single cytokine will play (Yirmiya and Goshen 2011). Transforming growth factor (TGF)-β, an important immune regulatory cytokine, is involved in signaling required for mouse

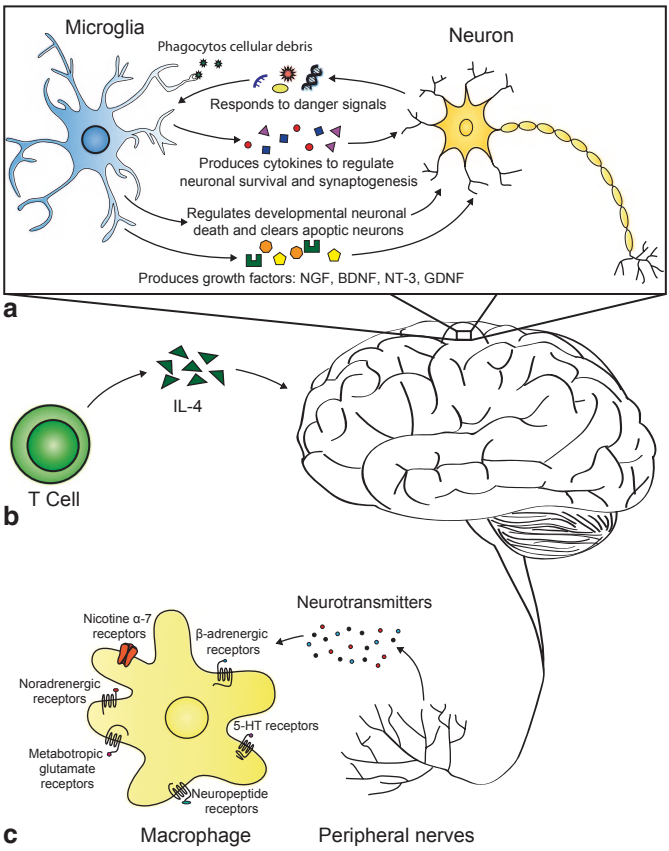


Fig. 6.1 a) Many immunological processes take part both in developing and maintaining the CNS. Microglia are the main contributor to many of these processes. As the resident phagocytic cell, microglia clear cellular debris and apoptotic neurons. They also further neural development by producing various growth factors and many cytokines that regulate neuronal survival and synaptogenesis. Microglia also respond to danger signals by producing reactive oxygen species and pro-inflammatory cytokines. Some of the cytokines that can affect the CNS include: IL-1β, TNF-α, IL-6 and TGF-β. These cytokines not only contribute to neuronal development but also play a role in learning and memory. **b)** Lymphocytes, part of the adaptive immune system have also been found to contribute to cognitive function. More specifically T cells have been demonstrated to contribute to visuospatial learning and enhance neurogenesis in the dentate gyrus. T cells important to cognitive function are believed to be found in the meningeal spaces where they produce cytokines; specifically IL-4 has been associated with visuospatial learning. **c)** Outside the CNS, neuro-immune interactions also take place. Many immune cells including macrophages express receptors for neurotransmistters. Some of these receptors include beta adrenergic, nicotine α-7, noradrenergic, metabotropic glutamate, neuropeptide and 5-hydroxytryptophan (5-HT) receptors. Signaling through these receptors can either enhance or suppress the immune response depending on the neurotransmitter and environmental conditions. Norepinephrine, for example, generally tends to have an inhibitory effect on pro-inflammatory cytokine production in macrophages but this can be reversed if other factors such as LPS are present. *NGF* nerve growth factor, *BDNF* brain derived neurotrophic factor, *NT-3* neurotrophin-3, *GDNF*, Glial cell line-derived neurotrophic factor, *IL* interleukin, *5-HT* 5-hydroxytryptophan

mesencephalic progenitors to differentiate into tyrosine hydroxylase $(TH)^+$dopaminergic neurons in vitro and in vivo (Roussa et al. 2006). When TGFβ2/TGFβ3 double knockout mice were examined it was found they have reduced numbers of TH^+ neurons in the ventral mesencephalon, however, in the locus coeruleus TH+ neurons were not significantly different from controls indicating that while TGFβ signaling may be important in the ventral mesencephalon it does not seem to contribute to ventral midbrain dopaminergic neuron development (Roussa et al. 2006).

6.1.2 Microglia

Microglia, the brain's resident phagocytic cells, play a central role in CNS development and maintenance through regulating developmental neuronal death and the clearing of apoptotic neurons (Wakselman et al. 2008; Takahashi et al. 2005).They also phagocytose cellular debris and respond to 'danger' signals through production of reactive oxygen species (ROS) and inflammatory cytokines (Neumann et al. 2009; Ron-Harel et al. 2011). The significance of this function is illustrated through the depletion of microglia from murine neonatal cerebellar slice cultures. This specific elimination of microglia leads to increased Purkinje cell survival due to the reduction of phagocytosis of caspase-3-expressing Purkinje cells (Marin-Teva et al. 2004). More recently *in vivo* studies have shown that microglia regulate neurogenesis in the cerebral cortex of primates, rodents and human fetal tissues (Cunningham et al. 2013). Cunningham et al. (2013) show microglia enter and colonize the cortical proliferative zones near the end of neurogenesis and phagocytose neural precursor cells. Manipulation of microglia in rats either by suppressing microglia using doxycycline or activating them using injection of LPS resulted in increased and decreased numbers of neural precursors cells, respectively, further demonstrating the critical role microglia and the immune system play in regulating neuronal numbers in early brain development.

In general, microglia activation increases expression of inflammatory cytokines and can have toxic effects on the surrounding cells; however, they are also vital for the down regulation of immune responses through autocrine feedback loops and production of anti-inflammatory cytokines (Garden and Moller 2006). Embryonic microglia are known producers of TNF-α and are key regulators of developmental apoptosis and synaptogenesis. Disrupting TNF-α signaling through use of anti-TNF-α antibodies or soluble TNF-α receptor (TNFR1) results in the decrease of AMPA-type glutamate receptors on hippocampal neurons and thereby modulate synaptic strength in these neurons (Beattie et al. 2002). TNF-α has been shown to upregulate expression of β3 integrins that help increase synaptic strength through stabilization of AMPARs (Cingolani et al. 2008). TNF-α is therefore suggested to have a central role for the homeostatic potentiation of synaptic strength during developmental synaptic refinement. Glial cells, in response to levels of activity in hippocampal cultures, have been shown to regulate TNF-α levels (Deverman and Patterson 2009).

Macrophage colony-stimulating factor (M-CSF) a growth factor for macrophages and microglia is necessary for proper development of certain areas of the brain. *M-CSF* mutant mice containing a null mutation in the M-CSF gene were shown to have auditory and visual processing impairment with failure of the newborn pups to respond to external cues and electrophysiologic abnormalities detected by intracortical recordings of brainstem auditory evoked potentials and visual evoked potentials (Michaelson et al. 1996). The effects of M-CSF are thought to be indirectly regulated by cytokine secreting microglia (Deverman and Patterson 2009). In addition to cytokines and chemokines, microglia produce many other regulatory and trophic factors promoting neuronal survival including: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin (NT)-3, basic fibroblast growth factor, and glial-derived neurotrophic factor (GDNF) (Garden and Moller 2006). Microglia not only influence neuronal cells but can also be influenced by neuronal activity through neurotransmitter receptors such as glutamate receptors and by astrocytes through purinergic receptors such as P2X4, P2X7, P2Y2, P2Y6 and P2Y12 (Biber et al. 2007; Hung et al. 2010; Ferrari et al. 2006).

6.1.3 Cytokines, Immune Cells and Cognitive Function

Cytokines and immune cells are supportive in brain function including neurogenesis and cognitive functioning. Lymphocytes, for example, have been found to have a supportive role in cognitive functioning (Kipnis et al. 2012). Experiments with severe combined immune deficient (SCID) mice, that do not have any T or B cells, display impairments in hippocampal dependent spatial learning and memory through analysis of the Morris water maze behavioral test (MWM) (Kipnis et al. 2004). Furthermore, nude mice (lacking only mature T cells) display similar impairments assessed by MWM which could be partially rescued with replenishment of T cells from wild-type mice, demonstrating the role of T cells more specifically in areas of visuospatial learning (Kipnis et al. 2004; Ron-Harel et al. 2008; Brynskikh et al. 2008). To provide further support of the role of T cells in learning and memory another study looked at rats that were either raised under normal environmental conditions or under enriched conditions in which neurogenesis was enhanced in the dentate gyrus. To confirm the role of T cells, mice were used in which monospecific T cells to either myelin basic protein (auto specific) (Tmbp) or ovalbumin (OVA) (non-CNS specific) (Tova) were used. Tmbp mice were found to have higher amounts of proliferating neurons compared with controls and preformed better in the MWM, while Tova had less proliferating neurons than controls (Ziv et al. 2006). To emphasize the importance of T cell/microglia interactions in neurogenesis the Tmbp mice were treated with a microglia blocking drug, minocycline, which significantly decreased neurogenesis in the dentate gyrus (Ziv et al. 2006). These findings support the role of T cells mediating neurogenesis and spatial learning through possible interaction with microglia.

Although T cells are not normally found in the CNS parenchyma under normal conditions, T-cell based support of behavioral plasticity is thought to take place in the meningeal spaces (Derecki et al. 2010; Schwartz and Shechter 2010). Depletion of T cells from the meningeal spaces results in impairments of learning and memory based on MWM results. Of the meningeal T cell population, $CD4^+$ $IL\text{-}4^+$ T cells were found to be the most important for spatial learning and memory as IL-4 deficient mice showed defects in MWM (Derecki et al. 2010). Other studies have also used T cell manipulations to demonstrate improved learning and memory (Ron-Harel and Schwartz 2009; Ron-Harel et al. 2008). Manipulation of T cells in aged mice through bone marrow transplantation improved spatial memory in these animals compared to young animals and was increased when compared with non treated aged animals (Ron-Harel et al. 2008). It has been suggested that assessing T cell immunity in old age could be used as a predictor of potential future memory loss and enhancing T-cell immunity could benefit age associated memory loss (Ron-Harel and Schwartz 2009).

Major histocompatibility complex (MHC) molecules are important cell surface molecules that interact with T cells involved in host immunity. It was long thought that the neural cells were among the small list of cells that did not express this family of surface proteins; however, in the late 1990's it was found that not only are these important immune molecules present on neurons but they actually influence synapse plasticity (Elmer and McAllister 2012). Mice with deficient signaling of class I MHC displayed impaired synapse plasticity (Huh et al. 2000). Peptides from within the cell are presented in MHC molecules and MHC I: peptide complexes, in an immunological context are scanned by cytotoxic T cells to detect abnormal conditions such as the presence of a viral infection or tumor. In the CNS it is thought that similar roles of MHC molecules are employed in presenting peptides in order to regulate normal developmental elimination of inappropriate synaptic connections, although the mechanism remains elusive (Boulanger 2009).

Communication between the immune system and CNS is not one way. Signaling is multidirectional and information can also pass from the CNS to the immune system. Norepinephrine (NE) released from sympathetic nerve terminals can signal to macrophages through beta adrenergic receptors (Kin and Sanders 2006). In general NE seems to have inhibitory effects on pro-inflammatory cytokine production such as TNF-α, IL-1β, and sometimes IL-6, based on data from splenic macrophages (Meltzer et al. 2004; Ignatowski et al. 1996; Nance and Sanders 2007). IL-6 production has been found to be both increased or decreased in response to NE depending on other signals and stimuli such as the presence of LPS (Nance and Sanders 2007). Other neuro-based receptors found on immune cells include noradrenergic receptors, nicotinic α-7 receptors, receptors for neuropeptides and hormones, metabotropic glutamate receptors (mGluRs) and receptors for monoamines serotonin and dopamine (Nance and Sanders 2007; Tracey 2002; Besedovsky and Rey 2007; Friedman and Irwin 1997; Pacheco et al. 2004). Signaling through these receptors can regulate and modulate immune function and may be important in response to stress and or in neuro-psychiatric disorders were imbalances in neuromodulators have been observed.

6.1.4 Neuroinflammation

While there are many beneficial roles of the immune system in CNS function, too much inflammation can be detrimental. Exposure to pathogens which activate immune responses to protect against infections can result in increased production of pro-inflammatory cytokines that contribute to sickness behavior, while both anxiety and depression have also been associated with inflammation (Irwin and Miller 2007). Pro-inflammatory cytokines including IL-1β, TNF-α and IL-6 can act on the brain causing sickness behaviors ranging from loss of appetite, lethargy to irritability (Dantzer et al. 2008). In addition to inflammation or as a result of infection, events causing stress, injury and ageing can also induce these same inflammatory mediators (Yirmiya and Goshen 2011). In experiments where IL-1β was injected intracerbroventricularly (i.c.v.) into the right lateral cerebral ventricle either 24 h or 1 h before training in the MWM, those rats injected for 1 h but not those injected 24 h before training showed impaired performance in spatial memory the next day (Oitzl et al. 1993) suggesting changes may be fast acting but also dose-dependent and transient. Furthermore, increased peripheral levels of IL-1β following infection with *Legionella pneumophila* or by direct administration of IL-1β daily also showed impaired spatial memory and learning in mice (Gibertini et al. 1995) suggesting there may be a conditioning effect with repeated prolonged exposure to cytokines. Transgenic mice that over express IL-1β show impairments in spatial memory that are particularly restricted to hippocampal dependent memory (Hein et al. 2010; Moore et al. 2009). Introduction of LPS also increases hippocampal IL-1 levels and induce similar impairments to special learning and memory (Nguyen et al. 1998). However, other study designs with different regimens of IL-1β administration did not show memory or learning impairments suggesting that the conditions and environmental factors contribute to memory and learning (Yirmiya and Goshen 2011). Additionally IL-1β associated neuroinflammation is linked in ageing and may play a role in age associated memory loss (Krabbe et al. 2004). Caspase-1 inhibitors when administered to aged mice over time reduced hippocampal IL-1β and helped to improve contextual memory (Gemma et al. 2005; Krabbe et al. 2004). In Alzheimer's disease increased levels of TNF-α, IL-6 and IL-1β have been detected in the serum and cerebral spinal fluid (Akiyama et al. 2000; Shaftel et al. 2008). Activation of microglia have been found in Alzheimer and other neurological diseases such as Parkinson's disease, multiple sclerosis and acquired immune deficiency syndrome dementia complex (Kim and de Vellis 2005). As stated above, the immune system orchestrates a vital and delicate balancing act necessary for the proper development and maintenance of the CNS. When there is imbalance in either direction, increased or decreased, appropriate functions of the CNS can become impaired.

6.1.5 Neuroinflammation in ASD

Recent studies have suggested that neuroinflammation occurs in individuals with ASD. Inflammation in post mortem brain specimens of a wide range of individuals with ASD age 4–45 years old have been observed, specifically, the cerebellum, anterior cingular gyrus and the midfrontal regions of the brain (Vargas et al. 2005). Neuroglial activation and presence of increased levels of inflammatory cytokines such as IFN-γ, IL-1β, IL-6, TNF-α and chemokines CCL-2 were found in brain tissue and CSF (Li et al. 2009; Morgan et al. 2010; Vargas et al. 2005). Additionally postmortem brain samples of patients with ASD were also found to have increased levels of glial fibrillary acidic protein (GFAP) in the frontal, parietal and cerebellar cortices (Laurence and Fatemi 2005). GFAP is expressed in activated astrocytes and is also a sign of inflammation. The cerebellum in particular showed the most prominent histological changes and microglial activation in individuals with ASD. In addition, some of the cerebellar tissues from individuals with ASD, but none of the control tissues had accumulation of perivascular macrophages and monocytes and deposition of complement membrane attack complexes which suggest that the neuroinflammation seen may be primarily driven by innate immune responses (Vargas et al. 2005). Furthermore, researchers found increases in TH1 with no differences in TH2 cytokines suggesting that ASD patients have increased neuroinflammatory immune response through the TH1 pathway (Li et al. 2009). Increases in TH1 cytokines such as IFNγ were not compensated by increases in IL-10 also suggesting a failure in immune regulation (Li et al. 2009). In addition to increases in cytokines, postmortem temporal cortex samples from ASD and general population controls were assessed for transcriptome differences and increases in expression of immune related genes were found in the ASD population (Garbett et al. 2008). In particular cytokine signaling and immune regulatory genes were altered, which included genes from the NFκB, IL-1r, Toll, IL-6, Caspase, TH1/TH2 and FAS pathways. Interestingly, the ASD samples had higher variability in transcriptome differences when compared to controls (Garbett et al. 2008). Furthermore, activation of microglial cells and perivascular macrophages measured by increased MHC II expression was seen in the cortical regions, white matter and most prominently in the cerebellum of patients with autism. This microglial and astroglial activation in the cerebellum was associated with degenerating purkinje cells, granule cells, and axons (Vargas et al. 2005). Altered microglial profiles found in post mortem brain samples of ASD patients showed an increase in average microglial somal volume and increase in microglial density in white and grey matter respectively and activation ranged from severe to mild in ASD brain specimens (Morgan et al. 2010). The data also suggested that microglial activation maybe particularly prominent in younger individuals, though more samples are needed to verify this (Morgan et al. 2010).

The specific inducer of microglia activation in ASD is unknown and whether dysfunction in immune pathways leads to neuroinflammation or if CNS impairments in ASD lead to immune dysregulation, or in fact an interplay between the two systems, is yet to be fully elucidated. Both environmental and genetic risk factors

are thought to play a role in ASD. Genetic contributions to ASD were first suggested in the 1980's after investigation of co-occurrences of rare syndromes and chromosomal disorders were observed with ASD (Blomquist et al. 1985). Moreover the increased occurrences of ASD in families shown in twin and sibling studies further provided evidence for a genetic component to ASD (Kates et al. 2004; Bailey et al. 1995; Constantino and Todd 2000; Steffenburg et al. 1989; Jorde et al. 1991). Candidate gene association studies and whole-genome linkage studies have been used to identify loci of interest and assess copy number variation. Even with a long list of putative contributing genetic mutations and syndromes associated with ASD, these only account for 10–20 % of cases (Abrahams and Geschwind 2008). Genetic risk factors for ASD include genes that affect both CNS and immune pathways. Immune related genes associated with ASD include: phosphoinisitide–3 kinase (PI3K) pathway proteins such as MET, PTEN, TSC1 and 2, as well as MHC II, complement 4B, and macrophage inhibitory factor (MIF) (Onore et al. 2012).

6.2 Maternal Immune Activation and ASD

6.2.1 Infection During Pregnancy

In addition to genetic contributions, environmental factors are also thought to play a role in ASD. Maternal immune activation (MIA) during pregnancy is one potential environmental factor that may increase the risk for developing ASD (Patterson 2009). Studies investigating viral and bacterial infections during pregnancy have shown associations with maternal infection and increases in ASD, including in 1964 when a rubella outbreak was connected with increased cases of autism (Chess et al. 1978). Other viruses that have been linked to congenital infection and associated with ASD include the herpes viruses: herpes simplex virus, cytomegalovirus, varicella and the paramyxovirus mumps (Libbey et al. 2005). The study of data from the Danish Medical Birth Register investigated 10,133 ASD diagnoses from children born from 1980 to 2005 looking at mothers who were hospitalized during pregnancy and found evidence to support association of viral infection during the first trimester and bacterial infection during the second trimester with increased risk of the child developing ASD (Atladottir et al. 2009). It is possible that genetically predisposed or susceptible individuals who encounter a prenatal infection may develop ASD due to high levels of cytokines or initiation of autoimmune processes resulting in increased maternal inflammation which could affect the developing fetal brain (Libbey et al. 2005). Additional evidence to infer maternal immune involvement in autism is data showing increased rates of autoimmunity in families with ASD (Croen et al. 2005; Atladottir et al. 2009). In support of a role of MIA, one study showed that mid-gestational findings of increased IFNγ, IL-4 and IL-5 in maternal serum significantly increased the risk of ASD (Goines et al. 2011).

6.2.2 Other Inflammatory Processes

In addition to increased frequencies of autoimmunity among families with individuals with ASD some reports have also identified fetal specific autoantibodies in the mothers of children with autism (Braunschweig et al. 2008; Croen et al. 2008). IgG maternal antibodies can cross the placenta and persist for up to 6 months after birth (Heininger et al. 2006). Antibodies with autoreactivity to fetal brain proteins were found at 37 kDA and 73 kDa molecular weights in approximately 12 % of mothers with an autistic child but no mothers of typically developing children or children with developmental delays other than ASD (Braunschweig et al. 2008); later another band with a molecular weight of 39 kDa was also found to be associated with ASD (Croen et al. 2008). Maternal antibodies are present in detectable levels at 18 weeks in the developing fetus and reach levels comparable to the mothers by 38 weeks of gestation (Croen et al. 2008). To further test the role these autoantibodies are playing in ASD, several studies have injected serum or purified IgG from mothers of children with ASD and mothers of controls into various animal models mid gestation. In one study pregnant mouse dams were intraperitoneally injected with purified IgG from mothers of children with autism disorders (MCAD) or from mothers of typically developing children. Injections were given daily during embryonic days 13–18, resulting in adolescent offspring from MCAD injected dams which displayed long-term behavioral differences compared with controls (Singer et al. 2009). In another study non-human primate, rhesus macaques were injected with purified IgG from mothers of children with ASD and from those of typically developed children. Animals were found to have higher amounts of stereotypical behaviors and increased motor activity than controls (Martin et al. 2008). These data suggest that dysfunction of the maternal immune system may play an active role in the pathology of some children who develop ASD.

6.2.3 Rodent Models of MIA

Other models that investigate the role of MIA include rodent models of immune activation of pregnant dams. IL-6 is an important cytokine involved in maternal immune influence of fetal development (Hsiao and Patterson 2011). Injecting IL-6 in the absence of other immune stimulus at embryonic day 12.5 is sufficient to cause behavioral changes in the offspring, particularly in measurements of prepulse inhibition of adult offspring (Smith et al. 2007). Other pro-inflammatory cytokines such as IL-1β, TNF-α and IFN-γ did not cause any changes in behaviors. Likewise, injection of neutralizing anti IL-6 antibodies administered when MIA was induced prevented development of behavioral abnormalities. IL-6 knockout mice also exhibited resistance to *in utero* MIA induced behavioral changes (Smith et al. 2007). Pregnant mice infected with the human influenza virus on embryonic day 9.5 had offspring who as adults displayed behavioral defects in prepulse inhibition and acoustic startle response (Shi et al. 2003). Additionally when polyinosinic –poly-

cytidylic acid (poly I:C), a viral mimic, was injected into dams at embryonic day 12.5 the offspring had similar behavioral defects as the influenza infected offspring suggesting that the behavioral abnormalities are indeed due to activation of the maternal immune system not the virus itself (Shi et al. 2003). Recent studies with poly IC induced MIA in mice show behavioral changes in three areas relevant to those seen in ASD which include impairments in communication, social interactions and repetitive behaviors (Malkova et al. 2012; Schwartzer et al. 2013). Male offspring of MIA mice were found to produce less ultrasonic vocalizations (a murine form of communication) in different social situations compared with controls. In addition, the offspring were also found to spend less time with novel mice and more time with a novel object when compared to saline controls indicating a difference in social interactions and finally the MIA offspring displayed more repetitive behaviors as measured by time spent self grooming and time spent burying marbles compared with controls (Malkova et al. 2012). In addition to poly I:C models, the use of the bacterial component lipopolysaccaride (LPS) as a mid-gestational activator of the maternal immune system has been tested and results in behavioral changes in offspring similar to those observed using poly I:C (Patterson 2009). In the latter model neuroglial activation and increased cytokine production has been shown that likely results in permanent elevation of cytokines in the brain that affect postnatal behaviors (Patterson 2009). These models together with epidemiological data of human infection during pregnancy demonstrate that the immune status of the mother is important for the developing fetus (Patterson 2009). Since not all mothers who are infected with a pathogen have offspring with ASD it is likely that genetic background acts as a factor to enhance ASD risk. Gene—environment interactions are thought to play a major role in ASD. One study examined these interactions by testing MIA in mice heterozygous for the tuberous sclerosis 2 (Tsc2) gene. Offspring of dams injected with poly I:C exhibited increased asocial behavioral abnormalities more than MIA alone suggesting a double hit of genetic and environmental factors results in severe behavioral defects (Ehninger et al. 2012). In addition to alteration in fetal neurodevelopment it is also possible that MIA alters peripheral immune responses as well. In one study of MIA, a TH17 skewing of T cells were seen in poly I:C maternally exposed mice compared with controls (Mandal et al. 2011).

6.3 Systemic Immune Activation in ASD

6.3.1 Peripheral Cytokines and Chemokines in ASD

Immune abnormities in ASD have been reported since 1977 (Stubbs and Crawford 1977). Since that initial report there have been a number of immune related problems described with some conflicting findings likely reflecting the heterogeneity of ASD. Elevated pro-inflammatory cytokines have been found in plasma of children with ASD aged 2–5 years old including IL-1β, IL-6, IL-8 and IL-12p40 (Ashwood et al.

2011b). Elevated amounts of chemokines MCP-1, RANTES and eotaxin were also found in children with ASD (Ashwood et al. 2011d). In both studies these elevated inflammatory mediators were associated with more impaired or aberrant behaviors. Other reports of inflammatory cytokines found elevations of IFNγ (Singh 1996), MIF (Grigorenko et al. 2008) and platelet derived growth factor BB (PDGF-BB) in plasma of children with ASD (Kajizuka et al. 2010). Both MIF and PDGF correlated with behavioral scores as well. In addition to increases in pro-inflammatory cytokines, decreases in TGFβ, a regulatory cytokine, were also found in children with ASD which were associated with worsening behavioral scores (Ashwood et al. 2008). In addition to plasma cytokine differences, there have also been reports of differences in immunoglobulin levels. One study reported that children with autism have reduced levels of plasma IgG and IgM which also correlated with increased behavioral severity (Heuer et al. 2011). Other studies have reported increases in serum proteins attributed mostly to increases in albumin; however, IgG, specifically IgG2 and IgG4 were also seen elevated in individuals with ASD and these increases in immunoglobulin correlated with behavioral abnormalities (Croonenberghs et al. 2002; Enstrom et al. 2009a). Autoantibodies to various and diverse targets have been reported in children with autism and could point to cellular damage that may be involved in increasing inflammation, revealing antigens otherwise hidden and/or epitope spreading (Onore et al. 2012).

6.3.2 Adaptive Responses in ASD

Adaptive immune responses in children with ASD also show increased cytokine production. Peripheral blood mononuclear cells (PBMC) isolated from the blood of children with ASD ages 2–5 were stimulated and compared to age matched controls. Unstimulated cells from children with ASD produced higher amounts of IL-8 when cultured overnight. After stimulation with phytohemagglutinin (PHA), cells from individuals with ASD produced larger quantities of GM-CSF, IL-13 and TNF-α (Ashwood et al. 2011c). A number of these increased cytokines also correlated to behavioral abnormalities. Increased production of TNF-α and IFN-γ were associated with more stereotyped behaviors. Increased impaired communications were associated with higher IFN-γ and IL-8 production. IL-12p40, a subunit of IL-12, correlated with worsening speech and increased hyperactivity (Ashwood et al. 2011c). This data suggest that perhaps an increased TH1 response may worsen behaviors. Both increases in IL-10 and IL-5 may help to improve behaviors—IL-10 increases were associated with better expressive language while increased IL-5 production correlated with improved fine motor skills. Besides increased production of cytokines, PBMC differences were also seen in T cell activation markers suggesting an altered activation of T cells which may contribute to the differences in cytokines produced (Ashwood et al. 2011c). Other studies have also looked at CD4 and CD8 T cells and have found a shift in TH1 and TH2 cytokines (Gupta et al. 1998).

Adhesion molecules play an important role in leukocyte migration and are involved in modulating immune—CNS connections via passage of T cells through epithelial barriers. Soluble adhesion molecules such as sPECAM, sL-selectin, and sP-selectin were found in lower amounts in high functioning ASD individuals when compared to controls (Iwata et al. 2008; Tsuchiya et al. 2007). Reports of improved behaviors during febrile outbreaks in children with ASD have also been described; these changes in behavior are transient and may be attributed to increased up-regulation of adhesion molecules allowing for more T cell-CNS interactions (Onore et al. 2012; Curran et al. 2007).

6.3.3 Innate Responses in ASD

Changes in innate immune responses have been described in children with ASD. Natural killer (NK) cells, normally involved in killing atypical host cells, have been found to have reduced ability to kill K562 target cells (an immortalized myelogenous leukemia cell line) in children with ASD (Warren et al. 1987; Enstrom et al. 2009b; Vojdani et al. 2008). Factors that may contribute to decreased NK cell activity may be attributed to production of lower amounts of perforin, granzyme B and IFN-γ following stimulation conditions in children with ASD (Enstrom et al. 2009b). Increased numbers of circulating monocytes have also been reported in ASD (Sweeten et al. 2003). Moreover, increased expression of activation markers on these monocytes suggest that these cells are in an activated state (Ashwood et al. 2011a). Indeed these cells have been found to have released increased inflammatory cytokines such as IL-1β, TNF-α and IL-6 in response to TLR2 and TLR4 stimulus. Increased production of IL-6 and IL-1β correlated with increased impairment of social behaviors in children with ASD (Enstrom et al. 2010). Monocytes under certain conditions can give rise to other myeloid cells such as dendritic cells, tissue macrophages and microglia (Djukic et al. 2006; Geissmann et al. 2010). Altered activation and responses in myeloid cells, therefore, have many implications for inflammation in both peripheral and CNS systems.

6.4 Gastrointestinal Abnormalities in ASD

6.4.1 GI Symptoms and Frequency in ASD

Recent studies have suggested that many children with ASD suffer from gastrointestinal (GI) symptoms, dysfunction and inflammation. Associations between ASD and GI symptoms were first reported in the early 1970s (Goodwin et al. 1971). Goodwin looked at 15 autistic children and found seven of them had GI issues.

Since then various other groups have reported the frequency of GI symptoms in the ASD population ranging from 17 to 86% (Erickson et al. 2005). The differences in reported GI abnormalities in children with ASD are in part due to the design of these studies with many lacking proper controls or were based on referral biases that only include children with ASD who have GI complaints. Other factors contributing to the wide range of reports include the heavily skewed amount of retrospective studies that rely on either medical records which mainly look at confirmed diagnosis of GI disease and may not necessarily include GI symptoms experienced in all patients or via a parental survey. Conversely, purely parental based surveys have the disadvantage of often involving memory recall of past GI problems whilst additional impairments in language in non-verbal children make it more difficult for parents to perceive pain in children with ASD. Differences between studies can also be attributed to non standardized GI surveys which define GI symptoms differently among the various studies (Mannion et al. 2013). Common gastrointestinal symptoms that are reported include: diarrhea, constipation, foul smelling stools, gaseousness, abdominal pain, and food regurgitation/reflux.

6.4.2 Nutrition and GI Immunity in ASD

Many of the reports of gastrointestinal symptoms in children with ASD have led some researchers to look into the role that nutritional imbalance may be playing in some of the GI symptoms reported. Studies examining the relationship between nutritional input and its effect on ASD; however, have not supported this hypothesis (Levy et al. 2007). Core features of autism such as repetitive behaviors and resistance to change may impact feeding behaviors and nutrition in children with ASD (Erickson et al. 2005). Overall these studies show that while children with ASD tend to have increased food selectivity but that selectivity does not seem to cause malnutrition and overall, nutrient intake is adequate in children with ASD (Raiten and Massaro 1986; Shearer et al. 1982; Ahearn et al. 2001; Field et al. 2003). Some reports concerning increased rates of food allergies among ASD populations have been reported (Horvath and Perman 2002). One such study seeking to address these concerns found that children with ASD had more responses to food allergens as measured by positive pin prick reactions (Lucarelli et al. 1995). In another study addressing these same concerns, children with ASD were compared to normal siblings and children with known dietary protein intolerances; elevated levels of IFNγ and TNFα were found in PBMC response to dietary proteins in both the ASD group and the known dietary intolerances group (Jyonouchi et al. 2002). Also of note in this same study a correlation between elevated IFNγ and TNFα responses with dietary proteins and elevated response to LPS was seen in children with ASD (Jyonouchi et al. 2002). This suggests that an imbalanced immune system may be playing a role in GI dysfunction.

6.4.3 GI Immunity

The gastrointestinal tract is the immune system's largest source of lymphoid tissue and is an important site of immune regulation (Turner and Goldsmith 2009). It is therefore conceivable that mucosal immune dysfunction could be playing a role in children with ASD who have GI symptoms. Immunohistochemical findings of children with ASD and GI problems showed an increase in CD8 T cells in duodenal and colonic samples (Torrente et al. 2002; Furlano et al. 2001), with increases also seen in γδ T cells in transverse colon samples (Furlano et al. 2001). In addition, decreases in peripheral T cell numbers were reported in children with ASD who have GI symptoms (Ashwood et al. 2003) and may reflect numbers of T cells translocating to the GI mucosa in this subset of ASD individuals. Other immunohistochemical findings revealed deposition of IgG and complement C1q co-localized on the basolateral enterocyte membrane in ASD GI samples (Torrente et al. 2002; Ashwood et al. 2003) suggesting a possible autoimmune component to ASD GI dysfunction. Other studies have illustrated findings of increased number of paneth cells in children with autism and gastrointestinal symptoms (Horvath and Perman 2002; Torrente et al. 2002; Horvath et al. 1999).

Common reports of children with ASD having "leaky gut" have been noted and one study in particular looked at 21 autistic children without known GI disease and found 9 with mucosal permeability; none of the 40 normal controls had permeability issues (D'Eufemia et al. 1996). Results of this study were confirmed when another group also found increased GI permeability in children with ASD and GI problems (Horvath 2000). In humans, disruption of mucosal barriers can occur in the absence of inflammation. Thus, increased mucosal permeability does not necessarily predict inflammation. Interestingly though, findings of atypical intestinal microbiota composition in ASD including increased findings of Clostridium species in stool samples of children with ASD, (Finegold et al. 2010; Finegold et al. 2004; Finegold et al. 2002) may elicit mucosal immune responses and GI dysfunction in some children with ASD and GI symptoms especially if bacteria are able to move across a more permeable intestinal barrier. Moreover, a small study developed on the reports of abnormal microbiota composition in children with ASD looked at 11 children with ASD, and treated with the antibiotic vancomycin, aiming to remove deleterious microbes with the antibiotic. Findings of this study include temporarily improved behavioral symptoms (Sandler et al. 2000). Another study addressed this issue by treating children with ASD and GI issues with oral human immunoglobulin (IG). The results showed that after administering oral IG for 8 weeks, 50% of the patients had improvements in GI severity; however, benefits for the treatment were not maintained at the 30 day follow up. Behavioral improvements were also seen from baseline through the end of the 8 week treatment (Schneider et al. 2006). Other studies that assessed treatment for GI and behavioral symptoms include treatment with secretin. Erickson et al. (2005) reported findings from 11 double blind and 2 open label studies testing secretin treatments in the ASD population. While one of the

open label studies reported behavioral improvements based on parental reporting, all double blind studies showed no differences between control (placebo) groups and secretin treatment groups (Erickson et al. 2005). These studies suggest that more research is needed to address the GI/immune/brain connection. There have been some studies that try to investigate the role GI inflammation has in altering behaviors and CNS function. One group of researchers infected AKR mice with the parasite *trichurs muris* and measured GI inflammation, brain biochemistry changes and behaviors related to anxiety. The authors found that chronic GI inflammation induces anxiety-like behavior in AKR mice (Bercik et al. 2010). Increased use and development of behavioral testing in mice now make it easier to measure behavioral changes associated with various induced and natural occurring conditions in mice.

6.4.4 Behavior and GI Dysfunction

Behavioral abnormalities associated with GI symptoms in autism include studies that have found children who had sleep abnormalities were also more likely to have GI problems (Maenner et al. 2012; Mannion et al. 2013; Ming et al. 2008). Mazurek et al. (2012) looked at 2973 children enrolled in autism treatment network and found that children with ASD and GI problems had higher levels of anxiety and sensory overresponsivity. The authors also suggest that the relationship of these three symptoms could include the involvement of the hypothalamic-pituitary-adrenal (HPA) axis and amygdala based circuits. It is of note that the HPA axis also regulates immune function (Mazurek et al. 2012; Herman and Cullinan 1997). The likelihood of a connection between mucosal immune irregularities and behavior in ASD is intriguing. While it is possible that immune dysfunction in the GI tract interacts with an already abnormal brain to aggravate behavioral symptoms or whether GI problems are just another manifestation of systemic immune abnormalities are questions still to be explored. Many studies examining animal and human subjects reveal elevated peripheral cytokines are able to cause striking changes in behavior (Patterson 2009). It still remains to be seen if similar phenomenon occurs with mucosal inflammation.

6.5 Conclusion

ASD is a complex and heterogeneous spectrum of behavioral disorders made even more apparent by the wide array of genetic and environmental factors and influences that have been linked to it. This heterogeneity has also impacted studies of the immune system with many findings that are often confusing and sometimes conflicting. Overall one theme that has remained constant is that, inflammation is associated with ASD. Findings of neuroinflammation in the CSF and brain tissue, peripheral immune abnormalities on both the innate and adaptive responses and the increasing number of studies on GI dysfunction within ASD all come to a consensus

that immune dysfunction can have persistent impact on behavior. More research is needed to further elucidate the mechanisms by which the immune system affects neurological and behavioral changes and to investigate immune modulating therapeutics for ASD.

References

Abrahams BS, Geschwind DH (2008) Advances in autism genetics: on the threshold of a new neurobiology. Nat Rev Genet 9:341–355

Ahearn WH, Castine T, Nault K, Green G (2001) An assessment of food acceptance in children with autism or pervasive developmental disorder-not otherwise specified. J Autism Dev Disord 31:505–511

Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, Cooper NR, Eikelenboom P, Emmerling M, Fiebich BL, Finch CE, Frautschy S, Griffin WS, Hampel H, Hull M, Landreth G, Lue L, Mrak R, Mackenzie IR, McGeer PL, O'Banion MK, Pachter J, Pasinetti G, Plata-Salaman C, Rogers J, Rydel R, Shen Y, Streit W, Strohmeyer R, Tooyoma I, Van Muiswinkel FL, Veerhuis R, Walker D, Webster S, Wegrzyniak B, Wenk G, Wyss-Coray T (2000) Inflammation and Alzheimer's disease. Neurobiol Aging 21:383–421

Ashwood P, Anthony A, Pellicer AA, Torrente F, Walker-Smith JA, Wakefield AJ (2003) Intestinal lymphocyte populations in children with regressive autism: evidence for extensive mucosal immunopathology. J Clin Immunol 23:504–517

Ashwood P, Enstrom A, Krakowiak P, Hertz-Picciotto I, Hansen RL, Croen LA, Ozonoff S, Pessah IN, Van de Water J (2008) Decreased transforming growth factor beta1 in autism: a potential link between immune dysregulation and impairment in clinical behavioral outcomes. J Neuroimmunol 204:149–153

Ashwood P, Corbett BA, Kantor A, Schulman H, Van de Water J, Amaral DG (2011a) In search of cellular immunophenotypes in the blood of children with autism. PLoS One 6:e19299

Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, Van de Water J (2011b) Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain Behav Immun 25:40–45

Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Water J (2011c) Altered T cell responses in children with autism. Brain Behav Immun 25:840–849

Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Water J (2011d) Associations of impaired behaviors with elevated plasma chemokines in autism spectrum disorders. J Neuroimmunology 232:196–199

Atladottir HO, Pedersen MG, Thorsen P, Mortensen PB, Deleuran B, Eaton WW, Parner ET (2009) Association of family history of autoimmune diseases and autism spectrum disorders. Pediatrics 124:687–694

Bailey A, Le Couteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E, Rutter M (1995) Autism as a strongly genetic disorder: evidence from a British twin study. Psychol Med 25:63–77

Beattie EC, Stellwagen D, Morishita W, Bresnahan JC, Ha BK, Von Zastrow M, Beattie MS, Malenka RC (2002) Control of synaptic strength by glial TNFalpha. Science 295:2282–2285

Bercik P, Verdu EF, Foster JA, Macri J, Potter M, Huang X, Malinowski P, Jackson W, Blennerhassett P, Neufeld KA, Lu J, Khan WI, Corthesy-Theulaz I, Cherbut C, Bergonzelli GE, Collins SM (2010) Chronic gastrointestinal inflammation induces anxiety-like behavior and alters central nervous system biochemistry in mice. Gastroenterology 139:2102–2112

Besedovsky HO, Rey AD (2007) Physiology of psychoneuroimmunology: a personal view. Brain Behav Immun 21:34–44

Biber K, Neumann H, Inoue K, Boddeke HW (2007) Neuronal 'On' and 'Off' signals control microglia. Trends Neurosci 30:596–602

Blomquist HK, Bohman M, Edvinsson SO, Gillberg C, Gustavson KH, Holmgren G, Wahlstrom J (1985) Frequency of the fragile X syndrome in infantile autism. A Swedish multicenter study. Clin Genet 27:113–117

Boulanger LM (2009) Immune proteins in brain development and synaptic plasticity. Neuron 64:93–109

Braunschweig D, Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Croen LA, Pessah IN, Van de Water J (2008) Autism: maternally derived antibodies specific for fetal brain proteins. Neurotoxicology 29:226–231

Brynskikh A, Warren T, Zhu J, Kipnis J (2008) Adaptive immunity affects learning behavior in mice. Brain Behav Immun 22:861–869

Carpentier PA, Palmer TD (2009) Immune influence on adult neural stem cell regulation and function. Neuron 64:79–92

Chess S, Fernandez P, Korn S (1978) Behavioral consequences of congenital rubella. J Pediatrics 93:699–703

Cingolani LA, Thalhammer A, Yu LM, Catalano M, Ramos T, Colicos MA, Goda Y (2008) Activity-dependent regulation of synaptic AMPA receptor composition and abundance by beta3 integrins. Neuron 58:749–762

Constantino JN, Todd RD (2000) Genetic structure of reciprocal social behavior. Am J Psychiary 157:2043–2045

Croen LA, Grether JK, Yoshida CK, Odouli R, Van de Water J (2005) Maternal autoimmune diseases, asthma and allergies, and childhood autism spectrum disorders: a case-control study. Arch Pediatr Adolesc Med 159:151–157

Croen LA, Braunschweig D, Haapanen L, Yoshida CK, Fireman B, Grether JK, Kharrazi M, Hansen RL, Ashwood P, Van de Water J (2008) Maternal mid-pregnancy autoantibodies to fetal brain protein: the early markers for autism study. Biol Psychiatry 64:583–588

Croonenberghs J, Wauters A, Devreese K, Verkerk R, Scharpe S, Bosmans E, Egyed B, Deboutte D, Maes M (2002) Increased serum albumin, gamma globulin, immunoglobulin IgG, and IgG2 and IgG4 in autism. Psychol Med 32:1457–1463

Cunningham CL, Martinez-Cerdeno V, Noctor SC (2013) Microglia regulate the number of neural precursor cells in the developing cerebral cortex. J Neurosci 33:4216–4233

Curran LK, Newschaffer CJ, Lee LC, Crawford SO, Johnston MV, Zimmerman AW (2007) Behaviors associated with fever in children with autism spectrum disorders. Pediatrics 120:e1386–1392

D'Eufemia P, Celli M, Finocchiaro R, Pacifico L, Viozzi L, Zaccagnini M, Cardi E, Giardini O (1996) Abnormal intestinal permeability in children with autism. Acta Paediatrica 85:1076–1079

Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW (2008) From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci 9:46–56

de la Mano A Gato A Alonso MI Carnicero E Martin C Moro JA (2007) Role of interleukin-1beta in the control of neuroepithelial proliferation and differentiation of the spinal cord during development. Cytokine 37:128–137

Derecki NC, Cardani AN, Yang CH, Quinnies KM, Crihfield A, Lynch KR, Kipnis J (2010) Regulation of learning and memory by meningeal immunity: a key role for IL-4. J Exp Med 207:1067–1080

Deverman BE, Patterson PH (2009) Cytokines and CNS development. Neuron 64:61–78

Djukic M, Mildner A, Schmidt H, Czesnik D, Bruck W, Priller J, Nau R, Prinz M (2006) Circulating monocytes engraft in the brain, differentiate into microglia and contribute to the pathology following meningitis in mice. Brain 129:2394–2403

Ehninger D, Sano Y, de Vries PJ, Dies K, Franz D, Geschwind DH, Kaur M, Lee YS, Li W, Lowe JK, Nakagawa JA, Sahin M, Smith K, Whittemore V, Silva AJ (2012) Gestational immune activation and Tsc2 haploinsufficiency cooperate to disrupt fetal survival and may perturb social behavior in adult mice. Mol Psychiatry 17:62–70

Elmer BM, McAllister AK (2012) Major histocompatibility complex class I proteins in brain development and plasticity. Trends Neurosci 35:660–670

Enstrom A, Krakowiak P, Onore C, Pessah IN, Hertz-Picciotto I, Hansen RL, Van de Water JA, Ashwood P (2009a) Increased IgG4 levels in children with autism disorder. Brain Behav Immun 23:389–395

Enstrom AM, Lit L, Onore CE, Gregg JP, Hansen RL, Pessah IN, Hertz-Picciotto I, Van de Water JA, Sharp FR, Ashwood P (2009b) Altered gene expression and function of peripheral blood natural killer cells in children with autism. Brain Behav Immun 23:124–133

Enstrom AM, Onore CE, Van de Water JA, Ashwood P (2010) Differential monocyte responses to TLR ligands in children with autism spectrum disorders. Brain Behav Immun 24:64–71

Erickson CA, Stigler KA, Corkins MR, Posey DJ, Fitzgerald JF, McDougle CJ (2005) Gastrointestinal factors in autistic disorder: a critical review. J Autism Dev Disord 35:713–727

Ferrari D, Pizzirani C, Adinolfi E, Lemoli RM, Curti A, Idzko M, Panther E, Di Virgilio F (2006) The P2X7 receptor: a key player in IL-1 processing and release. J Immunol 176:3877–3883

Field D, Garland M, Williams K (2003) Correlates of specific childhood feeding problems. J Pediatr Child Health 39:299–304

Friedman EM, Irwin MR (1997) Modulation of immune cell function by the autonomic nervous system. Pharmacol Ther 74:27–38

Finegold SM, Molitoris D, Song Y, Liu C, Vaisanen ML, Bolte E, McTeague M, Sandler R, Wexler H, Marlowe EM, Collins MD, Lawson PA, Summanen P, Baysallar M, Tomzynski TJ, Read E, Johnson E, Rolfe R, Nasir P, Shah H, Haake DA, Manning P, Kaul A (2002) Gastrointestinal microflora studies in late-onset autism. Clin Infect Dis 35:S6–S16

Finegold SM, Lawson PA, Vaisanen ML, Molitoris DR, Song Y, Liu C, Collins MD (2004) Anaerofustis stercorihominis gen. nov., sp. nov., from human feces. Anaerobe 10:41–45

Finegold SM, Dowd SE, Gontcharova V, Liu C, Henley KE, Wolcott RD, Youn E, Summanen PH, Granpeesheh D, Dixon D, Liu M, Molitoris DR, Green JA, 3rd (2010) Pyrosequencing study of fecal microflora of autistic and control children. Anaerobe 16:444–453

Furlano RI, Anthony A, Day R, Brown A, McGarvey L, Thomson MA, Davies SE, Berelowitz M, Forbes A, Wakefield AJ, Walker-Smith JA, Murch SH (2001) Colonic CD8 and gamma delta T-cell infiltration with epithelial damage in children with autism. J Pediatrics 138:366–372

Garbett K, Ebert PJ, Mitchell A, Lintas C, Manzi B, Mirnics K, Persico AM (2008) Immune transcriptome alterations in the temporal cortex of subjects with autism. Neurobiol Dis 30:303–311

Garden GA, Moller T (2006) Microglia biology in health and disease. J Neuroimmun Pharmacol 1:127–137

Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K (2010) Development of monocytes, macrophages, and dendritic cells. Science 327:656–661

Gemma C, Fister M, Hudson C, Bickford PC (2005) Improvement of memory for context by inhibition of caspase-1 in aged rats. Eur J Neurosci 22:1751–1756

Gibertini M, Newton C, Friedman H, Klein TW (1995) Spatial learning impairment in mice infected with Legionella pneumophila or administered exogenous interleukin-1-beta. Brain Behav Immun 9:113–128

Goines PE, Croen LA, Braunschweig D, Yoshida CK, Grether J, Hansen R, Kharrazi M, Ashwood P, Van de Water J (2011) Increased midgestational IFN-gamma, IL-4 and IL-5 in women bearing a child with autism: a case-control study. Mol Autism 2:13

Goodwin MS, Cowen MA, Goodwin TC (1971) Malabsorption and cerebral dysfunction: a multivariate and comparative study of autistic children. J Autism Child Schizophr 1(1):48–62

Goshen I, Avital A, Kreisel T, Licht T, Segal M, Yirmiya R (2009) Environmental enrichment restores memory functioning in mice with impaired IL-1 signaling via reinstatement of long-term potentiation and spine size enlargement. J Neuroscience 29:3395–3403

Goshen I, Kreisel T, Ounallah-Saad H, Renbaum P, Zalzstein Y, Ben-Hur T, Levy-Lahad E, Yirmiya R (2007) A dual role for interleukin-1 in hippocampal-dependent memory processes. Psychoneuroendocrinology 32:1106–1115

Grigorenko EL, Han SS, Yrigollen CM, Leng L, Mizue Y, Anderson GM, Mulder EJ, de Bildt A, Minderaa RB, Volkmar FR, Chang JT, Bucala R (2008) Macrophage migration inhibitory factor and autism spectrum disorders. Pediatrics 122:e438–445

Gupta S, Aggarwal S, Rashanravan B, Lee T (1998) Th1- and Th2-like cytokines in CD4 + and CD8 + T cells in autism. J Neuroimmunol 85:106–109
Hein AM, Stasko MR, Matousek SB, Scott-McKean JJ, Maier SF, Olschowka JA, Costa AC, O'Banion MK (2010) Sustained hippocampal IL-1beta overexpression impairs contextual and spatial memory in transgenic mice. Brain Behav Immun 24:243–253
Heininger U, Desgrandchamps D, Schaad UB (2006) Seroprevalence of Varicella-Zoster virus IgG antibodies in Swiss children during the first 16 months of age. Vaccine 24:3258–3260
Herman JP, Cullinan WE (1997) Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. Trends Neurosci 20:78–84
Heuer L, Braunschweig D, Ashwood P, Van de Water J, Campbell DB (2011) Association of a MET genetic variant with autism-associated maternal autoantibodies to fetal brain proteins and cytokine expression. Transl Psychiatry 1:e48
Horvath K (2000) Secretin treatment for autism. New Eng J Med 342:1216; author reply 1218
Horvath K, Perman JA (2002) Autism and gastrointestinal symptoms. Curr Gastroenterol Rep 4:251–258
Horvath K, Papadimitriou JC, Rabsztyn A, Drachenberg C, Tildon JT (1999) Gastrointestinal abnormalities in children with autistic disorder. J Pediatr 135:559–563
Hsiao EY, Patterson PH (2011) Activation of the maternal immune system induces endocrine changes in the placenta via IL-6. Brain Behav Immun 25:604–615
Huh GS, Boulanger LM, Du H, Riquelme PA, Brotz TM, Shatz CJ (2000) Functional requirement for class I MHC in CNS development and plasticity. Science 290:2155–2159
Hung J, Chansard M, Ousman SS, Nguyen MD, Colicos MA (2010) Activation of microglia by neuronal activity: results from a new in vitro paradigm based on neuronal-silicon interfacing technology. Brain Behav Immun 24:31–40
Ignatowski TA, Gallant S, Spengler RN (1996) Temporal regulation by adrenergic receptor stimulation of macrophage (M phi)-derived tumor necrosis factor (TNF) production post-LPS challenge. J Neuroimmunol 65:107–117
Irwin MR, Miller AH (2007) Depressive disorders and immunity: 20 years of progress and discovery. Brain Behav Immun 21:374–383
Iwata Y, Tsuchiya KJ, Mikawa S, Nakamura K, Takai Y, Suda S, Sekine Y, Suzuki K, Kawai M, Sugihara G, Matsuzaki H, Hashimoto K, Tsujii M, Sugiyama T, Takei N, Mori N (2008) Serum levels of P-selectin in men with high-functioning autism. Br J Psychiatry 193:338–339
Jorde LB, Hasstedt SJ, Ritvo ER, Mason-Brothers A, Freeman BJ, Pingree C, McMahon WM, Petersen B, Jenson WR, Mo A (1991) Complex segregation analysis of autism. Am J Hum Genet 49:932–938
Jyonouchi H, Sun S, Itokazu N (2002) Innate immunity associated with inflammatory responses and cytokine production against common dietary proteins in patients with autism spectrum disorder. Neuropsychobiology 46:76–84
Kajizuka M, Miyachi T, Matsuzaki H, Iwata K, Shinmura C, Suzuki K, Suda S, Tsuchiya KJ, Matsumoto K, Iwata Y, Nakamura K, Tsujii M, Sugiyama T, Takei N, Mori N (2010) Serum levels of platelet-derived growth factor BB homodimers are increased in male children with autism. Prog Neuropsychopharmacol Biol Psychiatry 34:154–158
Kates WR, Burnette CP, Eliez S, Strunge LA, Kaplan D, Landa R, Reiss AL, Pearlson GD (2004) Neuroanatomic variation in monozygotic twin pairs discordant for the narrow phenotype for autism. Am J Psychiatry 161:539–546
Kim SU, de Vellis J (2005) Microglia in health and disease. J Neurosci Res 81:302–313
Kin NW, Sanders VM (2006) It takes nerve to tell T and B cells what to do. J Leukocyte Biol 79:1093–1104
Kipnis J, Cohen H, Cardon M, Ziv Y, Schwartz M (2004) T cell deficiency leads to cognitive dysfunction: implications for therapeutic vaccination for schizophrenia and other psychiatric conditions. Proc Natl Acad Sci U S A 101:8180–8185
Kipnis J, Gadani S, Derecki NC (2012) Pro-cognitive properties of T cells. Nat Rev Immunol 12:663–669

Krabbe KS, Pedersen M, Bruunsgaard H (2004) Inflammatory mediators in the elderly. Exp Gerontol 39:687–699
Laurence JA, Fatemi SH (2005) Glial fibrillary acidic protein is elevated in superior frontal, parietal and cerebellar cortices of autistic subjects. Cerebellum 4:206–210
Levy SE, Souders MC, Ittenbach RF, Giarelli E, Mulberg AE, Pinto-Martin JA (2007) Relationship of dietary intake to gastrointestinal symptoms in children with autistic spectrum disorders. Biol Psychiatry 61:492–497
Li X, Chauhan A, Sheikh AM, Patil S, Chauhan V, Li XM, Ji L, Brown T, Malik M (2009) Elevated immune response in the brain of autistic patients. J Neuroimmunol 207:111–116
Libbey JE, Sweeten TL, McMahon WM, Fujinami RS (2005) Autistic disorder and viral infections. J Neurovirol 11:1–10
Lucarelli S, Frediani T, Zingoni AM, Ferruzzi F, Giardini O, Quintieri F, Barbato M, D'Eufemia P, Cardi E (1995) Food allergy and infantile autism. Panminerva Medica 37(3):137–141
Maenner MJ, Arneson CL, Levy SE, Kirby RS, Nicholas JS, Durkin MS (2012) Brief report: Association between behavioral features and gastrointestinal problems among children with autism spectrum disorder. J Autism Dev Disord 42:1520–1525
Malkova NV, Yu CZ, Hsiao EY, Moore MJ, Patterson PH (2012) Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism. Brain Behav Immun 26:607–616
Mandal M, Marzouk AC, Donnelly R, Ponzio NM (2011) Maternal immune stimulation during pregnancy affects adaptive immunity in offspring to promote development of TH17 cells. Brain Behav Immun 25:863–871
Mannion A, Leader G, Healy O (2013) An investigation of comorbid psychological disorders, sleep problems, gastrointestinal symptoms and epilepsy in children and adolescents with autism spectrum disorder. Res Autism Spect Disord 7:35–42
Marin-Teva JL, Dusart I, Colin C, Gervais A, van Rooijen N, Mallat M (2004) Microglia promote the death of developing Purkinje cells. Neuron 41:535–547
Martin LA, Ashwood P, Braunschweig D, Cabanlit M, Van de Water J, Amaral DG (2008) Stereotypies and hyperactivity in rhesus monkeys exposed to IgG from mothers of children with autism. Brain Behav Immun 22:806–816
Mazurek MO, Shattuck PT, Wagner M, Cooper BP (2012) Prevalence and correlates of screen-based media use among youths with autism spectrum disorders. J Autism Dev Disord 42:1757–1767
Meltzer JC, MacNeil BJ, Sanders V, Pylypas S, Jansen AH, Greenberg AH, Nance DM (2004) Stress-induced suppression of in vivo splenic cytokine production in the rat by neural and hormonal mechanisms. Brain Behav Immun 18:262–273
Michaelson MD, Bieri PL, Mehler MF, Xu H, Arezzo JC, Pollard JW, Kessler JA (1996) CSF-1 deficiency in mice results in abnormal brain development. Development 122:2661–2672
Ming X, Brimacombe M, Chaaban J, Zimmerman-Bier B, Wagner GC (2008) Autism spectrum disorders: concurrent clinical disorders. J Child Neurol 23:6–13
Moore AH, Wu M, Shaftel SS, Graham KA, O'Banion MK (2009) Sustained expression of interleukin-1beta in mouse hippocampus impairs spatial memory. Neuroscience 164:1484–1495
Morgan JT, Chana G, Pardo CA, Achim C, Semendeferi K, Buckwalter J, Courchesne E, Everall IP (2010) Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. Biol Psychiatry 68:368–376
Nance DM, Sanders VM (2007) Autonomic innervation and regulation of the immune system (1987–2007). Brain Behav Immun 21:736–745
Neumann H, Kotter MR, Franklin RJ (2009) Debris clearance by microglia: an essential link between degeneration and regeneration. Brain 132:288–295
Nguyen KT, Deak T, Owens SM, Kohno T, Fleshner M, Watkins LR, Maier SF (1998) Exposure to acute stress induces brain interleukin-1beta protein in the rat. J Neuroscience 18:2239–2246
Oitzl MS, van Oers H, Schobitz B, de Kloet ER (1993) Interleukin-1 beta, but not interleukin-6, impairs spatial navigation learning. Brain Res 613:160–163

Onore C, Careaga M, Ashwood P (2012) The role of immune dysfunction in the pathophysiology of autism. Brain Behav Immun 26:383–392

Pacheco R, Ciruela F, Casado V, Mallol J, Gallart T, Lluis C, Franco R (2004) Group I metabotropic glutamate receptors mediate a dual role of glutamate in T cell activation. J Biol Chem 279:33352–33358

Patterson PH (2009) Immune involvement in schizophrenia and autism: etiology, pathology and animal models. Behav Brain Res 204:313–321

Raiten DJ, Massaro T (1986) Perspectives on the nutritional ecology of autistic children. J Autism Dev Disord 16:133–143

Ron-Harel N, Schwartz M (2009) Immune senescence and brain aging: can rejuvenation of immunity reverse memory loss? Trends Neurosci 32:367–375

Ron-Harel N, Segev Y, Lewitus GM, Cardon M, Ziv Y, Netanely D, Jacob-Hirsch J, Amariglio N, Rechavi G, Domany E, Schwartz M (2008) Age-dependent spatial memory loss can be partially restored by immune activation. Rejuv Res 11:903–913

Ron-Harel N, Cardon M, Schwartz M (2011) Brain homeostasis is maintained by "danger" signals stimulating a supportive immune response within the brain's borders. Brain Behav Immun 25:1036–1043

Roussa E, Wiehle M, Dunker N, Becker-Katins S, Oehlke O, Krieglstein K (2006) Transforming growth factor beta is required for differentiation of mouse mesencephalic progenitors into dopaminergic neurons in vitro and in vivo: ectopic induction in dorsal mesencephalon. Stem Cells 24:2120–2129

Sandler RH, Finegold SM, Bolte ER, Buchanan CP, Maxwell AP, Vaisanen ML, Nelson MN, Wexler HM (2000) Short-term benefit from oral vancomycin treatment of regressive-onset autism. J Child Neurol 15:429–435

Schneider CK, Melmed RD, Barstow LE, Enriquez FJ, Ranger-Moore J, Ostrem JA (2006) Oral human immunoglobulin for children with autism and gastrointestinal dysfunction: a prospective, open-label study. J Autism Dev Disord 36:1053–1064

Schwartz M, Shechter R (2010) Protective autoimmunity functions by intracranial immunosurveillance to support the mind: the missing link between health and disease. Mol Psychiatry 15:342–354

Schwartzer JJ, Careaga M, Onore CE, Rushakoff JA, Berman RF, Ashwood P (2013) Maternal immune activation and strain specific interactions in the development of autism-like behaviors in mice. Transl Psychiatry 3:e240

Shaftel SS, Griffin WS, O'Banion MK (2008) The role of interleukin-1 in neuroinflammation and Alzheimer disease: an evolving perspective. J Neuroinflammation 5:7

Shearer TR, Larson K, Neuschwander J, Gedney B (1982) Minerals in the hair and nutrient intake of autistic children. J Autism Dev Disord 12:25–34

Shi L, Fatemi SH, Sidwell RW, Patterson PH (2003) Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. J Neurosci 23:297–302

Singer HS, Morris C, Gause C, Pollard M, Zimmerman AW, Pletnikov M (2009) Prenatal exposure to antibodies from mothers of children with autism produces neurobehavioral alterations: a pregnant dam mouse model. J Neuroimmunol 211:39–48

Singh VK (1996) Plasma increase of interleukin-12 and interferon-gamma. Pathological significance in autism. J Neuroimmunol 66:143–145

Smith SE, Li J, Garbett K, Mirnics K, Patterson PH (2007) Maternal immune activation alters fetal brain development through interleukin-6. J Neurosci 27:10695–10702

Steffenburg S, Gillberg C, Hellgren L, Andersson L, Gillberg IC, Jakobsson G, Bohman M (1989) A twin study of autism in Denmark, Finland, Iceland, Norway and Sweden. J Child Psychol Psychiatry 30:405–416

Stubbs EG, Crawford ML (1977) Depressed lymphocyte responsiveness in autistic children. J Autism Child Schizophr 7:49–55

Sweeten TL, Posey DJ, McDougle CJ (2003) High blood monocyte counts and neopterin levels in children with autistic disorder. Am J Psychiatry 160:1691–1693

Takahashi K, Rochford CD, Neumann H (2005) Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2. J Exp Med 201:647–657

Torrente F, Ashwood P, Day R, Machado N, Furlano RI, Anthony A, Davies SE, Wakefield AJ, Thomson MA, Walker-Smith JA, Murch SH (2002) Small intestinal enteropathy with epithelial IgG and complement deposition in children with regressive autism. Mol Psychiatry 7:375–382, 334

Tracey KJ (2002) The inflammatory reflex. Nature 420:853–859

Tsuchiya KJ, Hashimoto K, Iwata Y, Tsujii M, Sekine Y, Sugihara G, Matsuzaki H, Suda S, Kawai M, Nakamura K, Minabe Y, Yagi A, Iyo M, Takei N, Mori N (2007) Decreased serum levels of platelet-endothelial adhesion molecule (PECAM-1) in subjects with high-functioning autism: a negative correlation with head circumference at birth. Biol Psychiatry 62:1056–1058

Turner MS, Goldsmith JD (2009) Best practices in diagnostic immunohistochemistry: spindle cell neoplasms of the gastrointestinal tract. Arch Path Lab Med 133:1370–1374

Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA (2005) Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann Neurol 57:67–81

Vojdani A, Mumper E, Granpeesheh D, Mielke L, Traver D, Bock K, Hirani K, Neubrander J, Woeller KN, O'Hara N, Usman A, Schneider C, Hebroni F, Berookhim J, McCandless J (2008) Low natural killer cell cytotoxic activity in autism: the role of glutathione, IL-2 and IL-15. J Neuroimmunol 205:148–154

Wakselman S, Bechade C, Roumier A, Bernard D, Triller A, Bessis A (2008) Developmental neuronal death in hippocampus requires the microglial CD11b integrin and DAP12 immunoreceptor. J Neurosci 28:8138–8143

Warren RP, Foster A, Margaretten NC (1987) Reduced natural killer cell activity in autism. J Am Acad Child Adolesc Psychiatry 26:333–335

Widera D, Mikenberg I, Elvers M, Kaltschmidt C, Kaltschmidt B (2006) Tumor necrosis factor alpha triggers proliferation of adult neural stem cells via IKK/NF-kappaB signaling. BMC Neurosci 7:64

Yirmiya R, Goshen I (2011) Immune modulation of learning, memory, neural plasticity and neurogenesis. Brain Behav Immun 25:181–213

Ziv Y, Ron N, Butovsky O, Landa G, Sudai E, Greenberg N, Cohen H, Kipnis J, Schwartz M (2006) Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. Nat Neurosci 9:268–275

Chapter 7
Neuroimaging in Autism Spectrum Disorders

Danielle Baribeau and Evdokia Anagnostou

Abstract This chapter reviews the existing literature on neuroimaging in autism spectrum disorders. Research methodology and significant findings from structural and functional magnetic resonance imaging (MRI), magnetic resonance spectrometry (MRS) and positron emission tomography (PET) are described. Overall, despite significant heterogeneity in study results, a pattern emerges suggesting early brain overgrowth in the first few years of life, followed by dysmaturation in adolescence. Connectivity analyses reveal impaired long-range connectivity as well as increased local and/or subcortical connectivity in ASD. Such findings help to inform hypotheses regarding the etiopathogenesis of this condition, but have yet to yield practical clinical applications.

Keywords Autism spectrum disorder · Neuroimaging · Magnetic resonance imaging · Positron emission tomography · Magnetic resonance spectroscopy · Diffusion tensor imaging · Connectivity · Functional neuroimaging

Investigations using neuroimaging in Autism Spectrum Disorders (ASD) have centered on two primary goals: (1) to describe aberrant neurodevelopment in populations with ASD in attempts to further elucidate the etiopathogenesis of this condition; and (2) to identify potential neuroimaging biomarkers which may be predictive of a clinical diagnosis, prognosis or treatment response when applied to an individual. As of 2013, there is insufficient evidence to support the routine use of clinical neuroimaging during diagnostic evaluation of ASD. This chapter is therefore a summary of research literature to date.

Each section in this chapter will begin by highlighting different methodological approaches to neuroimaging in ASD, as it has developed over three decades of research, in order to introduce the reader to this emerging field and to provide context for interpretation of subsequent findings. Specifically, the majority of the literature has employed manipulation of magnetic resonance imaging (MRI) data, beginning

D. Baribeau (✉)
Department of Psychiatry, University of Toronto, Toronto, ON, Canada
e-mail: Danielle.baribeau@mail.utoronto.ca

E. Anagnostou
Department of Pediatrics, Bloorview Research Institute,
University of Toronto, Toronto, ON, Canada
e-mail: eanagnostou@hollandbloorview.ca

S. Hossein Fatemi (ed.), *The Molecular Basis of Autism,*
Contemporary Clinical Neuroscience, DOI 10.1007/978-1-4939-2190-4_7

in the 1980's with volumetric comparisons across the entire brain (Sect. 7.2) and in specific regions of interest (Sect. 7.6). Advancements in computational modeling and automated image processing paved the way for voxel based morphometry (VBM) and cortical thickness mapping, which will be discussed in detail in Sects. 7.3 and 7.4, respectively. VBM technology is also applied to study of the shape, thickness and pattern of folding of the cortical surface (Sects. 7.4 and 7.5) as well as to describe the structural connectivity patterns across the brain (Sect. 7.8). White matter analysis incorporating diffusion measurements subsequently permitted characterization of aberrant microstructure development in this condition, as will be discussed in Sect. 7.7. Functional neuroimaging techniques, incorporating functional MRI (fMRI, Sect. 7.10) and positron emission topography (PET, Sect. 7.11) will be surveyed, along with magnetic resonance spectroscopy (MRS, Sect. 7.9).

Within each section, significant, but often heterogeneous results will be discussed with respect to neurodevelopmental trajectories in ASD as well as the potential clinical utility of these findings. The chapter concludes with tentative recommendations regarding future areas of investigation. It is hoped that the development of more cost-effective and efficient neuroimaging technology, combined with the identification of highly sensitive, specific and predictive biomarkers, will yield neuroimaging protocols that may prove useful as a screening or diagnostic tool within the next decade.

7.1 Volumetric Analysis

In the late 1980's and early 1990's, MRI became more widely available in both clinical and research departments. Early trials of MRI in neuropsychiatric diseases required manual delineation of brain structures by technologists or investigators. Over time, it has become apparent that relative differences between patient and control populations can vary significantly depending on the age, sex, ethnicity, IQ, total brain volume and social economic status of the selected patient and control groups. Various efforts to control for these measures have been attempted, and remain a challenge in clinical research today.

Some of the first written reports of volumetric differences in patients with ASD were conducted on relatively low-resolution [0.5 Tesla (T)] MRI scanners. Ventricular enlargement in affected individuals was one of the first observations (Gaffney et al. 1987). As MRI technology advanced, studies conducted with 1.5 T scanners permitted more detailed structural analysis and comparisons. Increased total brain volume and increased ventricular volumes began to emerge more consistently in patients compared to controls, even after samples were controlled for IQ and height (Piven et al. 1995, 1996), and total intracranial volume (Hardan et al. 2001).

Increased total brain and ventricular volume was a finding that seemed more pronounced in young children with ASD compared to age matched controls (Aylward et al. 2002; Courchesne et al. 2001). Enlarged brain volumes correlated with larger head circumferences beginning at 1–2 years of age in patients with ASD, despite having average or below average head sizes at birth (Courchesne et al. 2003; Hazlett et al. 2005).

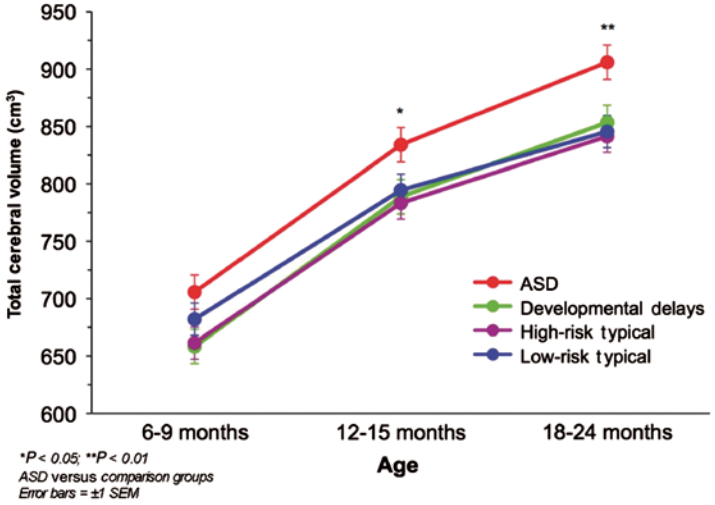


Fig. 7.1 Shen et al. showed how infants and toddlers who were subsequently diagnosed with ASD had greater total cerebral volumes (after removing non brain tissue) and increased rates of growth, as compared to children with other developmental delays or with typical development. (Reproduced with permission from Brain. Shen et al. 2013)

A meta-analysis of head circumference and volumetric MRI studies in ASD conducted prior to 2005 revealed a fairly consistent pattern of slight decreases in head circumference at birth, followed by volume enlargement in the first few years of life. During adolescence and adulthood, individuals with ASD did not differ significantly from their typically developing peers, however (Redcay and Courchesne 2005). Efforts to document abnormal growth rates using prospective and longitudinal data have observed accelerated growth trajectories in very young children (see Fig. 7.1; Schumann et al. 2010; Shen et al. 2013), as well as exaggerated volume losses in adolescence (Courchesne et al. 2011a).

Recently, a systematic review and re-analysis of head circumference and volumetric data suggests that biases in the selection of control populations may be falsely inflating differences in patients with ASD. The authors propose that had previous studies selected control samples from local communities, as well as accounted for known head circumference and brain volume confounders such as weight, ethnicity and social economic status, results would have been much less pronounced regarding early brain overgrowth in this disorder (Raznahan et al. 2013).

That being said, a recent publication sought to determine whether early neuroimaging biomarkers in infants at high risk for ASD might be predictive of a subsequent diagnosis. Shen et al. followed 55 infants (33 of whom were considered high risk given that they had a sibling with ASD and 22 of whom were considered low risk) with serial MRI scans between 6 and 24 months of age. They found that infants who were subsequently diagnosed with ASD had significantly greater extra axial fluid volumes (cerebrospinal fluid in the subarachnoid spaces), greater total brain volume (see Fig. 7.1), larger head circumferences, and larger lateral ventricle volumes. A ratio of extra-axial fluid volume to brain volume at 12–15 months of age that

was greater than 0.14 was predictive of a subsequent diagnosis of ASD with 78% sensitivity and 79% specificity. These findings have yet to be replicated, but the authors suggest that structural MRI monitoring in high risks infants merits further investigation (Shen et al. 2013).

7.2 Voxel Based Morphometry

As the resolution of MRI imaging technology continued to improve, computerized data-analysis software was also developed, which permitted more precise quantification of imaging data. Voxel based morphometry (VBM) is an approach which measures the specific grey matter content of each volumetric pixel (voxel), thereby permitting automated delineation of grey and white matter. Simultaneously, images can be 'warped' onto a common template, in order to more precisely enumerate the differences between cases and control data sets (Hernandez-Garcia and Buschkuehl 2012).

This technology has allowed for a more exact quantification of whole brain and regional volumes, removing the need for manual mapping. In addition, specific subregions can be investigated in greater detail, or the whole brain can be surveyed, in terms of grey matter density by voxel cluster. Alternatively, strategies to correlate structural MRI data from VBM with social and cognitive function have also been employed (Ecker et al. 2012).

There is significant heterogeneity in the literature regarding findings. Differences in image filtering techniques, corrections applied for motion artifacts, statistical thresholds given multiple comparisons, selection of brain templates, as well as demographic and clinical differences in patient samples have complicated the interpretation of study results. Several meta-analyses (Duerden et al. 2012; Nickl-Jockschat et al. 2012; Via et al. 2011; Yu et al. 2011; Cauda et al. 2011) have attempted to synthesize findings, but even results across meta-analyses are variable (see Table 7.1). Two meta-analytic approaches in VBM include the activation likelihood estimate (ALE) (Laird et al. 2005) and the signed-differential mapping (SDM) methods. Both ALE and SDM combine data from published MRI coordinates to generate probability maps identifying grey and white matter clusters where findings converge across studies.

Areas that seem to emerge most consistently in these meta-analyses include volume loss in the putamen (Duerden et al. 2012; Nickl-Jockschat et al. 2012; Yu et al. 2011), volume gains in the caudate (Duerden et al. 2012; Yu et al. 2011) and volume loss in the hippocampus and amygdala (Nickl-Jockschat et al. 2012; Via et al. 2011; Yu et al. 2011). Significant differences also emerge in the prefrontal cortex (see Table 7.1). These findings must be interpreted with the consideration that structural brain changes in ASD may in part be due to comorbid psychiatric symptoms (Juranek et al. 2006), as well as concomitant treatment with psychotropic medications (Chakos et al. 1994). These and other confounding factors cannot be controlled for

Table 7.1 Areas containing grey and white matter clusters of increased (+) or decreased (−) volume/density across voxel based morphometry meta-analyses in patients with ASD vs. controls. (L/R: left or right side, ALE: activation likelihood estimate, SDM: signed-differential mapping, ASP: Asperger's syndrome). Note: −R/L indicates an area of decreased volume/density of grey matter in this region on both the *left* and *right* side. −R/L and +R/L indicates subregions within the specified region that are both increased and decreased on both sides

	(Duerden et al. 2012)		(Nickl-Jockschat et al. 2012)	(Cauda et al. 2011)	(Via et al. 2011)	(Yu et al. 2011)	
Method	ALE		ALE	ALE	SDM	ALE	
Groups	Children	Adults	n/a	n/a	n/a	ASD	ASP
Frontal Lobe							
Precentral gyrus	−R/L		−L	−L			
Medial Frontal gyrus	−R/L	−R	−L			+R	−R
Superior frontal gyrus	−R +R	−R/L +R					−L −R
Middle frontal gyrus	−R/L	+R/L			+L	+R	
Inferior frontal gyrus	−L	−L			+L		
Temporal Lobe							
Superior temporal gyrus	−R/L +R/L	−R					
Middle temporal gyrus	−R/L +R/L	−R +R	−R	+R/L −R		+L	+R
Inferior temporal gyrus						−R +L	
Fusiform gyrus	−R/L +L			+R		−L +R	+R
Parietal Lobe							
Superior Parietal Lobule	−L				−R		
Inferior Parietal Lobule	−L +R			−R/L	−R		+R
Precuneus	−R/L +R	−R	+R	+R −R	−R/L	+R	−R
Primary Sensory Cortex	−L				−R	+R	
Occipital Lobe							
Temporal Occipital Regions			+R/L				
Middle Occipital Region	−L+R	−R					−L
Inferior Occipital Region			+R				

Table 7.1 (continued)

	(Duerden et al. 2012)		(Nickl-Jockschat et al. 2012)	(Cauda et al. 2011)	(Via et al. 2011)	(Yu et al. 2011)	
Method	ALE		ALE	ALE	SDM	ALE	
Groups	Children	Adults	n/a	n/a	n/a	ASD	ASP
Cuneus		−R/L					
Lingual Gyrus	+L		+R	+L		+L	
Insular cortex							
Any location	−R/L +R			+R −R		+R	
Cerebellum							
Any location	−R/L +R	−R/L +R/L	+R/L			−R/L +R	−R
Cerebellar Vermis			−R/L	+R			
Cerebellar Tonsil				−R/L			
Subcortical Structures							
Putamen	−L +L	−R/L	−R/L				−R
Caudate	+R			+R head −R tail		+R/L	
Thalamus	−R/L						
Limbic System and Associated Structures							
Hippocampus			−L		−R	−R	−R/L
Hippocampal Gyrus	−R/L	+L				+R	+R
Uncus						−R	+R
Amygdala	−R		−L	−R	−R		−R/L
Anterior Cingulate	−R +R	−R +L		+R		+R/L	
Posterior Cingulate	+R	−L		+R			

across meta-analyses. The functional implications of these areas will be discussed in more detail in Sect. 7.6 regarding regions of interest in ASD.

In addition to identifying regions of interests, the meta-analytic approaches described above have helped to elucidate age related differences in this condition. Findings span many heterogeneous regions, but similar to volumetric data, differences seem more pronounced and more numerous in the childhood/adolescent samples compared to adult samples (Duerden et al. 2012; Nickl-Jockschat et al. 2012).

Data from VBM analyses have also helped to inform the decision to condense previous diagnostic categories (i.e. autistic disorder, PDD-NOS, and Asperger's syndrome) into a common autism spectrum disorder in the DSM-5, given the absence of discrete biologically based disease categories (Via et al. 2011).

7.3 Cortical Thickness Mapping

In cortical thickness mapping, the technology from voxel based morphometry is applied specifically to the cerebral cortex, to permit more precise anatomic analysis of this area. Similar to whole brain VBM, in cortical thickness analysis, a computer is programmed to extract the cortical grey matter from underlying structures, and this data is transposed onto a common surface template. In this way, the cortical grey matter can be further quantified in terms of cortical thickness, and cortical surface area. These measurements are of interest in ASD, as they are thought to represent separate embryological processes, which may inform a more specific etiology in neurodevelopmental disorders (Lui et al. 2011). Cortical surface area, for example, is thought to reflect neural stem cell proliferation and migration into the ventricular zone, which occurs early in embryogenesis (Rakic 2009). Cortical thickness, however, fluctuates throughout the lifespan, and is representative of a dynamic process involving cell layering, axonal myelination, synaptic growth and pruning (Shaw et al. 2008). It is important to note that typical cortical development involves a process of gradually increasing thickness during early childhood, which reaches a maximum prior to the onset of puberty, followed by gradual thinning which extends into early adult life (Shaw et al. 2008). Neuroimaging findings suggestive of altered developmental trajectories would be consistent with the conceptual understanding that ASD is a neurodevelopmental disorder.

Similar to the heterogeneity in VBM data, the literature with respect to cortical thickness in ASD is again inconsistent, and seems to vary significantly depending on the age of the study population. Early trials in a small group of children (mean age of 10 years) with ASD compared to controls identified increased cortical thickness, particularly in the temporal lobe, despite comparable brain volumes overall (Hardan et al. 2006). Follow up longitudinal MRI data by the same research revealed that patients with ASD had greater decreases in cortical thickness over time, which correlated with severity of symptomatology. The small sample size, however, resulted in marginal statistical significance once multiple comparisons were accounted for. Despite this, hypotheses regarding exaggerated cortical thinning, excessive elimination of synapses or impaired arborization were put forward from this data (Hardan et al. 2009).

Follow up investigations looking at cortical thickness in ASD have aimed not only to quantify specific differences in larger sample sizes, but also to document the magnitude and direction of aberrant cortical growth rates, taking into account the important effects of age.

Looking at broad age range (10–60 years), Raznahan et al. (2010) examined cross sectional imaging data in males with ASD vs. controls. Contrary to earlier findings, individuals with ASD were found to have smaller cortical volumes and thickness than controls at younger ages. Additionally, the rate of cortical thinning with age was attenuated for affected individuals, particularly in the temporal regions. Surface area did not change with time in either group (Raznahan et al. 2010) (see Fig. 7.2). Scheel et al. (2011) found similar results looking cross-sectionally

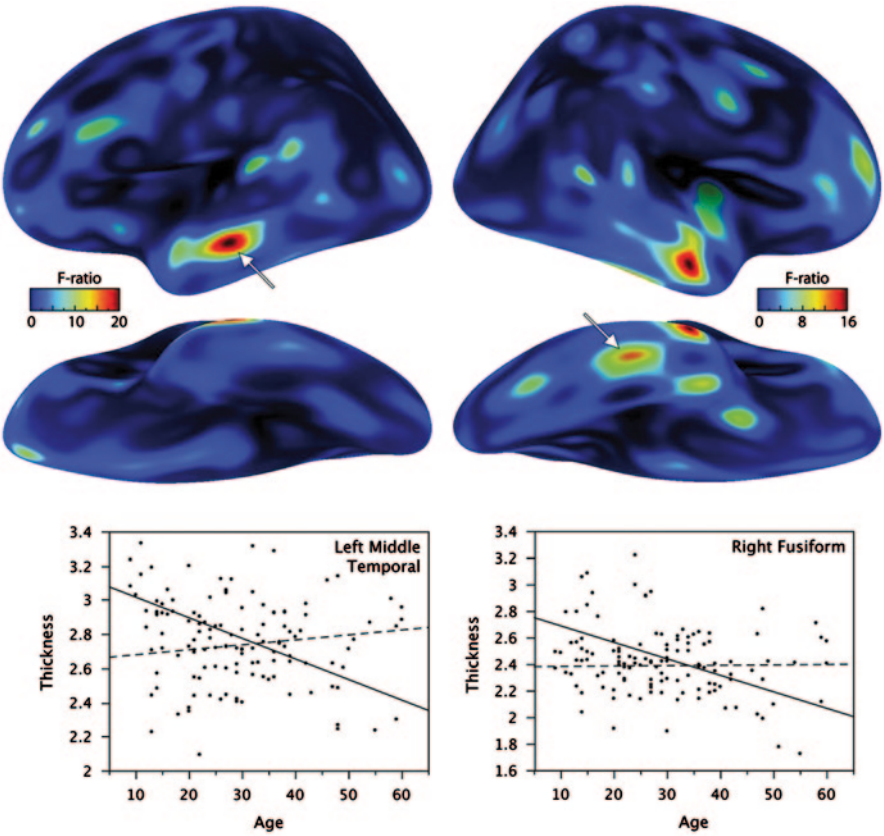


Fig. 7.2 Warmer colours on brain maps identify areas where age-by-group interactions were significant. Graphs show scatter plots of cortical thickness by age (10–60 years) in these specific regions (*dotted line*: ASD). (Reproduced with permission from Cerebral Cortex. Raznahan et al. 2010)

at patients with 'high functioning' autism between ages 20–55 (Scheel et al. 2011). Along these lines, Doyle-Thomas et al. (2013) found attenuated cortical thinning in patients with ASD across a wide age ranges as well (7–39 years) (Doyle-Thomas et al. 2013). Recently, Ecker et al. published a report on cortical thickness, volume and surface area in men with ASD ages 18–42 years. They identified many non-overlapping areas of both increased and decreased cortical thickness and surface area in patients with ASD compared to controls, particularly in frontal and temporal regions, although age correlates were not provided (Ecker et al. 2013a).

On the other hand, analysis of cortical thickness data focusing on more narrow age ranges, and including a greater proportion of younger participants, seems to have yielded findings more consistent with those initially described by Hardan et al. (i.e. exaggerated thinning). Wallace et al. found that males with ASD ages 12–23 years, had thinner cortices overall, and then showed increased rates of cortical thinning during adolescence, with differences again most pronounced in

the temporal regions (Wallace et al. 2010). Mak-Fan et al. (2012) looked at cross sectional data in children ages 6–15 years, and identified increased grey matter volume, cortical thickness and cortical surface area in younger children compared to controls, that then also underwent exaggerated thinning, surpassing controls on all measures between ages 10–12 years. Age effects were most pronounced in specific regions, including the medial precuneus and the left inferior frontal gyrus (Mak-Fan et al. 2012b).

In toddlers with ASD, cortical volume and surface area, (but not thickness) were found to be increased compared to controls at 2 years of age. The rate of cortical growth on longitudinal imaging between ages 2–5 years did not differ between groups, however, generating further questions regarding the specific chronology of aberrant neurodevelopment (Hazlett et al. 2011).

To summarize, a few studies suggest cortical overgrowth at very young ages; at age 2 this is marked by increased cortical volume and surface area, but potentially not thickness. School age children seem to display increased cortical thickness that is subsequently met with cortical dysmaturation throughout childhood and adolescence, suggestive mostly of exaggerated and then perhaps attenuated cortical thinning, although the specific magnitude and direction of this change varies depending on the study. The temporal lobe in particular is an area where rate of growth is most altered. Upcoming large scale, prospective neuroimaging trials may help to clarify some of this heterogeneity.

7.4 Surface Based Morphometry

In addition to cortical thickness and cortical surface area, the specific pattern of gyrification in the cortex can also be quantified with mathematical indices. The gyrification index (GI) for example, is the ratio of the total length of the folded cortical surface (extending into each sulcus) divided by the outer cortical surface length (see Fig. 7.3). The rationale for studying these indices is supported in part by early literature where visible structural abnormalities were noted in the gyri of a small group of patients with ASD. Also, as described above with respect to cortical thickness and surface area, specific patterns of gyrification may also be reflective of altered neurodevelopmental processes, or underlying differences in brain cytoarchitecture. For example, variations in neuron layers, cell density, and thalamocortical connections are noted on histology comparing tissue from sulci vs. gyri. Similarly, sulci formation in early life is hypothesized to be driven by tensile pull on areas with greater connectivity to distant regions [summarized in (White et al. 2003)]. Accordingly, altered functional connectivity may yield measurable structural changes in cortical morphology.

Early in the MRI literature, Piven et al. (1990) found significant structural abnormalities in the cortices of several adolescent males with ASD compared to controls. Of 13 patients, 5 displayed noteable polymicrogyria (multiple small cortical folds), and 2 had macrogyria (large cortical folds), as observed by radiologists blinded to

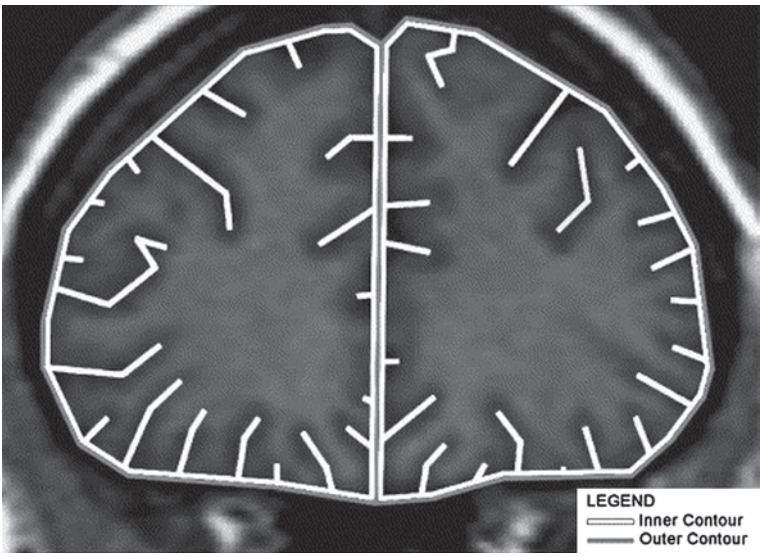


Fig. 7.3 The gyrification index was calculated by comparing the ratio of the inner cortical contour to the outer contour, on a coronal imaging slide. (Reproduced from Psychiatry Research. Hardan et al. 2004)

diagnosis (Piven et al. 1990). Control participants, on the other hand had typically appearing brains. With enlargement of sample sizes, the more striking structural abnormalities in the brains of patients with ASD were for the most part washed out, necessitating a more precise mathematical approach to quantify specific differences.

Hardan et al. (2004) for example, found preliminary evidence suggesting increased gyrification in the frontal lobes of adolescent but not adult patients with ASD compared to controls, as measured by statistical comparison of GI. Patterns emerged suggesting changes in cortical folding with age in affected patients but not in controls (Hardan et al. 2004). In a population of patients with high functioning autism, increased cortical folding was noted in the frontal lobe (Jou et al. 2010). More recently, high-resolution analysis on 3T scanners described increased cortical folding in the occipital and parietal regions of the brain in patients with ASD compared to controls, but that cortical surface area was comparable between the two groups. Both groups showed a decline in gyrification overtime (Wallace et al. 2013).

7.5 Structural Regions of Interest

Many investigators have sought to identify specific regions of interest (ROI), which may be structurally altered in ASD and serve as a disease biomarker. A variety of investigative approaches have been employed. Early trials were labor

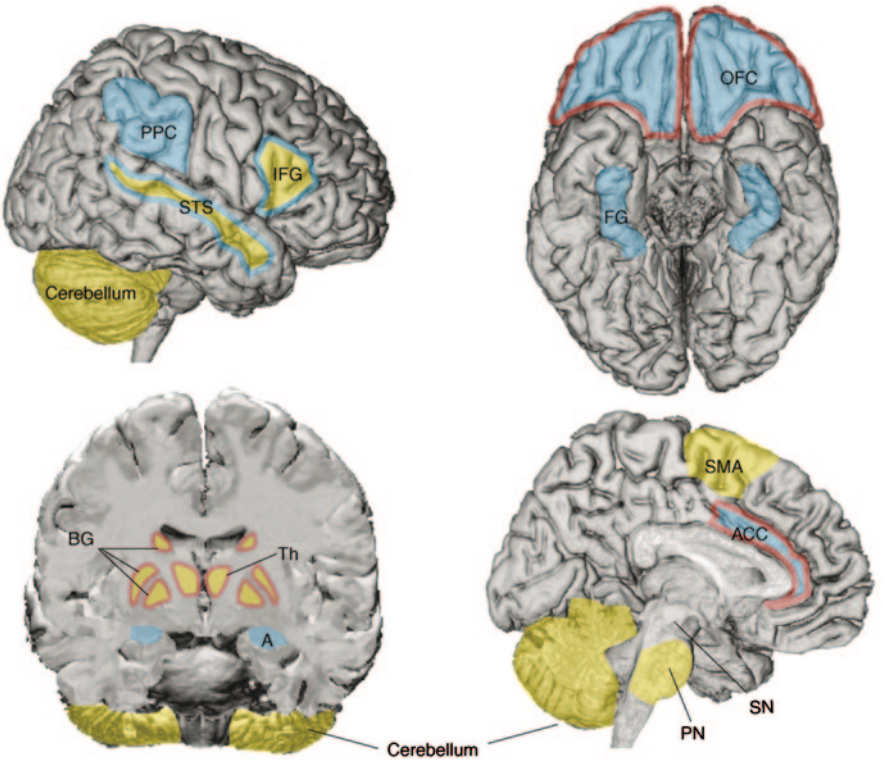
intensive, requiring manual tracing and measurement of specific regions. Since the advent of VBM, broad surveys of the brain, as conducted in the studies described in Sect. 7.4, allowed for simultaneous comparison across dozens of regions of interest or thousands of specific voxels, aiming to identify areas associated with the greatest differences between patient and control samples. Alternatively, the a priori specification of a hypothesis regarding structural differences in a particular region of interest has also been used, to allow for conservation of statistical power. Additionally, complex statistical approaches using mathematical models and machine learning algorithms have recently been developed to combine the predictive power of both methods.

Dozens of ROI have been implicated through VBM data (summarized in Table 7.1), but even across meta-analyses, the locations of proposed voxel clusters are variable, and show increases and decreases in grey matter density and volume in similar regions. Meta-analytic approaches reveal some agreement regarding volume loss in the putamen (Duerden et al. 2012; Nickl-Jockschat et al. 2012; Yu et al. 2011) and amygdala (Nickl-Jockschat et al. 2012; Via et al. 2011; Yu et al. 2011), and volume gains in the caudate (Duerden et al. 2012; Stanfield et al. 2008; Yu et al. 2011). Throughout the medical literature, the neuro-anatomical locations most often cited in relation to ASD include the cingulate cortex, limbic system, prefrontal cortex, temporal and parietal lobe structures, cerebellum and basal ganglia (see Fig. 7.4) (Cauda et al. 2011; Amaral et al. 2008). These regions will be discussed along with possible functional implications as they pertain to social communication deficits and repetitive behaviors. Regions of interest within white matter tracts will be discussed separately in subsequent sections.

7.5.1 Cerebellum

Some of the earliest MRI data in ASD noted structural differences in the ceberelli of patients with ASD, including hypoplasia of the cerebellar vermis (Courchesne et al. 1988). Some findings seemed to be accounted for primarily by variability in IQ (Piven et al. 1992). Subsequent trials, however, repeatedly observed slight volume reductions in several cerebellar vermi, in keeping with reduced numbers of Purkinje cells noted in this region on post mortem tissue analysis [see (Fatemi et al. 2012) for a review of cellular pathology]. Hyperplasia of the cerebellar vermi has also been observed in ASD, however, leading some to propose a bimodal distribution of both hypo and hyperplasia in this region (Courchesne et al. 1994). An overall pattern of slightly increased cerebellar volumes have been noted as well, which are proportionate to the overall larger cerebral volumes (Stanfield et al. 2008). Some studies, however, have found no overall differences or alternatively volume reductions, with age effects perhaps playing a role (Amaral et al. 2008; Scott et al. 2009).

The cerebellum is classically understood to be important in sensorimotor processing and balance; deficits in its function would be in keeping with subtle motor



Social impairment	Communication deficits	Repetitive behaviors
OFC – Orbitofrontal cortex ACC – Anterior cingulate cortex FG – Fusiform gyrus STS – Superior temporal sulcus A – Amygdala mirror neuron regions IFG – Inferior frontal gyrus PPC – Posterior parietal cortex	IFG- Inferior frontal gyrus (Broca's area) STS – Superior temporal sulcus SMA – Supplementary motor area BG – Basal ganglia SN – Substantia nigra Th – Thalamus PN – Pontine nuclei cerebellum	OFC – Orbitofrontal cortex ACC – Anterior cingulate cortex BG – Basal ganglia Th – Thalamus

TRENDS in Neurosciences

Fig. 7.4 Summary of neuro-anatomical locations implicated in ASD, and functional correlates of these structures. (Reproduced with permission from Trends Neurosci. Amaral et al. 2008)

impairments noted in patients with ASD (Gowen and Hamilton 2013). A growing consensus in the literature implicates the cerebellum's role in emotion, memory, attention, language and cognitive circuits, given that it is highly connected to many cortical regions and networks (Strick et al. 2009). Patients with cerebellar agenesis for example, display not only ataxia, but cognitive and affective symptoms as well (Schmahmann 2004). Along these lines, several studies have correlated structural differences in the cerebellum of patients with ASD to language impairment (Hodge et al. 2010) and social function (Catani et al. 2008).

7.5.2 Anterior Cingulate Cortex

The anterior cingulate cortex is a highly connected part of the frontal lobe, with functions related to autonomic regulation as well as social/ emotional processing, reward anticipation and error monitoring (Pfeifer and Peake 2012). On structural neuroimaging, increases (Doyle-Thomas et al. 2013; Jiao et al. 2010) and decreases (Ecker et al. 2013a; Greimel et al. 2013b; Hadjikhani et al. 2006) in volume and thickness of the anterior cingulate have been shown. Age effects have also been described in the region, (Doyle-Thomas et al. 2013; Scheel et al. 2011), which may account for some heterogeneity in the literature. Greater thickness in the anterior cingulate has been associated with poorer social outcomes in ASD (Doyle-Thomas et al. 2013). With functional neuroimaging, altered connectivity between the anterior cingulate, frontal lobe and striatal structures in ASD was associated with impaired response to social rewards and in impaired social communication (Delmonte et al. 2013). Similarly, deficits in structural connectivity in the anterior cingulate have been hypothesized to contribute to repetitive and rigid behaviors, given dysfunction in the error monitoring mechanism (Thakkar et al. 2008).

7.5.3 Amygdala

There is evidence of altered growth tragectories involving the limbic system, particularly centered on the amygdala in patients with ASD. Meta-analysis across VBM data suggest volume loss overall (see Table 7.1), however more recent data including younger age groups describe evidence of volume overgrowth (Bellani et al. 2013b). The amygdala is understood to play an important role in threat monitoring, memory formation and emotional reaction formation, with connections extending to many deep structures such as the thalami, hypothalami and brain stem nuclei, as well as cortical structures in the prefrontal and temporal regions (Catani et al. 2013). Interpretation of the literature with respect to the amygdala's role in ASD is complicated by findings that comorbid anxiety can result in structural changes in this region as well (Juranek et al. 2006).

The contributions of the amygdala to social cognition and behavior continue to be elucidated (Bellani et al. 2013b). There is some evidence that structural changes in this region may influence the formation of appropriate eye contact and gaze (Barnea-Goraly et al. 2014; Kliemann et al. 2012). Larger amygdala volumes correlated with greater social communication impairment in children with ASD across several studies (Munson et al. 2006; Schumann et al. 2009; Kim et al. 2010).

7.5.4 Basal Ganglia

The basal ganglia consist of a group of deep brain structures including the striatum (caudate nucleus and putamen), as well as the globus pallidus, substantia nigra,

subthalamic nucleus and the nucleus accumbens. The basal ganglia are classically understood to play a central role in voluntary motor control, but are also involved in procedural learning, reward processing and some social- emotional circuitry. Findings regarding structural changes in the basal ganglia of patients with ASD must be interpreted in light of possible confounders including neuroleptic medication use, which has been shown to independently increase striatal volumes (Chakos et al. 1994).

Increased volume and rate of growth of the caudate has been described in ASD (Langen et al. 2013), even after controlling for neuroleptic treatment. Volume loss in the putamen is shown across VBM meta-analyses in adults (see Table 7.1), but volume gains in this area have also been described in younger populations (Hua et al. 2013). From a functional perspective, enlargement of the caudate has been associated with repetitive behavior, rigidity and self-injury (Langen et al. 2007; Qiu et al. 2010; Wolff et al. 2013; Hollander et al. 2005; Langen et al. 2013). Changes in the shape of this structure have also been correlated with social communication deficits (Qiu et al. 2010).

The nucleus accumbens is another structure sometimes considered part of the basal ganglia system. It is important for perception and experience of reward (Kohls et al. 2013). Impaired connectivity/ activation of the nucleus accumbens in fronto-striatal circuitry may explain impaired processing of social rewards and punishment in patients with ASD (Delmonte et al. 2013; Delmonte et al. 2012).

7.5.5 Frontal, Temporal and Parietal Regions

The prefrontal cortex lies in the most anterior part of the brain; it is involved in cognition, attention, language as well as social and executive function. Sections within the prefrontal cortex are described anatomically, including the dorsolateral, ventromedial, frontopolar and orbitofrontal regions, for example. The prefrontal cortex is connected to temporal and parietal regions through long-range association fibers, together making up what has been described in the ASD literature as the "social brain network." Reduced volume and decreased activation in frontal and temporal regions (e.g. inferior frontal gyrus and middle temporal gyrus) have been associated with social communication impairment (Yamasaki et al. 2010), and impaired processing of facial expressions (Bastiaansen et al. 2011) in patients with autism, respectively. Age effects may play a role in interpreting finding in this region, however. In children with autism, for example, a significantly greater number of neurons have been observed in the prefrontal region on post mortem tissue analysis, in keeping with overall greater brain volume and weight in younger patients with ASD (Courchesne et al. 2011b).

The temporal lobe is important for language development and comprehension. It also houses the fusiform face area along its medial side, which has been well described to be an important structure involved in processing facial cues. Young children subsequently diagnosed with ASD show early deficits in activity in the

temporal lobe in response to language cues (Eyler et al. 2012). Increased volume in the fusiform gyrus correlated with impaired face processing in adults with ASD (Dziobek et al. 2010). The fusiform gyrus (as well as several other frontal and temporal structures) also showed weaker activity on fMRI in patients with ASD while looking at dynamic and static faces (Sato et al. 2012). Lastly, hypoactivation of the fusiform gyrus in ASD correlated with decreased gaze fixation on the eyes in pictures of faces (Dalton et al. 2005).

Hypotheses in ASD regarding deficits in 'theory of mind' and 'mirror neuron systems' also tend to be associated with frontal, temporal and parietal structures. Theory of mind, for example, is a developmental milestone whereby a child learns to distinguish his/her own thoughts and perceptions from those of others. Theory of mind has been demonstrated to be delayed or impaired in children with ASD, and is hypothesized to involve structures in the prefrontal cortex and the temporo-parietal junction (Kana et al. 2014). Similarly, the 'mirror neuron system' represents a group of neurons that activate not only when a participant performs an action, but also when that action is observed being performed by another. The mirror neuron system is thought to play an important role in learning, language development and theory of mind, and perhaps empathy, and therefore has been implicated in ASD, as well as in other neuropsychiatric conditions. The inferior frontal gyrus and the superior parietal lobule are structures most associated with the mirror neuron system (Hamilton 2013). Reduced activity or structural thinning in these regions have correlated with social communication deficits in ASD as well (Enticott et al. 2012; Hadjikhani et al. 2006).

7.6 White Matter: Volumetric Analysis and Diffusion Tensor Imaging

Earlier studies looking at white matter volume in ASD described a pattern of white matter overgrowth in young children but not adolescents compared to typically developing peers (Courchesne et al. 2004). Subsequent meta-analysis across VBM studies looking at white matter specifically described no difference overall in white matter volume, and no difference between child/adolescent and adult age groups, however data did not include very young children (<6 years). Some regional differences were noted, with increased white matter volume in the right arcuate fasciculus, the left uncinate and inferior fronto-occipital fasciculus, as well as volume losses in the cingulum and corpus callosum (Radua et al. 2011).

Beyond volumetric analysis, diffusion tensor imaging (DTI) is an approach permitting a more detailed analysis of the white matter microstructure of patients with neurodevelopmental disorders such as ASD. DTI uses MRI technology combined with vector algebra, to describe the magnitude and direction of diffusion of water molecules within each individual voxel in the brain. Differences in diffusivity patterns are represented by several specific parameters, and are thought to reflect

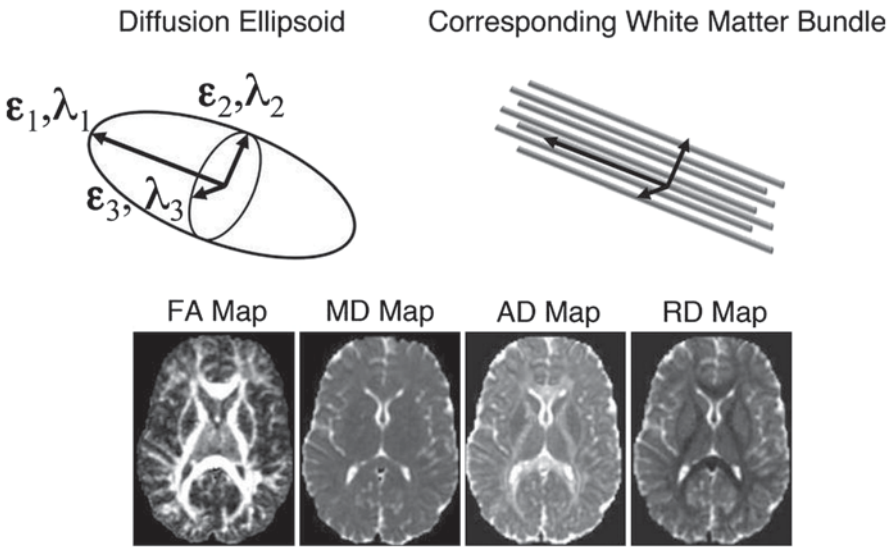


Fig. 7.5 Top panels show diffusion vectors. MD=(λ1+λ2+λ3)/3, RD=λ2+λ3)/2, AD=λ1, and FA= $\sqrt{\frac{3}{2}\frac{(\lambda 1-MD)^2+(\lambda 2-MD)^2+(\lambda 3-MD)^2}{\lambda 1^2+\lambda 2^2+\lambda 3^2}}$ *Bottom* panel shows sample MRI images selecting for specific diffusion measurements. (Reproduced with permission from Autism Research. Travers et al. 2012)

subcellular structural changes. It is important to understand these measurements in order to interpret the DTI literature in ASD.

Axial diffusivity (AD), for example, quantifies diffusion of water molecules occurring *parallel* to white matter fibers. Increased AD has been found in diseases involving axonal degeneration; in autism, AD is thought to reflect both the integrity and density of axons. Radial diffusivity (RD) is a measurement of diffusion traveling *perpendicular* to the white matter fibers. RD is increased in demyelinating diseases, and serves as a surrogate measure of white matter myelination across neurodevelopment. Mean diffusivity (MD) (also known as the apparent diffusion coefficient, ADC) summarizes the total average diffusion occurring in a region, in the absence of any directional gradient (Alexander et al. 2007; see Fig. 7.5).

A summary vector, termed "fractional anisotropy" (FA), describes the overall spherical shape of the other vectors combined. For example, an area where water molecules move freely in all directions would be described as "isotropic." An isotropic solution would yield in a three-dimensional diffusion vector that appears perfectly spherical, represented by an FA value close to zero. On the other hand, areas where diffusion is restricted in certain directions (e.g. in a white matter tract) are considered "anisotropic." The resulting three-dimensional vector would be more ellipsoid shaped, represented by an FA value closer to 1.

In neuroimaging of white matter tracts, higher FA is thought to be a sensitive but not specific marker of white matter integrity, including axonal myelination, fiber

diameter, and the integrity of cell membranes (Kubicki et al. 2007). In typically developing individuals, FA increases and MD decreases with age, as white matter gradually matures (Faria et al. 2010). The specific pathological correlates of DTI in ASD remain to be fully elucidated, however. From mouse models, induction of demyelination resulted in decreased FA and increased RD, while reduced axonal caliber correlated with lower AD values (Harsan et al. 2006). In mouse models of autism, impaired sociability of the BALB/CJ was associated with lower FA and higher MD on DTI. On pathology, the BALB/CJ mouse had more scattered, less densely packed and more poorly myelinated white matter fibers. The authors attributed higher MD values to the reduced axonal density in affected mice, while more poorly myelinated white matter fibers were thought to result in lower FA values (Kumar et al. 2012).

Dozens of articles have been published looking at DTI in patients with ASD compared to controls across age groups. Across the whole brain, most studies observe reduced FA and decreased MD [reviewed by (Travers et al. 2012)]. A recent systematic review and meta-analysis combines data from 14 DTI studies using regions of interest methods (with mean ages of participants between 3 and 32 years). Their results highlight some consistency in findings and are compared to volumetric data in Table 7.2. Fractional anisotropy seems to be lower in corpus callosum, left uncinate fasciculus and left superior longitudinal fasciculus in affected patients vs. controls, suggestive of reduced white matter integrity and possibly reduced myelination in these regions. Fewer studies have published data on MD, but significant increases emerge in the corpus callosum and the superior longitudinal fasciculi as well, which may suggest reduced axonal density of the fibers in these tracts (Aoki et al. 2013).

Table 7.2 Comparison of meta-analyses of white matter volume and diffusion tensor imaging findings by regions in ASD

	Meta-analysis on white matter volume	Meta-analysis on DTI
Study	(Radua et al. 2011)	(Aoki et al. 2013)
Mean age of patient group	21.4	15.2
Whole brain white matter	ND	–
Corpus Callosum	↓	↓ FA ↑ MD
Superior Longitudinal Fasciculus	–	↓ FA (L) ↑ MD
Arcuate Fasciculus	↑	–
Inferior Longitudinal Fasciulus	–	ND FA
Inferior Fronto-occipital Fasciculus	↑	ND FA
Cingulum	↓	ND FA
Uncinate Fasciulus	↑	↓ FA (L) ND MD

These findings may have functional implications related to the core features of ASD. The superior longitudinal fasciculus, for example, is a large white matter bundle coursing in a 'front to back' direction across the brain, relaying information between the frontal, temporal, parietal and occipital lobes. It is divided into several segments, which help to coordinate and relay information needed for movement, spatial navigation, occulomotor function, and importantly, language. Increased MD in the superior longitudinal fasciculus correlated with severity of language impairment in affected children and adolescents (Nagae et al. 2012), and lower FA scores in this region correlated with greater communication impairment on the ADOS and ADI-R (Poustka et al. 2012).

The uncinate fasciculus is an association fiber, which connects structures in the prefrontal cortex (specifically the orbital frontal cortex) with anterior temporal lobe structures as well as the amygdala. These regions are thought to be important for emotional regulation and processing, decision-making, and reward valuation and anticipation. In a study of behavioral interventions, increased adherence to therapy and improved clinical outcomes correlated with higher FA values in the uncinate fasciculus (Pardini et al. 2012). In infants, higher FA in the uncinate fasciculus at 6 months of age was predictive of greater social communication at 9 months of age (Elison et al. 2013).

The corpus callosum is the largest white matter structure in the brain, connecting the left and right cerebral hemispheres. It is involved in many cerebral functions including integration of sensory and motor information, as well as many higher cognitive tasks such as problem solving, executive function, planning, language, and social cognition and communication (Bellani et al. 2013a). Congenital absence of a corpus callosum (called 'agenesis of the corpus callosum') is a condition often associated with developmental delay and motor impairment, although at times can be asymptomatic as well. A screening survey on children with agenesis of the corpus callosum found that 45% were over the cut-off for autism spectrum disorder (Lau et al. 2013).

In addition to macroscopic and microscopic structural differences in white tracts, there is also evidence of dynamic white matter changes, with findings varying by regions and by age group. For example, Mak-Fan et al. (2012) observed that RD and MD measurements stayed stable between the ages 6–14 years in subjects with ASD, while control subjects showed gradual reductions in these measures as expected with age (Mak-Fan et al. 2012a). Similarly, Ameis et al. (2011) found several DTI measurements in individuals with ASD (including RD, AD and MD, but not FA), which deviated significantly from typically developing peers during childhood years but less so in adolescence (Ameis et al. 2011).

Overall, evidence from volumetric and DTI studies describe a pattern of altered white matter maturation, with findings perhaps more pronounced in younger subjects. Certain white matter tracts implicated in ASD seem to correlate with social, cognitive and language function, which are impaired in affected individuals. Despite these positive findings, individual studies report differences in neuroimaging biomarkers that are relatively small, (e.g. 1–2% range), and only in select regions. While results are statistically significant, it remains unclear to what extent

this information is clinically relevant. An approach that has been similarly applied across other neuroimaging modalities involves using computer algorithms to combine the predictive capacity of findings across multiple white matter regions, in hopes of generating a clinical test. Using a discriminant function on DTI data, one group was able to correctly classify patients vs. controls with greater than 90% sensitivity and specificity, and these results were reproduced using a separate patient sample (Lange et al. 2010). Large-scale neuroimaging studies will be required to validate these methods in populations with realistic prevalence rates, so that the true predictive capacity (i.e. positive predictive value) can be ascertained.

7.7 Structural Connectivity Analysis

In the previous section, altered structural connectivity is suspected given volumetric as well as microscopic differences in the white matter fibers that connect disparate brain regions. There are several other approaches that have been employed to study structural connectivity in ASD outside of white matter tract analysis as well.

One approach employed by Ecker et al. (2013b) has been to study cortico-cortical connectivity specifically. They hypothesized that the smaller neural networks within grey matter structures may be altered in autism as well. They applied a technique whereby the geometric formation of the cortical surface was mapped to identify and measure the shortest paths between two points, called 'mean separation distances' which serve as an estimate of the overall "wiring cost" of the brain (i.e. shorter separation leading to more efficient wiring). They found that patients with ASD had shorter lengths of connections between surface points. They suggest that their findings may reflect increased local connectedness at the expense of global connectedness in ASD (Ecker et al. 2013b).

An alternative approach has been to investigate specific brain networks involved in social communication impairment or repetitive interests and behaviors, to determine to what degree related structures co-vary in size. An assumption is made that the interconnecting brain systems have developed along similar timelines and in response to similar pressures, such that a change in one aspect of the system would be reflected throughout other areas. For example Dziobek et al. (2010) investigated structural changes in the lateral fusiform gyrus and in the amygdala, two regions that are known to be highly connected to each other and involved in assessing socially relevant information from facial cues. They looked at the degree to which volumetric changes in one structure co-varied with changes in the other. Interestingly, participants with ASD had negative covariance between both systems (i.e. patients with smaller amygdalas had increased cortical thickness in the fusiform gyrus), which correlated with greater problems in face processing. Controls on the other hand, had positive co-variance between these two structures, highlighting the impaired structural connectivity in this network in affected individuals. Similarly, Bernhardt et al. (2013) identified reduced co-variance suggestive of impaired structural connectivity in a network involved in theory of mind function, consisting of

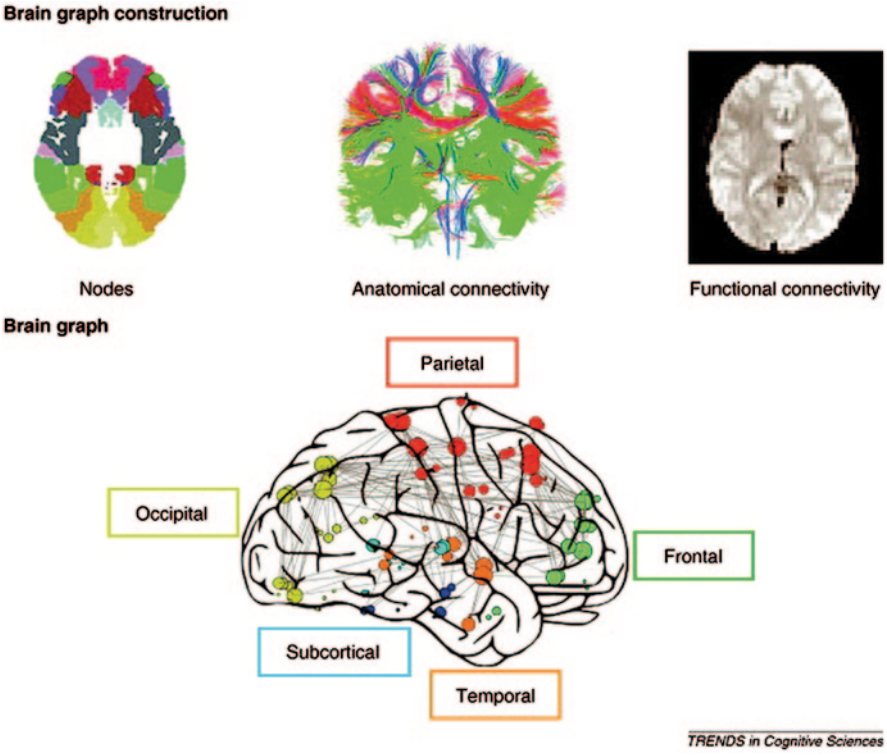


Fig. 7.6 Example of brain connectivity mapped using graph theory. The dots are considered 'nodes' of connectivity, while the lines are called 'edges' and representing connections between nodes. (Reproduced with permission from Trends in Cognitive Sciences. Bassett and Gazzaniga 2011)

the dorsal medial prefrontal cortex and the temporal parietal junction (Bernhardt et al. 2013). Looking across the whole brain, McAlonan et al. (2005) showed significantly fewer positive structural correlations in patients with ASD compared to controls, both in cortico-cortical networks and in cortical-subcortical networks (including limbic and striatal systems) (McAlonan et al. 2005).

In some studies looking at structural (and functional) connectivity across the whole brain, discrete mathematical approaches such as graph theory have been applied. In graph theory, certain highly connected areas are defined as 'nodes' and the connections to other areas are defined as 'edges' reflected in a network of dots and lines. Equations can thus model the degree of connectivity between local and distant regions, in absence of a priori defined hypotheses or specification of a region of interest (see Fig. 7.6). Using this technique, Shi et al. (2013) looked at structural connectivity in patients with ASD using correlations between defined regions in terms of cortical thickness. They observed decreased modularity overall (i.e. fewer clusters of interconnected nodes) in patients with ASD. As well, increased short-range connectivity was noted within the frontal lobe, while long-range connectivity

between frontal, temporal, limbic and parietal lobes was decreased (Shi et al. 2013). Graph theory and similar techniques are also discussed in Sect. 7.9 with respect to functional neuroimaging.

7.8 Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy (MRS) uses MRI technology to measure specific concentrations of brain metabolites. In a standard MRI, the electromagnetic emissions from protons permit quantification of the density of water molecules within a given region. In MRS, a water-suppressing signal is sent into the tissue to facilitate a more detailed analysis of other molecules containing hydrogen molecules, which have their own unique MRS signals normally masked by water signals. Examples of metabolites include creatine (a marker of energy metabolism), N-acetyl aspartate (NAA) (a marker of neuron integrity), choline (which is elevated in demyelination, but also marks membrane turnover), glutamate/ glutamine (which are neurotransmitters involved in excitatory neurotransmission) and myo-inisitol (a marker of myelin breakdown) (see Fig. 7.7 for a sample MRS reading).

A survey of the research results looking at MRS in ASD reveals many contradictory findings, as have been noted with other approaches. A review of the literature by Baruth et al. summarizes some areas of agreement (Baruth et al. 2013). Of note, most studies included in this review were conducted using 1.5 T MRI scanners, which may have inadequate resolution to sufficiently quantify metabolites. Studies employing more powerful 3T and 4T MRI scanners are just beginning to emerge in the ASD literature (Rojas et al. 2013; Joshi et al. 2013).

The most commonly studied metabolite in ASD is NAA. NAA gives off the largest signal of all the MRS metabolites. It is thought to be a marker of neuron integrity, is found within neuron cell bodies, axons and dendrites. Rising levels may also correlate with myelin synthesis, in line with increasing NAA levels throughout early childhood. Lower NAA levels are noted in conditions associated with neuron loss, including Alzheimer's dementia and stroke. Widespread loss of NAA has been reported both diffusely, and in many heterogeneous regions in autism (Baruth et al. 2013). A recent systematic review and meta-analysis by Ipser et al. (2012) of 20 MRS studies in ASD identified decreased concentrations of NAA most significantly in the cerebellum, parietal lobes, and anterior cingulate. Age effects were noted as well, with children showing more global losses in NAA (Ipser et al. 2012).

Creatine (Cr) is considered to be a marker of energy metabolism and storage, and may reflect the density of microglia. It has also been observed to increase throughout early childhood. Some studies report widespread reduction of Cr in ASD, while others note no change or else find reductions in specific regions only (ie. temporal lobes, prefrontal lobes). Some reports also observe increased Cr in deep structures like the caudate or amygdala (reviewed by (Baruth et al. 2013)). In the meta-analysis by Ipser et al. (2012), age effects were again noted with lower creatine

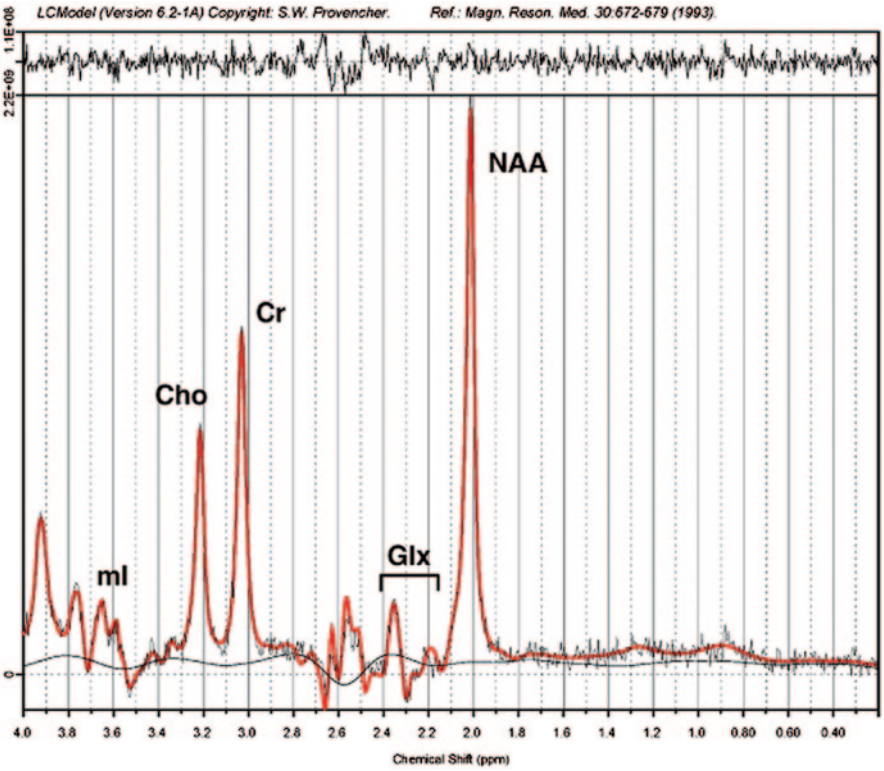


Fig. 7.7 Examples of signal peaks in proton MRS indicating concentrations of brain metabolites within a specific region. *Cho* choline; *Cr* creatine, *Glx* glutamate + glutamine + gamma-aminobutyric acid, *mI* myo-inositol, *NAA* N-acetylaspartate. (Baruth et al. 2013. Reproduced with permission from Autism Research)

concentrations in the occipital lobes in children, but higher levels in the temporal lobes in adults (Ipser et al. 2012).

In Fig. 7.7, Glx represents a combined peak measuring glutamate, glutamine (both involved in excitatory neurotransmission) and gamma-aminobutryirc-acid (GABA) (the major inhibitory neurotransmitter). With high-resolution magnetic fields (3–7T), these three metabolites can be separated out from the Glx peak. Study of these neurotransmitters is of particular relevance in ASD, as an imbalance in excitatory/ inhibitory neurotransmission is thought to contribute to altered neurodevelopment, and is in line with genetic findings of mutations in structural proteins at the excitatory synapse (e.g. SHANK-2 and neurexin) in affected individuals. Most studies have investigated Glx, without reliably parcellating this metabolite into its subcomponents, however. Some suggest widespread decreases in Glx, both in children and adults, as well in specific sub regions such as the anterior cigulate [reviewed by (Baruth et al. 2013)]. Horder et al. (2013) noted decreased Glx in the basal ganglia of patients with ASD, and lower levels of Glx

correlated with greater social communication impairment (Horder et al. 2013). On the other hand, using 3T MRS, increased GLX concentrations were noted in the auditory cortices of affected individuals (Brown et al. 2013). Using 4T MRS, higher glutamate levels were observed in the anterior cingulate of adolescent males (Joshi et al. 2013). Also using 3T MRS, a lower ratio of GABA to Cr was observed in the motor and auditory cortices of patients with ASD compared to controls (Gaetz et al. 2014; Rojas et al. 2013).

Other metabolites such as choline (related to membrane synthesis) and myo-inositol (involved in cell signaling, growth and maintenance of osmotic balance) have shown widespread and also regional decreases in some studies, as well as no difference or increases in others, with noteable age effects [reviewed by (Baruth et al. 2013)].

Given the possible contribution of age to MRS findings, Corrigan et al. (2013) recently published a longitudinal analysis using MRS in children with ASD, compared to typically developing controls and individuals with developmental delay, between 3 and 10 years of age. Interestingly, grey matter concentrations of NAA were found to be significantly lower than typically developing children at 3 years of age, but then increased to a level comparable to typically developing individuals by 10 years of age, while patients with developmental delay remain unchanged in NAA concentration. Several other metabolites were found to be lower in patients with ASD, with differences becoming less pronounced over time (Corrigan et al. 2013).

In summary, emerging research using MRS can provide additional information regarding the biochemical aspects of neurodevelopment in patients with ASD, and may potentially serve as a future biomarker of this condition. Further studies are needed to explore the important effects of age in adolescents and adults, correlations to histological findings, and potential use as a clinical biomarker.

7.9 Functional Magnetic Resonance Imaging

Functional neuroimaging in ASD has expanded our understanding of aberrant connectivity in brain networks by studying brain activation patterns. In functional MRI studies (fMRI), subtle differences in the magnetic field strength of oxygenated and deoxygenated blood (called the blood oxygen level dependent (BOLD) effect) serves as a surrogate marker of brain activity in response to a specific task, or in a brain at rest.

fMRI research across neuropsychiatric conditions have identified several resting brain activation networks [reviewed by (Heine et al. 2012; Lee et al. 2013)]. The default mode network (DMN) is the most extensively studied, and involves regions such as the anterior and posterior cingulate, and the temporo- parietal junction. This network is underactive during stimulus driven tasks, but becomes the most prominent form of brain activity when someone lies awake with eyes closed, during mind wandering and introspection.

Independent component analysis (ICA) or similar techniques look at overall activation patterns across the entire brain, and can provide data regarding the above-described networks. Alternatively, connections to and from an a priori defined region can also be studied in terms of functional connectivity, via the 'seed-technique'. As mentioned in the section on structural connectivity, discrete mathematics can be used to model the resulting activation networks in terms of 'nodes' (areas of increased connectivity represented by dots) and 'edges' (connections between nodes represented by lines) using graph theory (see Fig. 7.6; Dennis and Thompson 2013).

There is an abundance of literature looking at fMRI in patients with ASD. Some report a pattern of under connectivity in long-range circuits (Just et al. 2012), combined with increased local connectivity in short-range circuits, (Courchesne and Pierce 2005), similar to findings from structural neuroimaging data [i.e. (Shi et al. 2013)]. A closer look at the literature reveals a more complex picture, however.

Looking at task-based fMRI assessments, reduced synchronization across large scale networks has been shown during language comprehension and in response to auditory stimuli (Just et al. 2004; Williams et al. 2013; Dinstein et al. 2011), in studies of executive functioning (Just et al. 2007), visual spatial processing (Damarla et al. 2010), and in response to emotional cues (Abrams et al. 2013; Rudie et al. 2012). Increased functional connectivity during task-based assessments has also been shown, however (Shih et al. 2011; Shen et al. 2012; Redcay and Courchesne 2008). Some have proposed that increased functional connectivity in children with ASD is then met with aberrant development of cortical and subcortical networks, such that adults with ASD have reduced large-scale connectivity compared to controls (Uddin et al. 2013b).

Looking at resting state fMRI, there are contradictory findings in this literature as well. Some have suggested that methodological issues may be contributing (Muller et al. 2011). While under connectivity of large-scale networks is the most common finding [(Weng et al. 2010; Kennedy and Courchesne 2008) reviewed by (Uddin et al. 2013b)], studies in younger children also observe signs of increased connectivity in many resting state networks (Uddin et al. 2013a; Keehn et al. 2013; Redcay and Courchesne 2008). Through a seed-based approach, hyper connectivity in the posterior cingulate and retrosplenial cortex correlated with more severe social impairments in affected children (Lynch et al. 2013).

Recently a collaborative effort called the 'autism brain imaging data exchange' (ABIDE), has combined neuroimaging datasets across institutions to drastically increase the sample size in a joined fMRI connectivity analysis. They describe a predominance of lower intrinsic connectivity in cortico-cortical networks, particularly involving the default mode network, the paralimbic regions, the temporal lobe region and regarding connectivity between hemispheres. On the other hand hyper connectivity in sub cortical networks was also noted, specifically between the thalamus, globus pallidus and parietal sensorimotor regions (Di Martino et al. 2014).

7.10 Positron Emission Tomography

Positron emission tomography uses radioactive tracers that emit gamma rays, which are tied to biologically active molecules, in order to study the volumetric distribution of certain structural or metabolic processes in the body. Common examples of radionuclide tracers include fluorine-18, carbon-11, nitrongen-13 and oxygen-15, which all have different half lives. These isotopes are then tied to biologically active molecules (i.e. water, glucose, or to designer molecules targeting certain neurotransmitter receptors) to yield specific radiotracers. A commonly used radiotracer is fluorodeoxyglucose (FDG), which is a radioactive fluoride isotope combined with a molecule of glucose. FDG highlights glucose metabolism in an area, which is thought to be reflective of the level of neural activity. Along these lines, the amount of blood flow to a region can be quantified using water labeled with oxygen-25. In neuroimaging studies, tracers have also been developed to target dopamine receptors, GABA receptors, serotonin transporters and receptors, glutamate receptors, amyloid plaques, opioid receptors, and acetocholinesterase. In PET, the concentration and location of these selected tracers is measured using computed tomography (CT) scans. Images can provide both structural and functional information, depending on whether they were taken in a subject at rest, or following a particular stimulus or task, depending on the tracer used and the goals of the study. A high-resolution form of imaging known as single photon-emission tomography (SPECT) can also yield additional detail regarding tracer distribution in three dimensions. At times PET or SPECT images can be combined with MRI, to further highlight more precise anatomical locations. Both PET and SPECT require significant exposure to ionizing radiation, both from imaging technologies and from radiotracers. Therefore, research applying these approaches in young children or healthy volunteers must be carefully considered.

Very early literature applying PET using FDG in individuals with ASD identified a pattern of increased glucose metabolism across the brain (Heh et al. 1989; Rumsey et al. 1985), as well as more extreme fluctuations in metabolic rates between brain regions (Rumsey et al. 1985). Some evidence of metabolic under activity was noted in specific regions as well, including the anterior cingulate (Haznedar et al. 1997, 2000), caudate, putamen and thalamus (Haznedar et al. 2006). Hypoperfusion was observed in the temporal lobes of children with ASD (Zilbovicius et al. 2000; Dilber et al. 2013), while hyper-perfusion was localized to temporal, cerebellar and visual cortices, as well as several deep structures in adult populations with ASD (Pagani et al. 2012).

Regarding neuroreceptor distributions, lower levels of serotonin transporters have been observed in patients with ASD, both diffusely via PET imaging (Nakamura et al. 2010) and in the medial frontal cortex via SPECT imaging (Makkonen et al. 2008). Lower serotonin synthesis capacity and altered rate of development of serotonin synthesis capacity has been shown in children ages 2–14 years (represented by synaptic uptake of a radiotracer mimicking tryptophan) (Chandana et al. 2005). Lower serotonin transporter levels in the cingulate cortex correlated with

greater deficits in social cognition (Nakamura et al. 2010). Lower expression of 5-HT2 receptors in the cortex has also been noted in parents of affected individuals (Goldberg et al. 2009). Not all studies have observed differences in serotonin receptor or transporter concentrations, however. Girgis et al. (2011) found no significant difference in 5HT2 receptor or serotonin transporter concentrations between patients with Asperger's syndrome and typically developing controls (Girgis et al. 2011).

Deficits in cholinergic innervation in the fusiform gyrus were extrapolated from findings of lower acetocholinesterase activity in this region, and seemed to relate to social functioning as well (Suzuki et al. 2011). In three patients with ASD compared to three controls, $GABA_A$ receptor levels were lower across the brain, and in the amygdala and nucleus accumbens (Mendez et al. 2013).

Recently, a novel tracer targeted to microglia was applied in a PET study of adult males with ASD. Study of microglia is of interest to immune theories of autism, as increased number of microglia correlates with increased brain inflammation. The authors found overall increased binding of the microglia tracer across multiple brain regions including the cerebellum, midbrain, pons, fusiform gyri, the anterior cingulate and orbitofrontal cortex, and most markedly in the cerebellum (Suzuki et al. 2013).

Fragile X syndrome (FXS) is a genetic condition associated with ASD in one-third to one-quarter of cases (Kaufmann et al. 2004; Bailey et al. 2008). Results from investigations in populations with FXS have broad implications for idiopathic ASD. The metabotropic glutamate receptor theory (mGluR) of FXS proposes that deficits in the fragile X mental retardation protein (FMRP) has downstream effects in the form of exaggerated mGluR5 expression and activity at the synapse (Bear et al. 2004). Post mortem in-vitro analysis using radio-labeled tracers for mGluR5 applied to brain tissue from deceased patients with FXS identified increased expression of mGluR5 protein in the prefrontal cortex (Lohith et al. 2013). The authors suggest that future in vivo investigations using PET to quantify mGluR5 expression in patients with FXS and ASD may yield important information in terms of etiopathogenesis of these conditions, and may serve as a biomarker of treatment response, given the development of new compounds targeting this receptor (Lohith et al. 2013).

7.11 Summary, Interpretation and Recommendations

In summary, there is significant heterogeneity in findings from neuroimaging research in ASD. Looking at volumetric MRI data, a pattern emerges whereby early brain overgrowth in the first few years of life is subsequently met with signs of brain dysmaturation throughout childhood and adolescence. Along these lines, there is evidence of increased gyrification of the cerebral cortex, as well as early cortical overgrowth, followed by either exaggerated or attenuated cortical thinning, depending on the study and the age of included participants.

Investigations into structural connectivity reveal decreased white matter integrity in socio-emotional and language circuits. Signs of decreased long range-connectivity but increased short-range connectivity in cortical and subcortical networks emerges from both structural and functional neuroimaging data.

Certain regions of interest stand out across meta-analyses and reveal phenotypic correlates; the basal ganglia for example has been implicated in repetitive behaviors, while the amygdala, anterior cingulate, prefrontal and temporo-parietal regions seem to play a role in social communication and language impairment.

The magnitude and direction of altered neurodevelopment across modalities is an ongoing focus of investigation, but the importance of age as a confounding factor is a recurring theme. Analysis of aberrant 'brain growth curves' in this condition may contribute to hypothesis generation regarding specific pathology occurring at the synapse. For example, an imbalance between excitatory and inhibitory signaling leading to abnormal signal-to-noise ratios is a well-described hypothesis in ASD (Rubenstein and Merzenich 2003). Along these lines, neuroimaging data showing exaggerated cortical expansion, followed by early growth arrest and stabilization of cortical networks, would be in keeping with loss of inhibitory control during synaptic growth and development. Based on this pattern, some have suggested that brain growth trajectories in ASD appear to be 'shifted to the left' along the time axis (Courchesne 2004; Greimel et al. 2013a). Moving forward, expansion and longitudinal analysis of structural and functional neuroimaging data sets will help to clarify these important age effects.

Despite these positive findings, most studies report mean differences between cases and control groups in the range of 1–2%. These pooled group differences have yet to yield clinically useful information when applied at an individual level, however. The search for a sensitive and specific biomarker of ASD is a common goal. An ideal candidate biomarker would need to show high diagnostic accuracy in a large sample population where ASD is present at a realistic prevalence rate (i.e. 1 in 88), to permit calculation of positive predictive values. Additionally, this test would need to be able to distinguish ASD from other neuropsychiatric disorders. Currently, there are no individual neuroimaging metrics that satisfy the above-described criteria. Extra-axial fluid volume has shown some promise in one study (Shen et al. 2013).

Alternatively, several investigators have sought to optimize the potential predictive power of neuroimaging datasets through machine learning algorithms. In this technique, a computer is programmed to generate a discriminant function that identifies and consecutively combines the 'most different' neuroanatomical measurements across scans, to thereby stratify an image into a diagnostic category [for examples see (Lange et al. 2010) using DTI data; (Jiao et al. 2010) using cortical thickness data, and (Ecker et al. 2010) using multiple parameters]. With machine learning algorithms, several investigators have been able to correctly classify individuals with ASD vs. controls with greater than 80% specificity and sensitivity, and some have been able to replicate comparable predictive capacity to discern ASD from other neuropsychiatric conditions [e.g. ADHD in (Ecker et al. 2010)]. Again, these findings need to be replicated in large-population samples to ascertain

their positive predictive value and clinical utility. Computerized neuroimaging algorithms remain for most part a preclinical investigative tool- translation into the clinical setting will require a paradigm shift in diagnostic radiology. There is hope, however, that neuroimaging research may someday yield clinical diagnostics in ASD, given increased efforts towards collaboration across institutions [e.g. ABIDE (Di Martino et al. 2014)], a growing number of prospective and longitudinal neuroimaging trials, as well as the advent of more advanced neuroimaging technology.

References

Abrams DA, Lynch CJ, Cheng KM, Phillips J, Supekar K, Ryali S, Uddin LQ, Menon V (2013) Underconnectivity between voice-selective cortex and reward circuitry in children with autism. Proc Natl Acad Sci U S A 110:12060–12065

Alexander AL, Lee JE, Lazar M, Field AS (2007) Diffusion tensor imaging of the brain. Neurotherapeutics 4:316–329

Amaral DG, Schumann CM, Nordahl CW (2008) Neuroanatomy of autism. Trends Neurosci 31:137–145

Ameis SH, Fan J, Rockel C, Voineskos AN, Lobaugh NJ, Soorya L, Wang AT, Hollander E, Anagnostou E (2011) Impaired structural connectivity of socio-emotional circuits in autism spectrum disorders: a diffusion tensor imaging study. PLoS One 6:e28044

Aoki Y, Abe O, Nippashi Y, Yamasue H (2013) Comparison of white matter integrity between autism spectrum disorder subjects and typically developing individuals: a meta-analysis of diffusion tensor imaging tractography studies. Mol Autism 4:25

Aylward EH, Minshew NJ, Field K, Sparks BF, Singh N (2002) Effects of age on brain volume and head circumference in autism. Neurology 59:175–183

Bailey DB Jr, Raspa M, Olmsted M, Holiday DB (2008) Co-occurring conditions associated with FMR1 gene variations: findings from a national parent survey. Am J Med Genet A 146A:2060–2069

Barnea-Goraly N, Frazier TW, Piacenza L, Minshew NJ, Keshavan MS, Reiss AL, Hardan AY (2014) A preliminary longitudinal volumetric MRI study of amygdala and hippocampal volumes in autism. Prog Neuropsychopharmacol Biol Psychiatry 48:124–128

Baruth JM, Wall CA, Patterson MC, Port JD (2013) Proton magnetic resonance spectroscopy as a probe into the pathophysiology of autism spectrum disorders (ASD): a review. Autism Res 6:119–133

Bassett DS, Gazzaniga MS (2011) Understanding complexity in the human brain. Trends Cogn Sci 15:200–209

Bastiaansen JA, Thioux M, Nanetti L, van der Gaag C, Ketelaars C, Minderaa R, Keysers C (2011) Age-related increase in inferior frontal gyrus activity and social functioning in autism spectrum disorder. Biol Psychiatry 69:832–838

Bear MF, Huber KM, Warren ST (2004) The mGluR theory of fragile X mental retardation. Trends Neurosci 27:370–377

Bellani M, Calderoni S, Muratori F, Brambilla P (2013a) Brain anatomy of autism spectrum disorders I. Focus on corpus callosum. Epidemiol Psychiatr Sci 22:217–221

Bellani M, Calderoni S, Muratori F, Brambilla P (2013b) Brain anatomy of autism spectrum disorders II. Focus on amygdala. Epidemiol Psychiatr Sci 22:309–312

Bernhardt BC, Valk SL, Silani G, Bird G, Frith U, Singer T (2013) Selective disruption of sociocognitive structural brain networks in autism and alexithymia. Cereb Cortex 24:3258–3267

Brown MS, Singel D, Hepburn S, Rojas DC (2013) Increased glutamate concentration in the auditory cortex of persons with autism and first-degree relatives: a (1)H-MRS study. Autism Res 6:1–10

Catani M, Jones DK, Daly E, Embiricos N, Deeley Q, Pugliese L, Curran S, Robertson D, Murphy DG (2008) Altered cerebellar feedback projections in Asperger syndrome. Neuroimage 41:1184–1191

Catani M, Dell'acqua F, Thiebaut de Schotten M (2013) A revised limbic system model for memory, emotion and behaviour. Neurosci Biobehav Rev 37:1724–1737

Cauda F, Geda E, Sacco K, D'Agata F, Duca S, Geminiani G, Keller R (2011) Grey matter abnormality in autism spectrum disorder: an activation likelihood estimation meta-analysis study. J Neurol Neurosurg Psychiatry 82:1304–1313

Chakos MH, Lieberman JA, Bilder RM, Borenstein M, Lerner G, Bogerts B, Wu H, Kinon B, Ashtari M (1994) Increase in caudate nuclei volumes of first-episode schizophrenic patients taking antipsychotic drugs. Am J Psychiatry 151:1430–1436

Chandana SR, Behen ME, Juhasz C, Muzik O, Rothermel RD, Mangner TJ, Chakraborty PK, Chugani HT, Chugani DC (2005) Significance of abnormalities in developmental trajectory and asymmetry of cortical serotonin synthesis in autism. Int J Dev Neurosci 23:171–182

Corrigan NM, Shaw DW, Estes AM, Richards TL, Munson J, Friedman SD, Dawson G, Artru AA, Dager SR (2013) Atypical developmental patterns of brain chemistry in children with autism spectrum disorder. JAMA Psychiatry 70:964–974

Courchesne E (2004) Brain development in autism: early overgrowth followed by premature arrest of growth. Ment Retard Dev Disabil Res Rev 10:106–111

Courchesne E, Pierce K (2005) Why the frontal cortex in autism might be talking only to itself: local over-connectivity but long-distance disconnection. Curr Opin Neurobiol 15:225–230

Courchesne E, Yeung-Courchesne R, Press GA, Hesselink JR, Jernigan TL (1988) Hypoplasia of cerebellar vermal lobules VI and VII in autism. N Engl J Med 318:1349–1354

Courchesne E, Saitoh O, Townsend JP, Yeung-Courchesne R, Press GA, Lincoln AJ, Haas RH, Schriebman L (1994) Cerebellar hypoplasia and hyperplasia in infantile autism. Lancet 343:63–64

Courchesne E, Karns CM, Davis HR, Ziccardi R, Carper RA, Tigue ZD, Chisum HJ, Moses P, Pierce K, Lord C, Lincoln AJ, Pizzo S, Schreibman L, Haas RH, Akshoomoff NA, Courchesne RY (2001) Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. Neurology 57:245–254

Courchesne E, Carper R, Akshoomoff N (2003) Evidence of brain overgrowth in the first year of life in autism. JAMA 290:337–344

Courchesne E, Redcay E, Kennedy DP (2004) The autistic brain: birth through adulthood. Curr Opin Neurol 17:489–496

Courchesne E, Campbell K, Solso S (2011a) Brain growth across the life span in autism: age-specific changes in anatomical pathology. Brain Res 1380:138–145

Courchesne E, Mouton PR, Calhoun ME, Semendeferi K, Ahrens-Barbeau C, Hallet MJ, Barnes CC, Pierce K (2011b) Neuron number and size in prefrontal cortex of children with autism. JAMA 306:2001–2010

Dalton KM, Nacewicz BM, Johnstone T, Schaefer HS, Gernsbacher MA, Goldsmith HH, Alexander AL, Davidson RJ (2005) Gaze fixation and the neural circuitry of face processing in autism. Nat Neurosci 8:519–526

Damarla SR, Keller TA, Kana RK, Cherkassky VL, Williams DL, Minshew NJ, Just MA (2010) Cortical underconnectivity coupled with preserved visuospatial cognition in autism: Evidence from an fMRI study of an embedded figures task. Autism Res 3:273–279

Delmonte S, Balsters JH, McGrath J, Fitzgerald J, Brennan S, Fagan AJ, Gallagher L (2012) Social and monetary reward processing in autism spectrum disorders. Mol Autism 3:7

Delmonte S, Gallagher L, O'Hanlon E, McGrath J, Balsters JH (2013) Functional and structural connectivity of frontostriatal circuitry in Autism Spectrum Disorder. Front Hum Neurosci 7:430

Dennis EL, Thompson PM (2013) Mapping connectivity in the developing brain. Int J Dev Neurosci 31:525–542

Di Martino A, Yan CG, Li Q, Denio E, Castellanos FX, Alaerts K, Anderson JS, Assaf M, Bookheimer SY, Dapretto M, Deen B, Delmonte S, Dinstein I, Ertl-Wagner B, Fair DA, Gallagher L, Kennedy DP, Keown CL, Keysers C, Lainhart JE, Lord C, Luna B, Menon V, Minshew NJ, Monk CS, Mueller S, Muller RA, Nebel MB, Nigg JT, O'Hearn K, Pelphrey KA, Peltier SJ, Rudie JD, Sunaert S, Thioux M, Tyszka JM, Uddin LQ, Verhoeven JS, Wenderoth N, Wiggins JL, Mostofsky SH, Milham MP (2014) The autism brain imaging data exchange: towards a large-scale evaluation of the intrinsic brain architecture in autism. Mol Psychiatry 19:659–667

Dilber C, Caliskan M, Sonmezoglu K, Nisli S, Mukaddes NM, Tatli B, Aydinli N, Ekici B, Ozmen M (2013) Positron emission tomography findings in children with infantile spasms and autism. J Clin Neurosci 20:373–376

Dinstein I, Pierce K, Eyler L, Solso S, Malach R, Behrmann M, Courchesne E (2011) Disrupted neural synchronization in toddlers with autism. Neuron 70:1218–1225

Doyle-Thomas KA, Duerden EG, Taylor MJ, Lerch JP, Soorya LV, Wang AT, Fan J, Hollander E, Anagnostou E (2013) Effects of age and symptomatology on cortical thickness in autism spectrum disorders. Res Autism Spectr Disord 7:141–150

Duerden EG, Mak-Fan KM, Taylor MJ, Roberts SW (2012) Regional differences in grey and white matter in children and adults with autism spectrum disorders: an activation likelihood estimate (ALE) meta-analysis. Autism Res 5:49–66

Dziobek I, Bahnemann M, Convit A, Heekeren HR (2010) The role of the fusiform-amygdala system in the pathophysiology of autism. Arch Gen Psychiatry 67:397–405

Ecker C, Marquand A, Mourao-Miranda J, Johnston P, Daly EM, Brammer MJ, Maltezos S, Murphy CM, Robertson D, Williams SC, Murphy DG (2010) Describing the brain in autism in five dimensions–magnetic resonance imaging-assisted diagnosis of autism spectrum disorder using a multiparameter classification approach. J Neurosci 30:10612–10623

Ecker C, Suckling J, Deoni SC, Lombardo MV, Bullmore ET, Baron-Cohen S, Catani M, Jezzard P, Barnes A, Bailey AJ, Williams SC, Murphy DG (2012) Brain anatomy and its relationship to behavior in adults with autism spectrum disorder: a multicenter magnetic resonance imaging study. Arch Gen Psychiatry 69:195–209

Ecker C, Ginestet C, Feng Y, Johnston P, Lombardo MV, Lai MC, Suckling J, Palaniyappan L, Daly E, Murphy CM, Williams SC, Bullmore ET, Baron-Cohen S, Brammer M, Murphy DG (2013a) Brain surface anatomy in adults with autism: the relationship between surface area, cortical thickness, and autistic symptoms. JAMA Psychiatry 70:59–70

Ecker C, Ronan L, Feng Y, Daly E, Murphy C, Ginestet CE, Brammer M, Fletcher PC, Bullmore ET, Suckling J, Baron-Cohen S, Williams S, Loth E, Murphy DG (2013b) Intrinsic gray-matter connectivity of the brain in adults with autism spectrum disorder. Proc Natl Acad Sci U S A 110:13222–13227

Elison JT, Wolff JJ, Heimer DC, Paterson SJ, Gu H, Hazlett HC, Styner M, Gerig G, Piven J (2013) Frontolimbic neural circuitry at 6 months predicts individual differences in joint attention at 9 months. Dev Sci 16:186–197

Enticott PG, Kennedy HA, Rinehart NJ, Tonge BJ, Bradshaw JL, Taffe JR, Daskalakis ZJ, Fitzgerald PB (2012) Mirror neuron activity associated with social impairments but not age in autism spectrum disorder. Biol Psychiatry 71:427–433

Eyler LT, Pierce K, Courchesne E (2012) A failure of left temporal cortex to specialize for language is an early emerging and fundamental property of autism. Brain 135:949–960

Faria AV, Zhang J, Oishi K, Li X, Jiang H, Akhter K, Hermoye L, Lee SK, Hoon A, Stashinko E, Miller MI, van Zijl PC, Mori S (2010) Atlas-based analysis of neurodevelopment from infancy to adulthood using diffusion tensor imaging and applications for automated abnormality detection. Neuroimage 52:415–428

Fatemi SH, Aldinger KA, Ashwood P, Bauman ML, Blaha CD, Blatt GJ, Chauhan A, Chauhan V, Dager SR, Dickson PE, Estes AM, Goldowitz D, Heck DH, Kemper TL, King BH, Martin LA,

Millen KJ, Mittleman G, Mosconi MW, Persico AM, Sweeney JA, Webb SJ, Welsh JP (2012) Consensus paper: pathological role of the cerebellum in autism. Cerebellum 11:777–807

Gaetz W, Bloy L, Wang DJ, Port RG, Blaskey L, Levy SE, Roberts TP (2014) GABA estimation in the brains of children on the autism spectrum: measurement precision and regional cortical variation. Neuroimage 86:1–9

Gaffney GR, Kuperman S, Tsai LY, Minchin S, Hassanein KM (1987) Midsagittal magnetic resonance imaging of autism. Br J Psychiatry 151:831–833

Girgis RR, Slifstein M, Xu X, Frankle WG, Anagnostou E, Wasserman S, Pepa L, Kolevzon A, Abi-Dargham A, Laruelle M, Hollander E (2011) The 5-HT(2A) receptor and serotonin transporter in Asperger's disorder: a PET study with [^{11}C]MDL 100907 and [^{11}C]DASB. Psychiatry Res 194:230–234

Goldberg J, Anderson GM, Zwaigenbaum L, Hall GB, Nahmias C, Thompson A, Szatmari P (2009) Cortical serotonin type-2 receptor density in parents of children with autism spectrum disorders. J Autism Dev Disord 39:97–104

Gowen E, Hamilton A (2013) Motor abilities in autism: a review using a computational context. J Autism Dev Disord 43:323–344

Greimel E, Nehrkorn B, Schulte-Ruther M, Fink GR, Nickl-Jockschat T, Herpertz-Dahlmann B, Konrad K, Eickhoff SB (2013a) Changes in grey matter development in autism spectrum disorder. Brain Struct Funct 218:929–942

Greimel E, Nehrkorn B, Schulte-Ruther M, Fink GR, Nickl-Jockschat T, Herpertz-Dahlmann B, Konrad K, Eickhoff SB (2013b) Changes in grey matter development in autism spectrum disorder. Brain Struct Funct 218:929–942

Hadjikhani N, Joseph RM, Snyder J, Tager-Flusberg H (2006) Anatomical differences in the mirror neuron system and social cognition network in autism. Cereb Cortex 16:1276–1282

Hamilton AF (2013) Reflecting on the mirror neuron system in autism: a systematic review of current theories. Dev Cogn Neurosci 3:91–105

Hardan AY, Minshew NJ, Mallikarjuhn M, Keshavan MS (2001) Brain volume in autism. J Child Neurol 16:421–424

Hardan AY, Jou RJ, Keshavan MS, Varma R, Minshew NJ (2004) Increased frontal cortical folding in autism: a preliminary MRI study. Psychiatry Res 131:263–268

Hardan AY, Muddasani S, Vemulapalli M, Keshavan MS, Minshew NJ (2006) An MRI study of increased cortical thickness in autism. Am J Psychiatry 163:1290–1292

Hardan AY, Libove RA, Keshavan MS, Melhem NM, Minshew NJ (2009) A preliminary longitudinal magnetic resonance imaging study of brain volume and cortical thickness in autism. Biol Psychiatry 66:320–326

Harsan LA, Poulet P, Guignard B, Steibel J, Parizel N, de Sousa PL, Boehm N, Grucker D, Ghandour MS (2006) Brain dysmyelination and recovery assessment by noninvasive in vivo diffusion tensor magnetic resonance imaging. J Neurosci Res 83:392–402

Hazlett HC, Poe M, Gerig G, Smith RG, Provenzale J, Ross A, Gilmore J, Piven J (2005) Magnetic resonance imaging and head circumference study of brain size in autism: birth through age 2 years. Arch Gen Psychiatry 62:1366–1376

Hazlett HC, Poe MD, Gerig G, Styner M, Chappell C, Smith RG, Vachet C, Piven J (2011) Early brain overgrowth in autism associated with an increase in cortical surface area before age 2 years. Arch Gen Psychiatry 68:467–476

Haznedar MM, Buchsbaum MS, Metzger M, Solimando A, Spiegel-Cohen J, Hollander E (1997) Anterior cingulate gyrus volume and glucose metabolism in autistic disorder. Am J Psychiatry 154:1047–1050

Haznedar MM, Buchsbaum MS, Wei TC, Hof PR, Cartwright C, Bienstock CA, Hollander E (2000) Limbic circuitry in patients with autism spectrum disorders studied with positron emission tomography and magnetic resonance imaging. Am J Psychiatry 157:1994–2001

Haznedar MM, Buchsbaum MS, Hazlett EA, LiCalzi EM, Cartwright C, Hollander E (2006) Volumetric analysis and three-dimensional glucose metabolic mapping of the striatum and thalamus in patients with autism spectrum disorders. Am J Psychiatry 163:1252–1263

Heh CW, Smith R, Wu J, Hazlett E, Russell A, Asarnow R, Tanguay P, Buchsbaum MS (1989) Positron emission tomography of the cerebellum in autism. Am J Psychiatry 146:242–245

Heine L, Soddu A, Gomez F, Vanhaudenhuyse A, Tshibanda L, Thonnard M, Charland-Verville V, Kirsch M, Laureys S, Demertzi A (2012) Resting state networks and consciousness: alterations of multiple resting state network connectivity in physiological, pharmacological, and pathological consciousness states. Front Psychol 3:295

Hernandez-Garcia L, Buschkuehl M (2012) Advances in longitudinal MRI diagnostic tests. Expert Opin Med Diagn 6:309–321

Hodge SM, Makris N, Kennedy DN, Caviness VS Jr, Howard J, McGrath L, Steele S, Frazier JA, Tager-Flusberg H, Harris GJ (2010) Cerebellum, language, and cognition in autism and specific language impairment. J Autism Dev Disord 40:300–316

Hollander E, Anagnostou E, Chaplin W, Esposito K, Haznedar MM, Licalzi E, Wasserman S, Soorya L, Buchsbaum M (2005) Striatal volume on magnetic resonance imaging and repetitive behaviors in autism. Biol Psychiatry 58:226–232

Horder J, Lavender T, Mendez MA, O'Gorman R, Daly E, Craig MC, Lythgoe DJ, Barker GJ, Murphy DG (2013) Reduced subcortical glutamate/glutamine in adults with autism spectrum disorders: a [(1)H]MRS study. Transl Psychiatry 3:e279

Hua X, Thompson PM, Leow AD, Madsen SK, Caplan R, Alger JR, O'Neill J, Joshi K, Smalley SL, Toga AW, Levitt JG (2013) Brain growth rate abnormalities visualized in adolescents with autism. Hum Brain Mapp 34:425–436

Ipser JC, Syal S, Bentley J, Adnams CM, Steyn B, Stein DJ (2012) 1H-MRS in autism spectrum disorders: a systematic meta-analysis. Metab Brain Dis 27:275–287

Jiao Y, Chen R, Ke X, Chu K, Lu Z, Herskovits EH (2010) Predictive models of autism spectrum disorder based on brain regional cortical thickness. Neuroimage 50:589–599

Joshi G, Biederman J, Wozniak J, Goldin RL, Crowley D, Furtak S, Lukas SE, Gonenc A (2013) Magnetic resonance spectroscopy study of the glutamatergic system in adolescent males with high-functioning autistic disorder: a pilot study at 4T. Eur Arch Psychiatry Clin Neurosci 263:379–384

Jou RJ, Minshew NJ, Keshavan MS, Hardan AY (2010) Cortical gyrification in autistic and Asperger disorders: a preliminary magnetic resonance imaging study. J Child Neurol 25:1462–1467

Juranek J, Filipek PA, Berenji GR, Modahl C, Osann K, Spence MA (2006) Association between amygdala volume and anxiety level: magnetic resonance imaging (MRI) study in autistic children. J Child Neurol 21:1051–1058

Just MA, Cherkassky VL, Keller TA, Minshew NJ (2004) Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. Brain 127:1811–1821

Just MA, Cherkassky VL, Keller TA, Kana RK, Minshew NJ (2007) Functional and anatomical cortical underconnectivity in autism: evidence from an FMRI study of an executive function task and corpus callosum morphometry. Cereb Cortex 17:951–961

Just MA, Keller TA, Malave VL, Kana RK, Varma S (2012) Autism as a neural systems disorder: a theory of frontal-posterior underconnectivity. Neurosci Biobehav Rev 36:1292–1313

Kana RK, Libero LE, Hu CP, Deshpande HD, Colburn JS (2014) Functional brain networks and white matter underlying theory-of-mind in Autism. Soc Cogn Affect Neurosci 9:98–105

Kaufmann WE, Cortell R, Kau AS, Bukelis I, Tierney E, Gray RM, Cox C, Capone GT, Stanard P (2004) Autism spectrum disorder in fragile X syndrome: communication, social interaction, and specific behaviors. Am J Med Genet A 129A:225–234

Keehn B, Wagner JB, Tager-Flusberg H, Nelson CA (2013) Functional connectivity in the first year of life in infants at-risk for autism: a preliminary near-infrared spectroscopy study. Front Hum Neurosci 7:444

Kennedy DP, Courchesne E (2008) The intrinsic functional organization of the brain is altered in autism. Neuroimage 39:1877–1885

Kim JE, Lyoo IK, Estes AM, Renshaw PF, Shaw DW, Friedman SD, Kim DJ, Yoon SJ, Hwang J, Dager SR (2010) Laterobasal amygdalar enlargement in 6- to 7-year-old children with autism spectrum disorder. Arch Gen Psychiatry 67:1187–1197

Kliemann D, Dziobek I, Hatri A, Baudewig J, Heekeren HR (2012) The role of the amygdala in atypical gaze on emotional faces in autism spectrum disorders. J Neurosci 32:9469–9476

Kohls G, Perino MT, Taylor JM, Madva EN, Cayless SJ, Troiani V, Price E, Faja S, Herrington JD, Schultz RT (2013) The nucleus accumbens is involved in both the pursuit of social reward and the avoidance of social punishment. Neuropsychologia 51:2062–2069

Kubicki M, McCarley R, Westin CF, Park HJ, Maier S, Kikinis R, Jolesz FA, Shenton ME (2007) A review of diffusion tensor imaging studies in schizophrenia. J Psychiatr Res 41:15–30

Kumar M, Kim S, Pickup S, Chen R, Fairless AH, Ittyerah R, Abel T, Brodkin ES, Poptani H (2012) Longitudinal in-vivo diffusion tensor imaging for assessing brain developmental changes in BALB/cJ mice, a model of reduced sociability relevant to autism. Brain Res 1455:56–67

Laird AR, Fox PM, Price CJ, Glahn DC, Uecker AM, Lancaster JL, Turkeltaub PE, Kochunov P, Fox PT (2005) ALE meta-analysis: controlling the false discovery rate and performing statistical contrasts. Hum Brain Mapp 25:155–164

Lange N, Dubray MB, Lee JE, Froimowitz MP, Froehlich A, Adluru N, Wright B, Ravichandran C, Fletcher PT, Bigler ED, Alexander AL, Lainhart JE (2010) Atypical diffusion tensor hemispheric asymmetry in autism. Autism Res 3:350–358

Langen M, Durston S, Staal WG, Palmen SJ, van Engeland H (2007) Caudate nucleus is enlarged in high-functioning medication-naive subjects with autism. Biol Psychiatry 62:262–266

Langen M, Bos D, Noordermeer SD, Nederveen H, van Engeland H, Durston S (2013) Changes in the development of striatum are involved in repetitive behavior in autism. Biol Psychiatry 76:405–411

Lau YC, Hinkley LB, Bukshpun P, Strominger ZA, Wakahiro ML, Baron-Cohen S, Allison C, Auyeung B, Jeremy RJ, Nagarajan SS, Sherr EH, Marco EJ (2013) Autism traits in individuals with agenesis of the corpus callosum. J Autism Dev Disord 43:1106–1118

Lee MH, Smyser CD, Shimony JS (2013) Resting-State fMRI: a review of methods and clinical applications. Am J Neuroradiol 34:1866–1872

Lohith TG, Osterweil EK, Fujita M, Jenko KJ, Bear MF, Innis RB (2013) Is metabotropic glutamate receptor 5 upregulated in prefrontal cortex in fragile X syndrome? Mol Autism 4:15

Lui JH, Hansen DV, Kriegstein AR (2011) Development and evolution of the human neocortex. Cell 146:18–36

Lynch CJ, Uddin LQ, Supekar K, Khouzam A, Phillips J, Menon V (2013) Default mode network in childhood autism: posteromedial cortex heterogeneity and relationship with social deficits. Biol Psychiatry 74:212–219

Mak-Fan KM, Morris D, Vidal J, Anagnostou E, Roberts W, Taylor MJ (2012a) White matter and development in children with an autism spectrum disorder. Autism 17:541–557

Mak-Fan KM, Taylor MJ, Roberts W, Lerch JP (2012b) Measures of cortical grey matter structure and development in children with autism spectrum disorder. J Autism Dev Disord 42:419–427

Makkonen I, Riikonen R, Kokki H, Airaksinen MM, Kuikka JT (2008) Serotonin and dopamine transporter binding in children with autism determined by SPECT. Dev Med Child Neurol 50:593–597

McAlonan GM, Cheung V, Cheung C, Suckling J, Lam GY, Tai KS, Yip L, Murphy DG, Chua SE (2005) Mapping the brain in autism. A voxel-based MRI study of volumetric differences and intercorrelations in autism. Brain 128:268–276

Mendez MA, Horder J, Myers J, Coghlan S, Stokes P, Erritzoe D, Howes O, Lingford-Hughes A, Murphy D, Nutt D (2013) The brain GABA-benzodiazepine receptor alpha-5 subtype in autism spectrum disorder: a pilot [(11)C]Ro15–4513 positron emission tomography study. Neuropharmacology 68:195–201

Muller RA, Shih P, Keehn B, Deyoe JR, Leyden KM, Shukla DK (2011) Underconnected, but how? A survey of functional connectivity MRI studies in autism spectrum disorders. Cereb Cortex 21:2233–2243

Munson J, Dawson G, Abbott R, Faja S, Webb SJ, Friedman SD, Shaw D, Artru A, Dager SR (2006) Amygdalar volume and behavioral development in autism. Arch Gen Psychiatry 63:686–693

Nagae LM, Zarnow DM, Blaskey L, Dell J, Khan SY, Qasmieh S, Levy SE, Roberts TP (2012) Elevated mean diffusivity in the left hemisphere superior longitudinal fasciculus in autism spectrum disorders increases with more profound language impairment. Am J Neuroradiol 33:1720–1725

Nakamura K, Sekine Y, Ouchi Y, Tsujii M, Yoshikawa E, Futatsubashi M, Tsuchiya KJ, Sugihara G, Iwata Y, Suzuki K, Matsuzaki H, Suda S, Sugiyama T, Takei N, Mori N (2010) Brain serotonin and dopamine transporter bindings in adults with high-functioning autism. Arch Gen Psychiatry 67:59–68

Nickl-Jockschat T, Habel U, Michel TM, Manning J, Laird AR, Fox PT, Schneider F, Eickhoff SB (2012) Brain structure anomalies in autism spectrum disorder–a meta-analysis of VBM studies using anatomic likelihood estimation. Hum Brain Mapp 33:1470–1489

Pagani M, Manouilenko I, Stone-Elander S, Odh R, Salmaso D, Hatherly R, Brolin F, Jacobsson H, Larsson SA, Bejerot S (2012) Brief Report: alterations in cerebral blood flow as assessed by PET/CT in adults with autism spectrum disorder with normal IQ. J Autism Dev Disord 42:313–318

Pardini M, Elia M, Garaci FG, Guida S, Coniglione F, Krueger F, Benassi F, Emberti Gialloreti L (2012) Long-term cognitive and behavioral therapies, combined with augmentative communication, are related to uncinate fasciculus integrity in autism. J Autism Dev Disord 42:585–592

Pfeifer JH, Peake SJ (2012) Self-development: integrating cognitive, socioemotional, and neuroimaging perspectives. Dev Cogn Neurosci 2:55–69

Piven J, Berthier ML, Starkstein SE, Nehme E, Pearlson G, Folstein S (1990) Magnetic resonance imaging evidence for a defect of cerebral cortical development in autism. Am J Psychiatry 147:734–739

Piven J, Nehme E, Simon J, Barta P, Pearlson G, Folstein SE (1992) Magnetic resonance imaging in autism: measurement of the cerebellum, pons, and fourth ventricle. Biol Psychiatry 31:491–504

Piven J, Arndt S, Bailey J, Havercamp S, Andreasen NC, Palmer P (1995) An MRI study of brain size in autism. Am J Psychiatry 152:1145–1149

Piven J, Arndt S, Bailey J, Andreasen N (1996) Regional brain enlargement in autism: a magnetic resonance imaging study. J Am Acad Child Adolesc Psychiatry 35:530–536

Poustka L, Jennen-Steinmetz C, Henze R, Vomstein K, Haffner J, Sieltjes B (2012) Fronto-temporal disconnectivity and symptom severity in children with autism spectrum disorder. World J Biol Psychiatry 13:269–280

Qiu A, Adler M, Crocetti D, Miller MI, Mostofsky SH (2010) Basal ganglia shapes predict social, communication, and motor dysfunctions in boys with autism spectrum disorder. J Am Acad Child Adolesc Psychiatry 49:539–551

Radua J, Via E, Catani M, Mataix-Cols D (2011) Voxel-based meta-analysis of regional white-matter volume differences in autism spectrum disorder versus healthy controls. Psychol Med 41:1539–1550

Rakic P (2009) Evolution of the neocortex: a perspective from developmental biology. Nat Rev Neurosci 10:724–735

Raznahan A, Toro R, Daly E, Robertson D, Murphy C, Deeley Q, Bolton PF, Paus T, Murphy DG (2010) Cortical anatomy in autism spectrum disorder: an in vivo MRI study on the effect of age. Cereb Cortex 20:1332–1340

Raznahan A, Wallace GL, Antezana L, Greenstein D, Lenroot R, Thurm A, Gozzi M, Spence S, Martin A, Swedo SE, Giedd JN (2013) Compared to what? Early brain overgrowth in autism and the perils of population norms. Biol Psychiatry 74:563–575

Redcay E, Courchesne E (2005) When is the brain enlarged in autism? A meta-analysis of all brain size reports. Biol Psychiatry 58:1–9

Redcay E, Courchesne E (2008) Deviant functional magnetic resonance imaging patterns of brain activity to speech in 2–3-year-old children with autism spectrum disorder. Biol Psychiatry 64:589–598

Rojas DC, Singel D, Steinmetz S, Hepburn S, Brown MS (2013) Decreased left perisylvian GABA concentration in children with autism and unaffected siblings. Neuroimage 86:28–34

Rubenstein JL, Merzenich MM (2003) Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav 2:255–267

Rudie JD, Shehzad Z, Hernandez LM, Colich NL, Bookheimer SY, Iacoboni M, Dapretto M (2012) Reduced functional integration and segregation of distributed neural systems underlying social and emotional information processing in autism spectrum disorders. Cereb Cortex 22:1025–1037

Rumsey JM, Duara R, Grady C, Rapoport JL, Margolin RA, Rapoport SI, Cutler NR (1985) Brain metabolism in autism. Resting cerebral glucose utilization rates as measured with positron emission tomography. Arch Gen Psychiatry 42:448–455

Sato W, Toichi M, Uono S, Kochiyama T (2012) Impaired social brain network for processing dynamic facial expressions in autism spectrum disorders. BMC Neurosci 13:99

Scheel C, Rotarska-Jagiela A, Schilbach L, Lehnhardt FG, Krug B, Vogeley K, Tepest R (2011) Imaging derived cortical thickness reduction in high-functioning autism: key regions and temporal slope. Neuroimage 58:391–400

Schmahmann JD (2004) Disorders of the cerebellum: ataxia, dysmetria of thought, and the cerebellar cognitive affective syndrome. J Neuropsychiatry Clin Neurosci 16:367–378

Schumann CM, Barnes CC, Lord C, Courchesne E (2009) Amygdala enlargement in toddlers with autism related to severity of social and communication impairments. Biol Psychiatry 66:942–949

Schumann CM, Bloss CS, Barnes CC, Wideman GM, Carper RA, Akshoomoff N, Pierce K, Hagler D, Schork N, Lord C, Courchesne E (2010) Longitudinal magnetic resonance imaging study of cortical development through early childhood in autism. J Neurosci 30:4419–4427

Scott JA, Schumann CM, Goodlin-Jones BL, Amaral DG (2009) A comprehensive volumetric analysis of the cerebellum in children and adolescents with autism spectrum disorder. Autism Res 2:246–257

Shaw P, Kabani NJ, Lerch JP, Eckstrand K, Lenroot R, Gogtay N, Greenstein D, Clasen L, Evans A, Rapoport JL, Giedd JN, Wise SP (2008) Neurodevelopmental trajectories of the human cerebral cortex. J Neurosci 28:3586–3594

Shen MD, Shih P, Ottl B, Keehn B, Leyden KM, Gaffrey MS, Muller RA (2012) Atypical lexicosemantic function of extrastriate cortex in autism spectrum disorder: evidence from functional and effective connectivity. Neuroimage 62:1780–1791

Shen MD, Nordahl CW, Young GS, Wootton-Gorges SL, Lee A, Liston SE, Harrington KR, Ozonoff S, Amaral DG (2013) Early brain enlargement and elevated extra-axial fluid in infants who develop autism spectrum disorder. Brain 136:2825–2835

Shi F, Wang L, Peng Z, Wee CY, Shen D (2013) Altered modular organization of structural cortical networks in children with autism. PLoS One 8:e63131

Shih P, Keehn B, Oram JK, Leyden KM, Keown CL, Muller RA (2011) Functional differentiation of posterior superior temporal sulcus in autism: a functional connectivity magnetic resonance imaging study. Biol Psychiatry 70:270–277

Stanfield AC, McIntosh AM, Spencer MD, Philip R, Gaur S, Lawrie SM (2008) Towards a neuroanatomy of autism: a systematic review and meta-analysis of structural magnetic resonance imaging studies. Eur Psychiatry 23:289–299

Strick PL, Dum RP, Fiez JA (2009) Cerebellum and nonmotor function. Annu Rev Neurosci 32:413–434

Suzuki K, Sugihara G, Ouchi Y, Nakamura K, Tsujii M, Futatsubashi M, Iwata Y, Tsuchiya KJ, Matsumoto K, Takebayashi K, Wakuda T, Yoshihara Y, Suda S, Kikuchi M, Takei N, Sugiyama T, Irie T, Mori N (2011) Reduced acetylcholinesterase activity in the fusiform gyrus in adults with autism spectrum disorders. Arch Gen Psychiatry 68:306–313

Suzuki K, Sugihara G, Ouchi Y, Nakamura K, Futatsubashi M, Takebayashi K, Yoshihara Y, Omata K, Matsumoto K, Tsuchiya KJ, Iwata Y, Tsujii M, Sugiyama T, Mori N (2013) Microglial activation in young adults with autism spectrum disorder. JAMA Psychiatry 70:49–58

Thakkar KN, Polli FE, Joseph RM, Tuch DS, Hadjikhani N, Barton JJ, Manoach DS (2008) Response monitoring, repetitive behaviour and anterior cingulate abnormalities in autism spectrum disorders (ASD). Brain 131:2464–2478

Travers BG, Adluru N, Ennis C, Tromp do PM, Destiche D, Doran S, Bigler ED, Lange N, Lainhart JE, Alexander AL (2012) Diffusion tensor imaging in autism spectrum disorder: a review. Autism Res 5:289–313

Uddin LQ, Supekar K, Lynch CJ, Khouzam A, Phillips J, Feinstein C, Ryali S, Menon V (2013a) Salience network-based classification and prediction of symptom severity in children with autism. JAMA Psychiatry 70:869–879

Uddin LQ, Supekar K, Menon V (2013b) Reconceptualizing functional brain connectivity in autism from a developmental perspective. Front Hum Neurosci 7:458

Via E, Radua J, Cardoner N, Happe F, Mataix-Cols D (2011) Meta-analysis of gray matter abnormalities in autism spectrum disorder: should Asperger disorder be subsumed under a broader umbrella of autistic spectrum disorder? Arch Gen Psychiatry 68:409–418

Wallace GL, Dankner N, Kenworthy L, Giedd JN, Martin A (2010) Age-related temporal and parietal cortical thinning in autism spectrum disorders. Brain 133:3745–3754

Wallace GL, Robustelli B, Dankner N, Kenworthy L, Giedd JN, Martin A (2013) Increased gyrification, but comparable surface area in adolescents with autism spectrum disorders. Brain 136:1956–1967

Weng SJ, Wiggins JL, Peltier SJ, Carrasco M, Risi S, Lord C, Monk CS (2010) Alterations of resting state functional connectivity in the default network in adolescents with autism spectrum disorders. Brain Res 1313:202–214

White T, Andreasen NC, Nopoulos P, Magnotta V (2003) Gyrification abnormalities in childhood- and adolescent-onset schizophrenia. Biol Psychiatry 54:418–426

Williams DL, Cherkassky VL, Mason RA, Keller TA, Minshew NJ, Just MA (2013) Brain function differences in language processing in children and adults with autism. Autism Res 6:288–302

Wolff JJ, Hazlett HC, Lightbody AA, Reiss AL, Piven J (2013) Repetitive and self-injurious behaviors: associations with caudate volume in autism and fragile X syndrome. J Neurodev Disord 5:12

Yamasaki S, Yamasue H, Abe O, Suga M, Yamada H, Inoue H, Kuwabara H, Kawakubo Y, Yahata N, Aoki S, Kano Y, Kato N, Kasai K (2010) Reduced gray matter volume of pars opercularis is associated with impaired social communication in high-functioning autism spectrum disorders. Biol Psychiatry 68:1141–1147

Yu KK, Cheung C, Chua SE, McAlonan GM (2011) Can Asperger syndrome be distinguished from autism? An anatomic likelihood meta-analysis of MRI studies. J Psychiatry Neurosci 36:412–421

Zilbovicius M, Boddaert N, Belin P, Poline JB, Remy P, Mangin JF, Thivard L, Barthelemy C, Samson Y (2000) Temporal lobe dysfunction in childhood autism: a PET study. Positron emission tomography. Am J Psychiatry 157:1988–1993

Chapter 8
The Neuropathology of Autism

Manuel F. Casanova

Abstract Most researchers agree that autism spectrum disorders (ASD) comprise a group of developmental conditions whose pathological substratum resides in the brain. Despite the significance of neuropathological research in ASD, relatively few studies have been performed on the subject. The limited number of studies may be accounted, in part, by the scarcity of available tissues in different brain banks. Furthermore, variability within each patient population in regards to pre-agonal/agonal conditions, medications, comorbidity (e.g., seizures), and postmortem interval may all account for dissimilar findings among the limited number of reported studies. Only recently has a clear picture begun to emerge as to the neuropathological underpinnings of ASD. The presence of heterotopias, laminar effacement, and minicolumnopathy suggest that heterochronic divisions of periventricular germinal cells may provide for the asynchronous development of pyramidal cells and interneurons within the cerebral cortex. A similar defect within the rhombic lip may help explain brainstem and cerebellar malformations. Autism spectrum disorders are multifactorial conditions wherein a genetic proclivity and environmental stressors act at particular times during brain development to provide an autistic phenotype.

Keywords Neuropathology · Brain · Cerebral cortex · Minicolumns · Cortical modularity · Cerebellum · Brainstem · Axons · Brain weight

Neuropathology is the field of science that studies diseases of the nervous system by examining either surgical biopsies or autopsied brain tissue. The field itself is an amalgam of knowledge derived from neurology, neurosurgery, and neuroanatomy. The history of neuropathology can be divided into different eras each reflecting the major afflictions of society during those periods of time. In this regard, epilepsy and dementia have always garnered a large share of the attention. By comparison, the history of neuropathological investigations in autism has been characterized

M. F. Casanova (✉)
Department of Psychiatry and Behavioral Sciences, University of Louisville School of Medicine, 550 S Jackson St, Louisville, KY 40202, USA
e-mail: m0casa02@louisville.edu

S. Hossein Fatemi (ed.), *The Molecular Basis of Autism,*
Contemporary Clinical Neuroscience, DOI 10.1007/978-1-4939-2190-4_8

by a dearth of interest explainable by the scarcity of available materials to perform large case series, lack of appropriate diagnostic criteria, and confusion with related comorbidities. Indeed, many of the earliest reported cases lay buried in the literature of mental retardation and epilepsy. More recently, the rising prevalence rates of autism and the attendant societal, educational, and financial problems have lead to a burgeoning interest in regards to the condition. In addition, efforts by layman organizations in collecting autopsied brain tissues of autistic individuals have facilitated neuropathological research endeavors.

One of the leading organizations promoting neuropathological research into pervasive developmental disorders of children is the Autism Research Program (ATP). The Autism Tissue Program was established as an initiative of the National Alliance for Autism Research (now Autism Speaks), in 1998. Within the available series a limited number of cases have been examined with postmortem MRI using a protocol that optimizes imaging parameters for postmortem tissue (Schumann et al. 2001). The ATP also holds a limited number of cases with related disorders including tuberous sclerosis and chromosome 15q duplication. More information on the ATP and availability of tissue can be found on the web portal of the organization (http://www.atpportal.org), the National Database for Autism Research (NDAR, http://www.ndar.org) and in different publications (Pickett and London 2005; Briacombe et al. 2007).

8.1 Early History

The most comprehensive early review on the neuropathology of autism was undertaken by John Darby (1976). In addition to performing an extensive literature review, Darby made inquiries to a large number of academicians and was thus able to identify 33 cases with a diagnosis of autism that had some type of postmortem examination. Twenty nine of those cases had been previously reported in the literature. The significance of this series is limited due to many salient weaknesses in the study design. First of all, the diagnostic nature of these cases may be disputed as they were initially identified as having severe psychotic symptoms. In effect, Darby's cases were, in most instances, retrieved from a large bibliography of articles focusing on patients having either childhood psychosis or schizophrenia. The available tissues for the cases identified by Darby varied enormously; in some cases a small biopsy and a single slide were available for study while in others there was access to the whole brain. Unsurprisingly, there were significant differences in the clinical presentation of cases with the most common finding by light microscopy being cerebral lipidosis; a histological change that several decades ago made reference to intracellular lipofuscin accumulation. Nowadays the term is preferentially used for disorders of phospholipid metabolism. Darby concluded that the noticeable variety of presentations and neuropathological findings made autism a heterogeneous disorder. Many years after his initial publication, in a letter to the American Journal of Psychiatry, he described autism as a final

common pathway of behavioral expression for many organic disorders (Darby and Clark 1992).

Aarkrog (1968) reported the microscopic analysis of a frontal lobe biopsy showing thickening of the connective tissue within the leptomeninges. Similar nondescriptive findings were provided by Williams and colleagues in a series of 4 individuals who exhibited an autistic phenotype. Among the patients reported, one had phenylketonuria and another had a probable diagnosis of Rett's syndrome. Both macro- and microscopic examination, including Golgi examination, were normal (Williams et al. 1980).

The first study involving cell counts (pyramidal and non-pyramidal neurons as well as glia) in autism was reported by Coleman et al. (1985) in one autistic individual and two age-matched controls. Tissues were embedded in celloidin to prevent shrinkage artifacts and then stained with cresyl violet (a nuclear, nucleolar and ribosomal stain), hematoxylin-eosin (a nuclear stain with a cytoplasmic counterstain), luxol fast blue (a myelin stain), and the Bodian silver impregnation method (a stain that emphasizes the visualization of neurofibrils, axons and dendrites). There were no significant findings in cell numbers. Small pockets of eosinophilic (ischemic) neurons at the base of gyri in the autistic individual suggested the possibility of an ulegyric process. In ulegyria scarring occurs in the depth of sulci most often as a result of hypoxic-ischemic brain injury during the perinatal period. The pockets of cells in Coleman's case report should be considered the sequela of hypoperfusion, a pathophysiological mechanism that, in this case, is in agreement with the cause of death, i.e., infection and shock leading to disseminated intravascular coagulation.

8.2 Modern History

The modern history of neuropathological studies in autism begins with the classic studies of Margaret Bauman and Tom Kemper. The database used for their studies stemmed from a collection of whole-brain serial sections that had been embedded in celloidin following a protocol previously devised by Paul Yakovlev. Slides were viewed under a comparison microscope that allowed side-by-side analysis of specimens and qualitative observations derived from their visual survey.

The initial case for Bauman and Kemper (1985) was a 29 year-old autistic male with a history of delayed development and stereotypical behaviors. He had a short attention span, showed no eye contact, and did not respond to his name. At age 21 he had a major motor seizure and an EEG examination revealed a photoconvulsive pattern. He died by accidental drowning at the age of 29 years. Postmortem findings were primarily confined to subcortical regions with smaller than normal neurons being described in the hippocampus, entorhinal cortex, and amygdala. Purkinje cell loss was evident in the cerebellum for which no evidence of reactive gliosis was present, albeit examination was based on Nissl stained slides.

Since their original descriptions, Bauman and Kemper added nine additional cases to their series (Bauman and Kemper 1985, 2005; Bauman 1991; Kemper and Bauman 1993; Raymond et al. 1996). Although the survey was based primarily on qualitative visual inspection, areas of suspected abnormality were further quantitated for packing (cell) density and neuronal size. Four of the ten examined patients had seizures and had been treated with various anticonvulsant medications. Gross examination of the brains revealed no abnormalities. As in the initial case, most findings were found within the subcortical regions. Reduced neuronal size and increased cell density were reported for the hippocampus, entorhinal cortex, amygdala, medial septal nuclei and mammillary bodies. Within the nucleus of the diagonal band of Broca cells and olivary nuclei cells were described as larger in the brains of younger patients. All patients exhibited Purkinje cell loss without evidence of retrograde abnormalities in the olivary nuclei. Other subcortical regions, including striatum, pallidum, hypothalamus and basal forebrain revealed no histologic abnormalities.

The hippocampi of two of the patients in Bauman and Kemper series, a boy aged 9 and a girl aged 7, were further studied by the rapid Golgi technique and compared against 2 controls (Raymond et al. 1996). The reasons for examining these two cases instead of their whole series, the anatomical level used for sampling, and number of slides analyzed were not described in the study. Both autistic cases had adequate staining of the CA1 subfield, but only 1 case was suitable for visualizing the pyramidal cells of the CA4 subfield. According to the authors, the CA4 subfield in one of the autistic children showed a reduction in size of the cell soma. Both autistic individuals; however, showed a simplification of the dendritic arborization in the CA1 and CA4 subfields.

Bauman and Kemper along with their colleagues also studied the hippocampus using immunocytochemistry for calbindin (CB), calretinin (CR) and parvalbumin (PV) (Lawrence et al. 2010). The patient sample consisted of 5 autistic individuals and an equal number of age matched controls. Results showed increased density of CB immunoreactive neurons in the dentate gyrus, increased CR positive interneurons in area CA1, and an increase in PV interneurons in the CA1 and CA3 subfields of the hippocampus. The study failed to consider whether agonal (meaning pertaining to death or dying) and/or preagonal conditions influenced their findings, nor did they examine for the presence of concomitant gliosis. The significance of the study is arguable given the large number of confounding variables that may cause misestimating anatomical elements in human postmortem tissue.

Many of the above related neuropathological findings have not been reproduced in more recent studies. Fatemi et al. (2002) found no differences in Purkinje cell density between their autistic and controls individuals ($N=5$ per group). Bailey et al. (1998) did not find any consistent neuronomorphometric differences among the different CA subfields of their patients. In similar manner, Schumann and Amaral (2005, 2006) using modern stereological methods could not corroborate the presence of decreased neuronal size and increased packing density in the amygdala of autistic individuals. In effect, cell counts were lower in autistic individuals thus giving opposite results to the previous reports based on qualitative observations.

One major difference between the series of Schumann and Amaral (2006) and Bauman and Kemper (1994, 2005) was the exclusion in the former of individuals with seizure disorders.

Many years after Bauman and Kemper's original report, Bailey et al. (1998) reported a comprehensive neuropathological study on the brains of 6 mentally handicapped autistic individuals. Although Bailey and colleagues found some abnormalities involving the brainstem (i.e., locus coeruleus, stria medullaris and arcuate nuclei) they were the first to emphasize a dysplastic process within the cerebral cortex and a possible migratory abnormality. Indeed, several of their cases showed clustering of individual neurons (ectopias) within the white matter and abnormalities in lamination within the frontal cortex.

8.3 Cerebral Cortex

Bauman and Kemper reported on abnormalities of the cerebral cortex in autistic individuals but the same did not occupy their main focus of attention. The anterior cingulate gyrus was described as poorly laminated in 5 out of 6 cases in their series (Bauman and Kemper 1994). The same researchers pursued their original findings by performing sterelogical quantitation of neuronomorphometric indices on the anterior cingulate gyrus in 9 autistic individuals and 4 controls. The series were matched for what was called "age-range" but not for age (mean age for autistics was 27.9 and 38.8 for controls) nor for hemisphere side. Results revealed decreased cell size in layers I–III and layers V–VI of area 24b and in cell packing density in layers V–VI of areas 24c.

Sections through the anterior cingulate gyrus have also been taken to study cell size and density of von Economo (spindle) neurons (VEN) (Simms et al. 2009). Because of their long connections and location (i.e., anterior cingulate, fronto-insular, and dorsolateral prefrontal cortex) these cells have been implicated in many cognitive abilities and disabilities of humans. The study by Simms et al. (2009) revealed no significant between group differences for any of the parameters studied. Despite lack of significant findings the authors undertook a *post hoc* analysis that defined subgroups of autistic individuals (3 with increased and 3 with reduced VEN densities). Other studies on VENs have found either no differences (Kennedy et al. 2007) or a significant higher ratio of VEN to pyramidal cells in autistics as compared to controls (Santos et al. 2011). The significance of the latter study by Santos and colleagues is difficult to gauge as average section thickness of cut tissue was not standardized in the control series; varying from 200 to 500 μm with an average of 300 μm. Since the section thickness in the series of autistic individuals was 200 μm, the findings of the study may be an artifact of how the tissue was prepared for examination.

Hutsler et al. (2007) evaluated thickness and lamination in postmortem tissue of 8 ASD patients and an equal number of age matched controls. The study found no significant differences for cortical thickness or for abnormalities in lamination.

However, qualitative observations revealed cell clustering in lamina I and the subplate region. A later study by the same group revealed an indistinct boundary between layer VI and the white matter possibly reflecting the presence of an increased number of neurons beneath the cortical plate (Avino and Hutsler 2010; Hutsler and Avino 2013). It was believed that the presence of supernumerary neurons within the subplate region was the result of a migratory defect or failed apoptosis.

Courchesne et al. (2011) analyzed the cell number and size of neurons within the prefrontal cortex of 7 children with autism and 6 controls. Criteria used in quantitation (i.e., a high cytoplasm to nucleus ratio and the presence of a prominent nucleoli) excluded interneurons. The authors reported 70% more neurons in the dorsolateral cortex and a 29% increase in the mesial prefrontal cortex. There were no differences in the size of the cells. Prior to Courchesne, neuronal density in the dorsolateral prefrontal cortex was described as being 34% higher in autistic individuals as compared to controls (Casanova et al. 2006a). The findings in the latter study were not confined to the frontal cortex but were also seen in other areas of the brain. Casanova et al. (2006a) attributed their finding to an increased number of minicolumns (see below).

8.4 Cortical Modularity

Arrays of vertically arranged pyramidal cells spanning the width of the cerebral cortex were noted by early anatomists of the late nineteenth century. Von Economo and Koskinas (1925) believed that the pyramidal cell arrays were closely related to the so-called radii, that is, pencil shaped bundles of myelinated fibers (also known as bundles of Meynert) within the cerebral cortex (Campbell 1905). Other authors have similarly reported a correlation between pyramidal cell arrays and both apical dendritic bundles and double bouquet cells. Thus, both pyramidal cell arrays as well as the axonal bundles or horsetails from double bouquet cells have shown a one-to-one correspondence to myelinated axonal bundles (DeFelipe et al. 1990; Casanova et al. 2008). The previously described composite of cells and their projections suggested their presence and interaction within a unifying anatomical structure which Lorente de Nó (1938) designated as an elementary unit of cortical organization. Based on thousands of microelectrode impalements, Mountcastle (1978) described a similarly oriented unit based on physiological means. He denominated this unit as a mini- or microcolumn.

Pyramidal cells are usually intermixed with smaller neurons, many of which lack a longitudinal axis. This limitation has often made it difficult to perform qualitative estimates of minicolumns based on inter-pyramidal cell array widths. Considering the fact that myelin staining is less prone to both postagonal and processing artifacts (Chan and Lowe 2002) and that myelinated axonal bundles do not bifurcate some authors have indicated that “the radial arrangement of the myeloarchitecture in many cortical regions often mirrors the suggestion of columnar arrangements

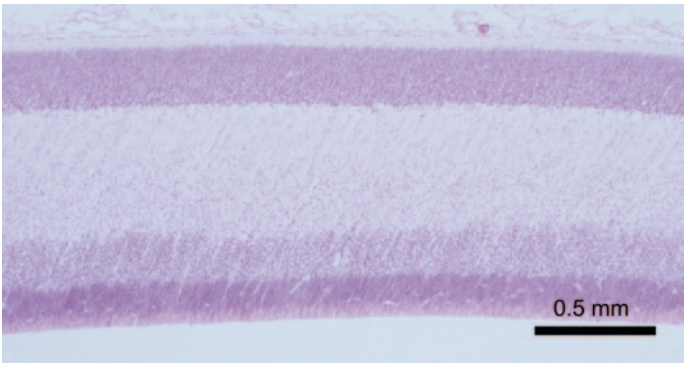


Fig. 8.1 Periventricular germinal cells line the ventricular cavity, seen at the *bottom* of the image. Asymmetric divisions of germinal cells provide for daughter cells that migrate to the cortex following a glial scaffold. Once in the cortex the neuroblasts will continue their differentiation to pyramidal cells. These excitatory cells provide the core of the cell minicolumn. Inhibitory cells, in their majority, will develop in synchrony with pyramidal cells but migrate to the cortex following a tangential path

better than Nissl preparations" (von Bonin and Mehler 1971). Still, despite these arguments, most studies on minicolumnarity rely on Nissl stains.

Pyramidal cell arrays in humans have often been examined by Nissl stain and reported distances between these units range between 30 and 80 μm depending on brain region. These units originate from the ontogetic minicolumn (Fig. 8.1) and as such are one cell-wide (Casanova et al. 2007). For this reason some of the initial algorithms developed to detect minicolumns assumed that the separation between minicolumns was of the same order of magnitude as the separation between pyramidal cell somas (Buxhoeveden et al. 2000). While this assumption may hold true for supragranular layers it does not appear to hold for those laminae where granular or stellar neurons abound.

Using a semiautomated computerized imaging method to analyze minicolumnar morphometry based on pyramidal cell arrays, Casanova et al. (2002a) studied Brodmann areas 9, 21, and 22 in postmortem tissues of autism spectrum disorder (ASD) patients. The population consisted of 9 ASD individuals (7 males and 2 females; mean age of 12 years) and 11 control subjects (7 males and 4 females; mean age of 14 years). Photomicrograph images were processed with a Gaussian gray-scale distribution and thresholded in order to define cellular kernels (Otsu 1979). A Euclidean minimum spanning tree was used to parcellate the kernels so as to minimize the total length of all of the lines joining them (Fig. 8.2). Minicolumnar width was defined as the mean distance between the tangential axes of pyramidal cell arrays. Other measured parameters included the relative dispersion of cells around the radial axis of each minicolumn, and the mean intercellular distance within minicolumns. Results of the study showed a significant reduction in minicolumnar widths with the greatest reduction found within their peripheral neuropil compartment.

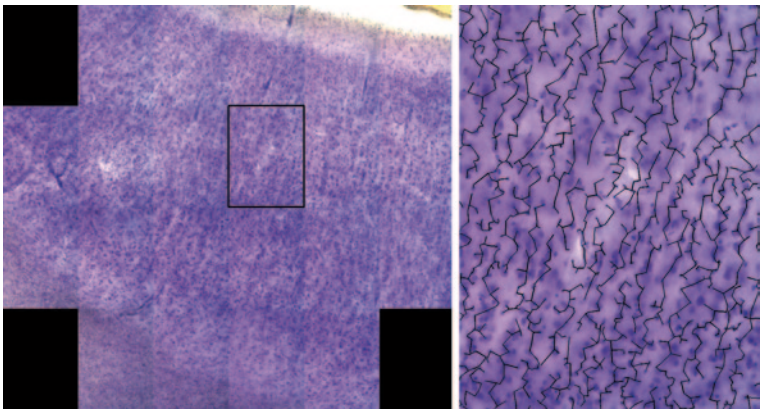


Fig. 8.2 Panel on left shows a Nissl stained cross section of the cerebral cortex. The inset area, measuring 0.8 mm × 1.1 mm, is magnified on the *right* panel wherein a computerized algorithm identifies the presence of minicolumns. The method eliminates minicolumnar fragments usually less than 8 cells in depth

Casanova et al. (2002b) used the same patient population (*vide supra*) and analyzed the photomicrographs using a modification of the method developed by Schleicher et al. (2005) to assess thresholded images according to the gray level index (GLI). Intercolumnar distances, as defined by the intercluster width of GLI maxima values, were decreased and the mean number of clusters increased per given image. The results were consistent with previous findings indicating a greater number of narrower minicolumns in ASD.

The prior studies were followed by a multicenter international attempt to corroborate the minicolumnar findings. The study centered on an independent sample provided by the Autism Tissue Program (6 patients DSM-IV-TR and ADI-R diagnosed and an equal number of age matched controls). Serial whole hemispheric coronal sections embedded in celloidin were available for examination. Digital photomicrograph montages were taken of cortical Brodmann areas 3, 4, 9, and 17. Computerized image analysis with both the Euclidean minimum spanning tree and the GLI method were done blind to diagnoses. Minicolumnar widths were significantly diminished across all examined areas. Mean neuronal (cell body) and nucleolar cross sections were smaller in autistic individuals as compared to controls. Analysis of inter- and intracluster distances of edges obtained from a Delaunay triangulation suggested an increased number of minicolumns each one containing a normal number of cells. The authors indicated that the smaller cell bodies created a bias in corticocortical connectivity favoring shorter connections at the expense of longer ones. Also, the findings of an increased number of minicolumns, each one of appropriate cell density, suggested an increased number of symmetrical divisions of periventricular germinal cells during embryogenesis (Casanova 2013).

The nature of the proposed minicolumnopathy was furthered characterized by comparing minicolumnar widths across cortical laminae (Casanova et al. 2010).

The study used postmortem material from 7 ASD individuals and an equal number of age matched neurotypicals sampled at 9 different locations: Brodmann areas 3b, 4, 9, 10, 11, 17, 24, 43, and 44. Each area was assessed at supragranular, granular and infragranular levels. Minicolumnar width diminution was equally observed across all examined levels. The findings suggested to the authors the involvement of an anatomical element in common to all levels. Given that the described minicolumnopathy is marked by a diminution of the peripheral neuropil space (i.e., the compartment holding most of the interneurons), a possible abnormality of the inhibitory elements surrounding the minicolumns is suggested.

The presence of inhibitory elements within the peripheral neuropil space of minicolumns has drawn the analogy to a strong vertical flow or shower curtain of inhibition (Szentágothai and Arbib 1975; Mountcastle 1998). Many of the symptoms (e.g., seizures, hyper- hypo sensitivities) attributed to an excitatory/inhibitory imbalance within the cerebral cortex of autistic individuals may be rooted in an abnormality of the peripheral surround of minicolumns. Electrophysiological experiments indirectly corroborate the presence of such a deficit in ASD. Casanova (2013) has proposed that the most parsimonious explanation to the minicolumnar findings is the forced and abnormal division of periventricular germinal cells during brain development. Heterochronic divisions of germinal cells would provide for the migration of neuroblasts to the cortical plate at a time when their positioning off from the radial glial scaffold would not be coordinated with the tangentially derived interneurons (Casanova et al. 2013).

8.5 Cerebellum

Neuropathological reports have often stressed the presence of histological abnormalities of the cerebellum in autistic individuals. Many of these abnormalities are of a dysplastic nature, suggesting a deviation during brain development in the organization of this anatomical structure. In general, dysplasias are often noted in the context of widespread cerebral malformations (Soto-Ares et al. 2000). Clinical information is seldom useful for diagnosing cerebellar dysplasias and classification is largely based on morphological assessments performed either by structural imaging methods or postmortem examination (Patel and Barkovich 2002).

Within the cerebellar cortex, Purkinje cells are linearly arranged and buttressed between a cell-poor molecular layer and the underlying granular layer. Several studies have called attention to diminished numbers of Purkinje cells in autism. Given that these cells are the only source of efferent projections of the cerebellar cortex, it is claimed that this abnormality could account for some autistic symptoms. Bauman and Kemper's (1985) original case had bilateral Purkinje cell loss in the neocerebellum. Purkinje cell loss was more widespread when taking into consideration the ten cases in their series (Bauman 1991; Kemper and Bauman 1993; Bauman and Kemper 2005). Cell loss was observed in the posterolateral neocerebellar and archicerebellar cortices. Only two autistic individuals within their series showed

pallor when staining for granule cells. Contrary to neuroimaging studies of vermal abnormalities in autism, Bauman and Kemper have reported no histological abnormalities in this region (Arin et al. 1991; Bauman and Kemper 1996). The lack of concomitant gliosis and absence of retrograde olivary cell loss made Bauman and Kemper suggest that Purkinje cell loss is most consistent with a lesion early during brain development.

Bauman and Kemper (1985) conclusions of an early developmental lesion have been questioned. Screening for the presence of gliosis using Nissl staining is unreliable and should be avoided. More recent studies using immunocytochemistry or Western blotting for GFAP suggest the presence of astrocytosis in the cerebellum of autistic individuals (Bailey et al. 1998; Laurence and Fatemi 2005; Vargas et al. 2005). GFAP belongs to a family of intermediary filament proteins that is expressed during the process of reactive astrocytosis and glial scar formation (Sofroniew and Vinters 2010). This upregulation of GFAP expression in autism is characterized by the presence of hypertrophic cell bodies and processes in a narrow and compact spread along the Purkinje cell layer. There is no reorganization of tissue architecture and no intermingling of processes across different astrocytic domains to suggest a glial scar formation or a severe focal lesion. Thus, the presence of a GFAP astrocytic response appears to be a reactive process to the loss of Purkinje cells.

Many autistic individuals suffer from epilepsy and may receive the anticonvulsant phenytoin as treatment. In addition some patients may engage in self-injurious behaviors, such as head banging, leading to contusions. Both seizures and phenytoin can provide for cerebellar atrophy and reduced Purkinje cell densities as a result of excitatory damage by the cerebro-cerebellar pathways (Crooks et al. 2000). Fronto-temporal contusions are associated to posterior lobe atrophy via a posttraumatic mechanism rather than to excitatory damage (Crooks et al. 2000). It seems possible that Purkinje cell loss in autism is, in part, the result of either or all of the above related conditions rather than a neurodevelopmental condition. In effect, Bailey et al. (1998) claimed that Purkinje cell loss during development was puzzling in lieu of the normal development of the cerebellar cortex. Moreover, these investigators also claimed that olivary cell loss is hard to assess as the cells normally show large variations in density and lie relatively far apart. It is therefore unsurprising that a later study by the Bauman and Kemper group comparing immunocytochemistry and Nissl staining indicated the possibility that agonal conditions and postmortem handling could account for inadequate Nissl staining and the lower Purkinje cell counts reported in their prior studies (Whitney et al. 2008).

8.6 Brainstem

Rodier et al. (1996) examined sections though the lower cranial nuclei of a single autistic individual. There were multiple abnormalities including a shortened distance between the trapezoid body and the inferior olive, and an absence of both superior olivary and facial nuclei. The authors concluded that, at least for the reported

case, there was some type of injury that occurred around the time of neural tube closure. Congenital facial palsy is uncommon and when present it may be characteristic of the Moebius syndrome. Approximately 40% of children with Moebius syndrome show symptoms typical of autism spectrum disorders (Gillberg and Steffenburg 1989).

Kulesza and Mangunay (2008) pursued Rodier's finding and studied the superior olivary nucleus of 5 autistic subjects and 2 non-age matched controls. According to the ATP's portal, one of the cases used in this study had a diagnosis of Fragile X-syndrome. Kuleszca and Mangunay (2008) concluded that there were significant morphological differences in the medial superior olivary nuclei in each of their 5 autistic cases as compared to controls. The age of their two controls (26 and 29 years) did not match the ages of the younger autistic patients (8 and 13 years). A more recent study by Thevarkunnel et al. (2004) showed no abnormalities of the superior olivary or facial nuclei in autistic individuals. Details of this work remain unknown as the original abstract presentation has not been published in a peer-review journal.

Another nucleus of the brainstem that has received attention in autism is the locus coeruleus. In Bailey et al. (1998) study on 6 autistic patients, one was described as having a widely dispersed nuclei and another one as having loosely grouped neurons. Martchek et al. (2006) used non-age matched hemisections (also not matched for hemisphere side) to perform a stereological study of this nucleus. They reported no significant between group differences in total cell counts, volume or numerical density of the locus coeruleus.

8.7 Axons

Hof et al. (1991) reported the neuropathological findings of a microcephalic autistic patient with premature closure of the cranial sutures. During life the patient exhibited a variety of self-injurious behaviors including head banging, eye-gouging, and self-biting. The gyral pattern at autopsy was normal but the brain exhibited generalized atrophy. At microscopic examination neurofibrillary tangles were found in the superficial layers of the perirhinal and entorhinal cortex and layers II and III of the neocortex. There was no evidence of concomitant amyloid accumulation. Given the severity of the patient's self-injurious behaviors the authors suggested that the presence of neurofibrillary tangles was the results of a degenerative condition promoted by repeated brain trauma, i.e., dementia pugilistica. If this was the case, the patient must have exhibited thinning of the corpus callosum and diffuse axonal injury. Curiously the neurofibrillary tangle distribution in this case followed the same distribution as people with Alzheimer's rather than mimic the location of contusive injuries (Jordan 2009).

Weidenheim et al. (2001) reported 2 cases of neuroaxonal dystrophy with secondary autism. Axonal swellings forming varicosities or spheroids were found within catecholaminergic and serotonergic nuclei. According to the authors, the topography

of neuropathological changes suggested that limbic and other deep grey matter structures play an important role in the pathophysiology of autistic behaviors.

The density and thickness of axonal projections were studied using stereology in 5 autistic individuals and 4 age matched controls (Zikopoulos and Barbas 2010). Sampled sites included the white matter below the anterior cingulate cortex, orbitofrontal cortex, and lateral prefrontal cortex. Increased axonal density was reported below the anterior cingulate cortex and an excessive number of thinned axons below the orbitofrontal cortex. The findings indicate that at least anatomically there seems to be a bias towards shorter projections in autism (Casanova et al. 2006b).

The temporal lobes and telencephalic subcortical structures (i.e., medial and lateral forebrain bundles) were examined for serotonergic axons with immunocytochemistry (Azmitia et al. 2011). Thirteen autistic individuals and nine age matched controls were used in this study. Dystrophic serotonin positive axons were described in both forebrain bundles, amygdala, and in the piriform, superior temporal, and parahippocampal cortices. In analyzing the results the investigators did not take into account the preagonal and agonal conditions of the patients, length of fixation or postmortem interval.

8.8 Brain Weight

The fact that the brain, even after death, has the capacity to react to its environment is poorly appreciated by the research community. Within minutes after death autolysis, resulting from the release of enzymes within cells, is evident as part of the postmortem decomposition process. Within less than 4 h the gray matter shows a rapid increase in water and sodium and a fall in potassium content.

Later on bacterial proliferation leads to putrefaction. Several factors influence how rapidly these processes take place including environmental temperature, obesity, and the presence of septicemia.

All of the above mentioned factors influence fresh brain weight and my lead to spurious conclusions when taking this parameter as an index of pathology. It is therefore of importance that studies of fresh brain weights take into consideration the possibility of postmortem edema before attributing heftier specimens to either true macrocephaly or *in vivo* brain swelling. Usually convolutional flattening along with diminished ventricular size could suggest an *in vivo* phenomenon. However, histologic examination is of little use in ascertaining the presence of edema (Yates et al. 1975). It is usually agreed that neuroimaging studies *in vivo* allow for a more accurate determination of brain swelling than does post-mortem assessment: "The assessment of diffuse brain swelling at autopsy on the basis of convolutional flattening, obliteration of sulci and small symmetrical ventricles is complicated by the fact that some swelling of the brain is normally present post mortem. Neuroimaging *in vivo* allows more accurate and sensitive measurement of brain swelling than does macroscopic post-mortem assessment" (Blumbergs et al. 2008, p. 767).

Brain weight is also collected after formalin fixation. Depending on time of fixation and concentration of fixative solution (usually 10% formalin solution,

the equivalent of a 3.7% formaldehyde solution) there will be a variable increase in brain weight. This increase will be more noticeable in children as compared to adults (Ludwig 2002). The findings are of significance when researchers perform studies on cellular density measurements instead of total cell counts.

Most studies on brain weight in autism have failed to disclose the conditions under which this parameter was collected. In some cases it is not possible even to say whether fresh or post-fixation brain weights were used alone or mixed together within the same sample. Furthermore, useful clinical indices to judge the validity of brain weights are usually not reported, e.g., cause of death, duration of terminal illness, terminal state of hydration (Itabashi et al. 2007). Given all of the above mentioned limitations the few available studies on postmortem brain weight in autism are of arguable significance.

In Williams et al. (1980) study the brains of their 4 autistic subjects (4 to 33 years of age) fell within 2 standard deviations of the mean for age. Hof et al. (1991) patient was microcephalic with a brain weigh of 773 g. Bauman and Kemper (1994) collected fresh brain weights from 12 autistic children, aged 5–13 years, and 8 adults, aged 18–54 years. The investigators concluded that their data were in agreement with that of others suggesting an early acceleration followed over the course of ageing by an apparent deceleration in brain weight. The conclusions may stand to be corrected as the patient series included 3 obvious outliers.

Bailey et al. (1993) reported heavier brain weights in 3 out of their 4 autistic subjects (age range 4–27 years). Microscopic examination did not reveal an increase in neuronal density. Two additional cases, with tissue available for microscopic examination, were added to their series (Bailey et al. 1998). Three of the six patients evidence brain swelling without herniation most possibly as a result of postmortem edema. One of the latter brains also showed signs of putrefaction.

Courchesne et al. (1999) reported on brain weights of 5 new cases and 16 previously reported ones and compared them to 6 normative series. They concluded that brain weights for autistic subjects are usually within the normal range with rare cases of megalencephaly. The series was later pursued with a metanalysis on 55 specimens (Courchesne et al. 1999; Redcay and Courchesne 2005). The researchers concluded that there is divergence in brain weights early on in life that tends to normalize with ageing.

8.9 A Locus Minoris Resistentiae

A multitude of studies presently suggest that the brains of autism spectrum disorder individuals suffer from a developmental malformation. The underlying process appears widespread affecting the cerebral cortex, brainstem and cerebellum. Reports of non-universality have been attributed to the limited sampling of some studies (Hutsler et al. 2007).

Bailey et al. (1998) reported cortical dysgenesis in five out of seven of their cases. Hutsler et al. (2007) described an overabundance of cells within the cortical

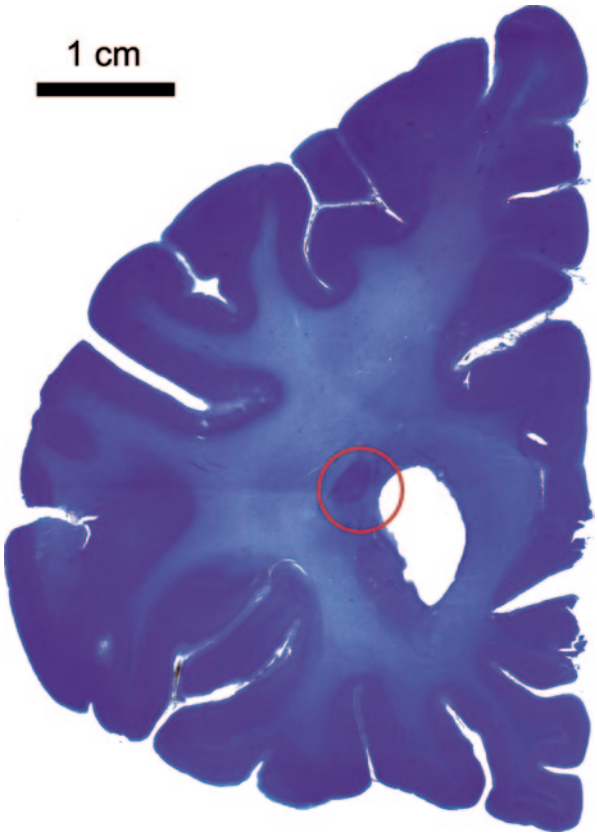


Fig. 8.3 Cellodin embedded Nissl stained section of the human brain. The coronal section is within the prefrontal lobes. A large cluster of neurons (*circled*) has failed to migrate to the cortex and now rests near the ventricle. Similar clusters of cells, known as heterotopias, are often related to cortical malformations (dysplasias) and seizures

white matter. All nine cases reported by Bauman and Kemper (2005) showed clustering of neurons in the inferior olive and in one case an ectopic cluster of cells adjacent to the inferior peduncle. Wegiel et al. (2010) have described the presence of focal heterotopias that vary widely as to location among a significant portion of autistic individuals (Fig. 8.3). More recently, Casanova et al. (2013) described the presence of circumscribed dysplastic foci throughout the cerebral cortex of autistic individuals.

The generality of findings, from heterotopias to cortical malformations (including a minicolumnopathy), indicate the presence of a migrational defect in autism.

Defects of neuronal migration involve different steps including the division of precursors from a germinal field, ongoing migration through the white matter, penetration of the subplate, and their progression through the cortical plate. The literature supports abnormalities for each of these steps. In autism migrational abnormalities are suggested from accounts of cortical dysplasias, thickening of the cortex, variations in neuronal density, minicolumnar alterations, the presence of neurons in the molecular layer as well as in the white matter, irregular laminar

patterns, poor gray-white matter differentiation, and ectopic foci of cells (Schmitz and Rezaie 2008).

8.10 Conclusions

This chapter summarizes the literature of neuropathological findings in autism. The proper interpretation of neuropathological findings has to take into account limitations in both research designs and techniques. Autopsied or biopsied tissue offers a cross section of an ongoing disease process for which the initial stages may have already escaped examination. This is of importance especially in neurodevelopmental conditions when tissue is available only many years after onset of the insult. In this regard both adaptive changes (those provided by the plasticity of the brain) and reactive processes may provide for neuropathological findings that are secondary to the core features of the condition. This limitation, a mixture of findings denoting primary and secondary aspects of pathology, is proper to the temporal nature of an unfolding pathological process. Changes may fortuitously be discovered in the biased and often limited sampling strategy of a neuropathologist. However, it is clear that many areas of the brain act within networks to combine different stimuli into a behavioral response. Sometimes the patency of these networks may be better judged *in vivo* with electrophysiological and neuroimaging techniques than with neuropathology. These limitations are not unique to autism, but they appear inordinately important to this condition given the small sample sizes within postmortem series. In this regard, we should always take into consideration the possible role of comorbidities (e.g., seizures and consequent hypoxia) and medications (e.g., anticonvulsant, neuroleptics) when reporting neuropathological findings in autism.

Despite many controversies, there appear to be a consensus of findings in autism that may reflect on the core pathology of the disorder. Indeed, many described abnormalities are clearly visible and robustly demonstrable. As an example, most researchers agree that there is variability in brain volume that appears to be age dependent. There is also evidence of migrational abnormalities as reflected in both heterotopias and dysplastic cortical abnormalities (e.g., minicolumnopathy). Neuroimaging studies have consistently demonstrated a smaller corpus callosum despite larger than average brains. Both neuroimaging and neurophysiological studies indicate alterations in the blueprint of corticocortical connectivity in the brains of autistic individuals. It is therefore of importance that these valuable findings are taken into account when providing a working framework explaining the pathophysiology of the condition. Although many details of this framework are presently missing, the findings thus far reported provide the fingerprint of a neurodevelopmental condition.

Autism is a complex multifactorial trait. This author has previously proposed that it is the result of a “triple hit” wherein a genetic proclivity and environmental stressors act at particular times during brain development to provide the autistic phenotype. Although variability for each of these events may provide for clinical

heterogeneity, a basic mechanism may underlie most of the reported pathology. The author's own studies and those available in the medical literature help illustrate the possibility of heterochronic division of germinal cells giving rise to abnormalities of corticogenesis. Within this framework, reported abnormalities such as those of a minicolumnopathy and/or heterotopias offer useful correlates to sensory abnormalities and seizures often reported in patients within the autistic spectrum.

References

Aarkrog T (1968) Organic factors in infantile psychoses and borderline psychoses: retrospective study of 45 cases subjected to pneumoencephalography. Dan Med Bull 15:283–288

Arin DM, Bauman ML, Kemper TL (1991) The distribution of Purkinje cell loss in the cerebellum in autism. Neurology 41(3 Suppl. 1):307

Avino TA, Hutsler JJ (2010) Abnormal cell patterning at the cortical gray-white matter boundary in autism spectrum disorders. Brain Res 1360:138–146

Azmitia EC, Singh JS, Hou XP, Wegiel J (2011) Dystrophic serotonin axons in postmortem brains from young autism patients. Anat Rec 294:1653–1662

Bailey A, Luthert P, Bolton P, Le Couteur A, Rutter M, Harding B (1993) Autism and megalencephaly. Lancet 341:1225–1226

Bailey A, Luthert P, Dean A et al (1998). A clinicopathological study of autism. Brain 121:889–905

Bauman ML (1991) Microscopic neuroanatomic abnormalities in autism. Pediatrics 87:791–796

Bauman M, Kemper TL (1985) Histoanatomic observations of the brain in early infantile autism. Neurology 35:866–874

Bauman ML, Kemper TL (1994) Neuroanatomical observations of the brain in autism. In: Bauman ML, Kemper TL (eds) The neurobiology of autism. Johns Hopkins University Press, Baltimore, pp 119–145

Bauman ML, Kemper TL (1996) Observations on the Purkinje cells in the cerebellar vermis in autism. J Neuropathol Exp Neurol 55:613

Bauman ML, Kemper TL (2005) Structural brain anatomy in autism: what is the evidence? In: Bauman ML, Kemper TL (eds) The neurobiology of autism, 2nd edn. Johns Hopkins University Press, Baltimore, pp 121–135

Blumbergs P, Reilly P, Vink R (2008) Trauma. In: Love S, Louis DN, Ellison DW (eds) Greenfield's neuropathology. Hodder Arnold, London, pp 733–832

Briacombe MB, Pickett R, Pickett J (2007) Autism postmortem neuroinformatic resource: the autism tissue program (ATP) informatics portal. J Autism Dev Disord 37:574–579

Buxhoeveden D, Switala AE, Roy E, Casanova MF (2000) Quantitative analysis of cell columns in the cerebral cortex. J Neurosci Methods 97:7–17

Campbell AW (1905) Histological studies on the localization of cerebral function. Cambridge University Press, Cambridge

Casanova MF (2013) The minicolumnopathy of autism. In: Buxbaum JD, Hof PR (eds) The neuroscience of autism spectrum disorders. Academic, London, pp 327–333

Casanova MF, Buxhoeveden DP, Switala A, Roy E (2002a) Minicolumnar pathology in autism. Neurology 58:428–432

Casanova MF, Buxhoeveden DP, Switala AE, Roy E (2002b) Neuronal density and architecture (gray level index) in the brains of autistic patients. J Child Neurol 17:515–521

Casanova MF, Van Kooten IAJ, Switala AE et al (2006a) Minicolumnar abnormalities in autism. Acta Neuropathol 112:287–303

Casanova MF, Van Kooten I, Switala AE et al (2006b). Abnormalities of cortical minicolumnar organization in the prefrontal lobes of autistic patients. Clin Neurosci Res 6:127–133

Casanova MF, Trippe J 2nd, Switala AE (2007) A temporal continuity to the vertical organization of the human neocortex: a study spanning prenatal development and aging. Cereb Cortex 17:130–137

Casanova MF, Konkachbaev AI, Switala AE, Elmaghraby AD (2008) Recursive trace line method for detecting myelinated bundles: a comparison study with pyramidal cell arrays. J Neurosci Methods 168:367–372

Casanova MF, El-Baz A, Vanbogaert E, Narahari P, Switala A (2010). A topographic study of minicolumnar core width by lamina comparison between autistic subjects and controls: possible minicolumnar disruption due to an anatomical element in-common to multiple laminae. Brain Pathol 20:451–458

Casanova MF, El-Baz AS, Kamat SS et al (2013) Focal cortical dysplasias in autism spectrum disorders. Acta Neuropathol Commun 1(1):67

Chan KK, Lowe J (2002) Techniques in neuropathology. In: Bancroft JD, Gamble M (eds) Theory and practice of histological techniques. Churchill Livingstone, London, pp 371–414

Coleman P, Romano J, Lapham L, Simon W (1985) Cell counts in cerebral cortex of an autistic patients. J Autism Dev Disord 15:245–255

Courchesne E, Müller RA, Saitoh O (1999) Brain weight in autism: normal in the majority of cases, megalencephalic in rare cases. Neurology 52:1057–1059

Courchesne E, Mouton PR, Calhoun ME et al (2011). Neuron number and size in prefrontal cortex of children with autism. JAMA 306:2001–2010

Crooks R, Mitchel T, Thorn M (2000) Patterns of cerebellar atrophy in patients with chronic epilepsy: a quantitative neuropathological study. Epilepsy Res 41:63–73

Darby JK (1976) Neuropathologic aspects of psychosis in children. J Autism Child Schizophr 6:339–352

Darby JK, Clark L (1992) Autism syndrome as a final common pathway of behavioral expression for many organic disorders. Am J Psychiatry 149:146

DeFelipe J, Hendry S, Hashikawa T, Molinari M, Jones EG (1990) A microcolumnar structure of monkey cerebral cortex revealed by immunocytochemical studies of double bouquet cell axons. Neuroscience 37:655–673

Fatemi SH, Halt AR, Realmuto G Earle J, Kist DA, Thuras P, Merz A (2002) Purkinje cell size is reduced in cerebellum of patients with autism. Cell Mol Neurobiol 22:171–175

Gillberg C, Steffenburg S (1989) Autistic behavior in Moebius syndrome. Acta Paediatr Scand 78:314–316

Hof PR, Knabe R, Bovier P, Bouras C (1991) Neuropathological observations in a case of autism presenting with self-injury behavior. Acta Neuropathol 82:321–326

Hutsler JJ, Avino TA (2013) Sigmoid fits to located and characterized cortical boundaries in human cerebral cortex. J Neurosci Methods 212:242–246

Hutsler JJ, Love T, Zhang H (2007) Histologic and magnetic resonance imaging assessment of cortical layering and thickness in autism spectrum disorders. Biol Psychiatry 61:449–457

Itabashi HH, Andrews JM, Tomiyasu U, Erlich SS, Sathyavagiswaran L (2007) Forensic neuropathology: a practical review of the fundamentals. Elsevier, New York

Jordan BD (2009) Brain injury in boxing. Clin Sports Med 28:561–578

Kemper TL, Bauman ML (1993) The contribution of neuropathologic studies to the understanding of autism. Neurol Clin 11:175–187

Kennedy DP, Semendeferi K, Courchesne E (2007) No reduction of spindle neuron number in frontoinsular cortex in autism. Brain Cogn 64:124–129

Kulesza RJ, Mangunay K (2008) Morphological features of the medial superior olive in autism. Brain Res 1200:132–137

Laurence JA, Fatemi SH (2005) Glial fibrillary acidic protein is elevated in superior frontal, parietal and cerebellar cortices of autistic subjects. Cerebellum 4:206–210

Lawrence YA, Kemper TL, Bauman ML, Blatt GJ (2010) Parvalbumin-, calbindin-, and calretinin-immunoreactive hippocampal interneurn density in autism. Acta Neurol Scand 121:99–108

Lorente de Nó R (1938) The cerebral cortex: architecture, intracortical connections, and motor projections. In: Fulton JF (ed) Physiology of the nervous system. Oxford University Press, London, pp 291–339

Ludwig J (2002) Handbook of autopsy practice. Humana Press, Totowa

Martchek M, Thevarkunnel S, Bauman M, Blatt G, Kemper T (2006) Lack of evidence of neuropathology in the locus coeruleus in autism. Acta Neuropathol 111:497–499

Mountcastle VB (1978) An organizing principle for cerebral function: the unit module and the distributed system. In: Edelman GM, Mountcastle VB (eds) The mindful brain: cortical organization and the group-selective theory of higher brain function. MIT Press, Cambridge, pp 7–51

Mountcastle VB (1998) Perceptual neuroscience: the cerebral cortex. Harvard University Press, Cambridge

Otsu N (1979) A threshold selection method from grey-level histograms. IEEE Trans Syst Man Cybern 9:62–66

Patel S, Barkovich AJ (2002) Analysis and classification of cerebellar malformations. AJNR 23:1074–1087

Pickett J, London E (2005) The neuropathology of autism: a review. J Neuropathol Exp Neurol 64:925–935

Raymond GV, Bauman ML, Kemper TL (1996) Hippocampus in autism: a Golgi analysis. Acta Neuropathol 91:117–119

Redcay E, Courchesne E (2005) When is the brain enlarged in autism? A metaanalysis of all brain size reports. Biol Psychiatry 58:1–9

Rodier PM, Ingram JL, Tisdale B, Nelson S, Romano J (1996) Embryological origins for autism: developmental abnormalities of the cranial nerve motor nuclei. J Comp Neurol 370:247–261

Santos M, Uppal N, Butti C et al (2011) Von Economo neurons in autism: a stereological study of the frontoinsular cortex in children. Brain Res 1380:206–217

Schleicher A, Palomero-Gallagher N, Morosan P, Eickhoff SB, Kowalski T, de Vos K, Amunts K, Zilles K (2005) Quantitative architectural analysis: a new approach to cortical mapping. Anat Embryol 210:373–386

Schmitz C, Rezaie P (2008) The neuropathology of autism: where do we stand? Neuropathol Appl Neurobiol 34:4–11

Schumann CM, Amaral DG (2005) Stereological estimation of the number of neurons in the human amygdaloid complex. J Comp Neurol 491:320–329

Schumann CM, Amaral DG (2006) Stereological analysis of amygdala neuron number in autism. J Neurosci 26:7674–7679

Schumann CM, Buonocore MH, Amaral DG (2001) Magnetic resonance imaging of the postmortem brain. J Autism Dev Disord 31:561–568

Simms ML, Kemper TL, Timbie CM, Bauman ML, Blatt GJ (2009) The anterior cingulate cortex in autism: heterogeneity of qualitative and quantitative cytoarchitectonic features suggests possible subgroups. Acta Neuropathol 118:673–684

Soto-Ares G, Delmaire C, Deries B, ValleeL, Pruvo JP (2000) Cerebellar cortical dysplasia: MR findings in a complex entity. AJNR 21:1511–1519

Sofroniew MV, Vinters HV (2010) Astrocytes: biology and pathology. Acta Neuropathol 119:7–35

Szentágothai J, Arbib MA (1975) Conceptual models of neural organization. MIT Press, Cambridge

Thevarkunnel S, Martchek MA, Kemper TL, Bauman ML, Blatt GJ (2004) A neuroanatomical study of the brainstem nuclei in autism. Abstr Soc Neurosci 1028.10

Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA (2005) Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann Neurol 57:67–81

Von Bonin G Mehler W (1971) On columnar arrangement of nerve cells in cerebral cortex. Brain Res 27:1–9

Von Economo CF, Koskinas GN (1925) Die Cytoarchitektonik der Hirnrinde des erwachsenen Menschen. Springer, Wien

Wegiel J, Kuchna I, Nowicki K et al (2010) The neuropathology of autism: defects of neurogenesis and neuronal migration, and dysplastic changes. Acta Neuropathol 119:755–770

Weidenheim KM, Goodman L, Dickson DW, Gillberg C, Rastam M, Rapin I (2001) Etiology and pathophysiology of autistic behavior: clues from two cases with an unusual variant of neuroaxonal dystrophy. J Child Neurol 16:809–819

Whitney ER, Kemper TL, Bauman ML, Rosene DL, Blatt GJ (2008) Cerebellar Purkinje cells are reduced in a subpopulation of autistic brains: a stereological experiment using calbindin-D28K. Cerebellum 7:406–416

Williams RS, Hauser SI, Purpura DP, DeLong GR, Swisher CN (1980) Autism and mental retardation: neuropathologic studies performed in four retarded persons with autistic behavior. Arch Neurol 37:749–753

Yates AJ, Thelmo W, Pappius HM (1975) Postmortem changes in the chemistry and histology of normal and edematous brains. Am J Pathol 79:555–564

Zikopoulos B, Barbas H (2010) Changes in prefrontal axons may disrupt the network in autism. J Neurosci 30:14595–14609

Chapter 9
Pharmacology of Autism Spectrum Disorder

Donald E. Greydanus, Gabriel Kaplan and Dilip R. Patel

Abstract ASD is a complex and common neurobehavioral disorder with no identified single specific biologic etiology. Instead there appear to be diverse abnormalities of brain networks with cellular, biochemical, and genetic factors. Though behavioral therapy is a major component to management of individuals with ASD, psychopharmacology has emerged as an important therapeutic tool. Pharmacology is targeted at associated disorders such as sleep disorders, aggression, anxiety, depression, repetitive behaviors, and hyperactivity. This chapter reviews current agents noted by research trials to be of benefit. Additional medications under current research are also briefly noted.

Keywords Pharmacology · ASD · SSRIs · Antipsychotics · Mood Stabilizers

Autism spectrum disorder (ASD) is a complex neurobehavioral entity that requires a multi-disciplinary and multi-level management approach (Silver and Rapin 2012). Research shows that children with ASD have abnormalities at the brain network level as well as cellular (i.e., cerebellar Purkinje cells) and biochemical abnormalities. Brain network abnormalities include excess or redundant connections in neighboring central nervous system (CNS) regions at the expense of other long-distance connections. Biochemical dysfunctions are identified in neurotransmitter levels, immune system function, and reaction to drugs. A variety of etiologies have been proposed which share elaborate genetic and molecular systems of considerable heterogeneity as well as environmental influence (Tuchman 2013; Tchaconas and Adesman 2013; Butler et al. 2012).

D. E. Greydanus (✉) · D. R. Patel
Department of Pediatric & Adolescent Medicine, Western Michigan University School of Medicine, 1000 Oakland Drive, D48G, Kalamazoo, 49008-1284 MI, USA
e-mail: donald.greydanus@med.wmich.edu

D. R. Patel
e-mail: dilip.patel@med.wmich.edu

G. Kaplan
Rutgers New Jersey Medical School, 535 Morris Avenue, Springfield, 07081 NJ, USA
e-mail: drgkaplan@gmail.com

S. Hossein Fatemi (ed.), *The Molecular Basis of Autism,*
Contemporary Clinical Neuroscience, DOI 10.1007/978-1-4939-2190-4_9

The diagnostic criteria for ASD were significantly revised in the recently published Fifth Edition of the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association 2013). An advanced release of proposed new criteria elicited considerable controversy amongst lay groups because of the concern that some patients would not meet the new diagnostic parameters and would thus lose benefit eligibility (Weblink #1 n. d.; Weblink #2 n. d.). On the other hand, initial studies appear to show that most patients would still be diagnosed with ASD (Huerta et al. 2012). At any rate, since there is no unique biologic etiology known at this time, there is no specific biologic treatment that is available to improve the core features of ASD. There are, however, ASD associated features that can be improved to a variable and individual extent by utilizing pharmacologic agents. Although such agents are not indicated as first line treatment options, they play an important role in the management of selected patients who have failed behavioral approaches (Kaplan and McCracken 2012).

Current pharmacologic management involves utilization of mediations to target the following symptoms: *sleep dysfunction (sleep disorders), aggression, repetitive behaviors, hyperactivity, depression,* and/or *anxiety*. Careful initial and on-going assessments are needed to identify these symptoms as well as co-morbid disorders, understand what impact they have on the individual, and identify any potential drug/drug interactions with other medications the patient may be on. It is also important to carefully manage other disorders that may be co-occurring such as epilepsy, sleep disorders and ADHD (Silver and Rapin 2012; Robinson 2012; Taurines et al. 2012). As research remains limited especially with regard to the optimal medications for adolescents and young adult populations with ASD (Dove et al. 2012), it is hoped that advances in pharmacology in the twenty-first century will lead to new and improved options. The aim of this chapter is to review medications that are available at this time.

9.1 Sleep Disorders

The brain of *Homo sapiens* is a complex and wondrous structure of biology that has evolved over millions of years (Bullock et al. 2005). It contains over 100 billion neurons (100×10^{12}) and over 10,000 synapses per neuron ($> 10^{18}$ synapses). The human brain has about 100,000 km of fibers, 1 trillion or more glial cells and holds equivalent of 1.25 terabytes (TB) of digital information. As this highly complex system evolved to allow modern human existence, three specific stages of consciousness emerged: wake, non-REM (rapid eye movement) sleep, and REM sleep (Greydanus et al. 2008). Even though the brain is very active during deep sleep, it requires many hours of REM-sleep (rapid-eye-movement sleep phase) every 24 h to function optimally during the awake or non-sleep phase of life. This is true of every human being regardless of medical conditions that may be present including ASD.

Table 9.1 lists the normal sleep requirement for pediatric individuals from newborns to adolescents. There is considerable variability from person to person but all

Table 9.1 Normal sleep requirements per 24 h of pediatric patients. (Tuchman 2013)

Newborn: 16–20 h
Infants: 14–15 h
Toddlers (1–3 years): 12 h
Pre-school (3–5 years): 11–12 h
School age (6 to 12 years): 10–11 h
Adolescents: 9 h

Table 9.2 Partial list of sleep disorders. (Tuchman 2013; Tchaconas and Adesman 2013)

Insomnia
Excessive daytime sleepiness
Narcolepsy
Klein-Levin Syndrome
Post-traumatic hypersomnolence
Obstructive sleep apnea (OSA) or obstructive sleep apnea Syndrome
Parasomnias
Rhythmic movement disorders (i.e., head banging, head rolling, body rocking)
Bruxism
Sleep talking
Sleep walking (somnambulism)
Nightmares
Sleep terrors (pavor nocturnus)
Nocturnal enuresis
Restless Leg Syndrome (Periodic limb movement disorder)
Others

must confirm to their personal sleep requirements for maximum physical and emotional health. As sleep science has emerged over the past few decades, it has become understood that many people do not meet their proper sleep requirement and this includes children and adolescents (Chhangani et al. 2011). Many pediatric patients have sleep disorders or dysfunction (Table 9.2) that complicates their overall health.

A variety of sleep disorders can be found in children as well as adolescents with ASD (Kotagal and Broomall 2012; Richdale 1999; Cortesi et al. 2010; Bruni 2007; Paavonen et al. 2008; Young et al. 2007). Sleep disorders in this population can be present in up to 83 % of adolescents with ASD (Bullock et al. 2005) and can be due to a number of behavioral and biological factors. *Behavioral* factors involved with sleep dysfunction include poor sleep hygiene in which the individual does not learn to establish a regular and normal sleep cycle. Heightened sensitization to environmental stimuli may be found in those with ASD (Kotagal and Broomall 2012).

There can also be *circadian* or biologic factors causing sleep dysfunction which can involve the CNS master clock that is identified as the suprachiasmatic nucleus

of the anterior hypothalamus (Silver and Rapin 2012). Abnormal melatonin secretion may be found in some individuals with ASD who have sleep dysfunction (Silver and Rapin 2012; Kotagal and Broomall 2012). Sleep problems are also common in conditions comorbid with ASD such as ADHD, mood disorders, anxiety disorders, epilepsy, and other developmental disorders (Kotagal and Broomall 2012).

Medications themselves may be a cause of sleep dysfunction and these include anticonvulsants, antihistamines, antidepressants (i.e.,selective serotonin reuptake inhibitors [SSRIs] or tricyclic antidepressants [TCAs],corticosteroids, opioids, and stimulants). Some induce daytime sedation (i.e., anticonvulsants, antihistamines, or opioids) while others have stimulant effects with resultant insomnia (i.e., stimulants, corticosteroids). SSRIs can produce activating effects with sleep interruption. Insomnia due to delayed sleep onset can be found with use of alcohol or nicotine (tobacco).

A thorough sleep disorder assessment is needed to correctly identify what sleep dysfunction (s) is occurring. It is important to identify what medication (s) the child or adolescent is taking and what role such agents may be having in causing or worsening underlying sleep dysfunction. A formal sleep study may be useful including a diagnostic nocturnal polysomnogram. The next step is the application of behavioral interventions seeking to improve any sleep dysfunction that is identified (Greydanus et al. 2008; Chhangani et al. 2011; Kotagal and Broomall 2012). Improvement of altered sleep hygiene and/or co-morbid sleep disorders is an important step in biological management of this complex patient.

There are no current FDA-guidelines available for specific guidance in pharmacologic management of children with ASD who have co-morbid sleep disorders. Insomnia is the most common sleep disorder that is found and some recent research suggests that melatonin is safe and effective in doses up to 6 mg per day (Kaplan and McCracken 2012; Anderson et al. 2008; Tordjman et al. 2013). Table 9.3 lists various pharmacologic agents that have been used in the treatment of insomnia in pediatric patients; each agent has side effects and must be very cautiously prescribed if melatonin is not beneficial (Chhangani et al. 2011).

Tables 9.4 and 9.5 outline pharmacologic agents used in pediatric patients in general who have a sleep disorder. Table 9.6 lists agents that may be useful in

Table 9.3 Medications used for the management of insomnia. (Tchaconas and Adesman 2013)

Melatonin
Melatonin agonist (ramelteon)
Alpha-2 agonists (clonidine)
Antihistamines (diphenhydramine, doxylamine)
Chloral hydrate
Tricyclic antidepressants (amitriptyline, nortriptyline, doxepine)
Other antidepressants (trazodone, mirtazapine)
Benzodiazepines (temazepam, triazolam, flurazepam, estazolam, quazepam, clonazepam, lorazepam)
Nonbenzodiazepines (zolpidem, zalepon, eszopiclone)

Table 9.4 Medications used to manage narcolepsy: Daytime sleepiness

Class	Agent	Dose	Side effects
Psychostimulants	Methylphenidate (MPH) Mixed amphetamines Dextroamphetamine	10–60 mg/d; start with 5–10 mg 2x/d; no more than 20 mg in a single dose or 60–80 mg/d; dosing can be a single AM dose or 3x/d. Dose similar to MPH dose	Both MPH and amphetamines: insomnia, reduced appetite, loss of weight, abdominal pain, headache, depression, rebound symptoms, FDA black box warning on sudden death; tolerance; controlled Scheduled II substance with abuse risk; others
Wakefulness promoting agent (long-acting)	Modafinil	Start with 100–200 mg once a day in the morning; some need a morning and noon dose; maximum dose is 400 mg/d	Not a controlled drug; FDA approved for narcolepsy > 14 years of age; headache, nausea, nervousness, rhinitis, diarrhea, back pain, insomnia, dizziness, dyspepsia

Used with permission from Greydanus et al. 2008, pp. 199–222

Table 9.5 Medications used to manage narcolepsy: cataplexy, sleep paralysis, hypnagogic hallucinations

Class	Agent	Oral dose	Side effects
Selective serotonin reuptake inhibitors	Fluoxetine	10–20 mg/d (up to 60 mg/d)	Insomnia, headache, nausea
Other antidepressants	Venlafaxine	37.5 to 150 mg/d	Sedation, dry mouth, headache, nausea
Tricyclic antidepressants	Imipramine Protriptyline Clomipramine	50–200 mg/d 10–40 mg/d 25–50 mg/d	Confusion, constipation, dizziness, sedation, dry mouth, tremor, urinary retention, weight gain
Benzodiazepines	Clonazepam	0.25 mg to 2 mg orally at night	Adverse effects: sedation, ataxia, confusion; if stopped too soon: rebound reactions. Schedule IV controlled substance
Miscellaneous	Sodium oxybate	6–9 g/d divided in 2 equal doses	FDA-approved for cataplexy; difficult to obtain unless one is a sleep expert; sedation, headache, nausea, dizziness, high abuse potential

Used with permission from Greydanus et al. 2008, pp. 199–222

Table 9.6 Sedating medications used at bedtime to manage insomnia in ADHD (given orally)

Alpha-2-agonists: Clonidine (0.1–0.3 mg)
Tricyclic antidepressants: Imipramine (50–75 mg)
Other antidepressants (Trazodone: 25–50 mg)
Exogenous melatonin (3–6 mg)
SSRIs: Paroxetine (20–30 mg)
Mirtazapine: (7.5–15 mg)

Table 9.7 Four essential criteria for restless leg syndrome: URGE

1.	***U***: **u**rge to move legs due to unpleasant sensations
2.	***R***: worsening during periods of **r**est
3.	***G*****: g**ets better with movement
4.	***E***: worse in the **e**vening

those with comorbid ADHD. As already noted, one should be aware of potential adverse effects of these or any medications prescribed to patients with ASD and sleep disorders because these patients have heightened reactions to use of pharmacologic agents.

Obstructive sleep apnea and restless leg syndrome (RLS) may occur in children with ASD though their precise prevalence is not known at this time (Miano and Ferri 2010). However, the presence of these two medical conditions that can result in sleep disturbances should be evaluated as there are some additional therapeutic options. Obstructive sleep apnea (OSA) has a prevalence of 2–5 % in children and is characterized by periodic episodes of upper-airway obstruction (partial or complete) that leads to hypoxia, hypercapnea, and sleep dysfunction (Chhangani et al. 2011; Greydanus et al. 2008). There is limited pharmacologic management of OSA and therapy usually utilizes adenotonsillectomy and if necessary, nasal continuous positive airway pressure (CPAP) (Chhangani et al. 2011; Greydanus et al. 2008).

RLS (Table 9.7) can be difficult to manage in children and adolescents. Treatment in pediatric patients is based on watchful waiting in mild conditions and seeking to improve sleep hygiene as well as avoid sleep deprivation. Factors which worsen the RLS should be removed or reduced and these include SSRIs, dopamine antagonists, antihistamines, caffeine, nicotine, and alcohol (Chhangani et al. 2011; Greydanus et al. 2008). The affected areas (i.e., as lower leg muscles) can be massaged along with application of hot or cold packs while moderate exercise may also be helpful. Pharmacologic agents used in adults with RLS include iron supplementation, opioids, anticonvulsants, benzodiazepines, and dopaminergic agents (Robinson 2012).

9.2 Aggression

Perhaps the symptom eliciting the most concern in children or adolescents with ASD is that of *aggression* and other dangerous behavior that continues despite the implementation of behavioral management approaches (Kaplan and McCracken 2012; Nazeer 2011). Aggression is theorized to involve serotonergic, adrenergic, dopaminergic, and opioid systems in the human being (Nazeer 2011) and its impact is a common and potentially serious issue in those with ASD as revealed in research. For example, in a review of 1380 patients with ASD, aggression to caregivers was described in 68 % and in 49 % to others (i.e., non-caregivers) (Nazeer 2011). Pharmacologic therapy is initiated in attempts to biologically ameliorate

Table 9.8 Pharmacologic agents used to manage aggression in pediatric patients with ASD

Antipsychotics
a. Haloperidol
b. Risperidone
c. Aripiprazole
d. Olanzapine
e. Ziprasidone
Methylphenidate
Divalproex
Naltrexone

patterns of aggression, severe irritability, and self-injury. Use of these agents, listed in Table 9.8, should always be combined with intensive behavioral therapy.

9.2.1 Antipsychotics

Initial research with antipsychotics for aggressive children with ASD involved the use of haloperidol which demonstrated positive effects that were significantly better than placebo (Campbell et al. 1978; Mandell et al. 2008). Aggressive Behavior was improved along with such issues as negativity, lability of affect, and angry affect. Haloperidol is a classic antipsychotic with a high degree of negative effects including unacceptable sedation along with one-third developing dystonias and withdrawal dyskinesias. Use of these older (first generation) antipsychotics has been replaced by use of the newer (second generation) atypical antipsychotics which may have a lower incidence of tardive dyskinesia (Anderson et al. 1984; Campbell et al. 1997; Posey et al. 2008; Caccia 2013). Research has mainly focused on aripiprazole and risperidone (Nazeer 2011).

Regarding aripiprazole, one controlled study of patients 6 to 17 years of age with ASD noted that severe aggression and irritability were significantly improved over placebo using three doses: 5, 10, and 15 mg per day (Marcus et al. 2009). Another research study of this same age and diagnosis cohort also found improvement over 8 weeks that reached a gradually increasing dose with a mean of 8.6 mg per day at the end of an eight week study (Owen et al. 2009). Both studies noted adverse effects of sedation as well as weight gain and the 8 week study also noted the presence of extrapyramidal symptoms (Owen et al. 2009). In 2009 the US Food and Drug Administration (FDA) approved the use of aripiprazole for those with ASD who are 6 to 17 years of age and had considerable irritability. Clinicians generally use a maintenance dose between 5 and 15 mg per day (Kaplan and McCracken 2012).

The United States NIMH RUPP ASD Network studied risperidone and concluded that a mean dose of 2.08 mg/day provided significant reduction in aggression (including self-injury and severe tantrums) in their study of 101 pediatric patients with

Table 9.9 Potential side effects of atypical antipsychotics

Allergic reactions (including rash)
Cardiovascular adverse effects
Hyperglycemia
Hyperlipidemia
Hyperprolactinemia (with galactorrhea)
Lethargy
Neuroleptic malignant syndrome
Parkinsonism
Sedation
Tardive dyskinesia
Weight gain

ASD aged 5 to 17 years of age (McCracken et al. 2002; RUPP[a] 2005a). A relapse of these symptoms occurred in most if the medication was stopped at 6 months of age and use of the medication resulted in a significant weight gain of 5.6 kg over 6 months. No evidence of tardive dyskinesia or dystonia was seen in this research. An added benefit of the use of atypical antipsychotics in children with ASD is a significant reduction in hyperactivity (*vida infra*) (McCracken et al. 2002; Sharma and Shaw 2012).

A meta-analysis of post-2000 RCT studies using risperidone in patients with ASD revealed a large mean-effect size of 1.21 (Sharma and Shaw 2012). In 2006 the US FDA approved it in those with ASD aged 5 to 17 years of age for the management of ASD irritability that includes rapidly changing moods, temper tantrums, deliberate self-injury, and aggression; the maximum daily dose was 3 mg. It is not clear how long to place these children and adolescents on atypical antipsychotics but positive benefits may be seen for 6 to 12 months (Kaplan and McCracken 2012).

Observing for potential side effects (Table 9.9) is important and this includes monitoring each visit for such adverse effects as sedation, lethargy, and weight gain. The clinician should monitor every 6 months for the development of tardive dyskinesia as well as Parkinsonism; also, one should order laboratory studies every 6 months for screening of potential hyperglycemia and hyperlipidemia (Kaplan and McCracken 2012). The Abnormal Involuntary Movement Scale (AIMS) can be used to monitor abnormal movement development (Guy 1976). The clinician can also screen every few visits for symptoms of hyperprolactinemia (i.e., galactorrhea, gynecomastia, menstrual irregularity, sexual dysfunction) and obtain a prolactin level if indicated (Roke et al. 2012). The pharmacokinetics of these agents can be unique and complex for children and adolescents based on individualized factors including demographic variants, phenotype, and drug interactions (Caccia 2013).

Research is limited in the effectiveness of other atypical antipsychotics but there is some early evidence that olanzapine and ziprasidone may be helpful while quetiapine has not been shown to be helpful in ASD children in terms of amelioration of aggression and related symptoms (Potenza et al. 1999; Malone et al. 2007; Martin

et al. 1999). Research is seeking novel drugs for management of ASD that have improved drug profiles over atypical antipsychotics (Politte and McDougle 2014). However, antipsychotics remain a commonly used class of psychotropic drugs used in the management of children and adolescents with ASD (Schubart et al. 2013).

9.2.2 Stimulants

In addition to a well-researched role of methylphenidate (MPH) for improvement of hyperactivity and attention span dysfunction in those with ADHD, there is some limited research suggesting that MPH can improve symptoms of aggression in children aged 5 to 11 years with ASD (Handen et al. 2000; Quintana et al. 1995). These studies are small and of limited duration; one noted an increase in unacceptable adverse effects from MPH which included abnormal movement, agitation, and changes of mood (Handen et al. 2000). Thus, the role of MPH is not well established and is considered in selective situations such as when aggression is associated with impulsivity and hyperactivity (Nazeer 2011). There is no research evidence at this time for the use of amphetamines in those with ASD.

9.2.3 Divalproex

There is also limited research on the use of the anticonvulsant divalproex for ASD children or adolescents with aggression. One study has shown modest improvement in use of this mood stabilizer in 55 patients with ASD who were 5 to 17 years of age (Hollander et al. 2010). Limited side effects were recorded in these patients and those with benefit tended to have higher valproate blood levels than those who did not develop improvement in aggression. This chemical has a number of actions including enhancing neurotransmission of gamma-amino-butyric acid (GABA), blocking voltage-gated sodium channels as well as T-type calcium channels, and also serving as a histone deacetylase inhibitor. Increased appetite, liver dysfunction, and blood dyscrasias can develop with this agent. Divalproex is not approved by the US FDA for this indication. Other mood stabilizers under research include lamotrigine and levetiracetam (Nazeer 2011).

9.2.4 Others

Limited research has noted some positive response in pediatric patients with ASD and aggression who were placed on clonidine (alpha-2 adrenergic agonist) and naltrexone (opioid receptor antagonist) (McCracken et al. 2002; Parikh et al. 2008). These agents are not FDA approved for this indication and more research is needed in this regard.

9.3 Repetitive Behaviors

A classic feature of ASD is the presence of restricted repetitive and stereotyped behaviors such as rocking and other limb movements. When severe, these can interfere with activities and may necessitate treatment. Though behavior therapies are the first line of management, pharmacologic agents may be necessary for these severe restricted repetitive behaviors (RRBs) not responding to such therapy. Agents which have been used include selective serotonin reuptake inhibitors (SSRIs), atypical antipsychotics, and divalproex (Kaplan and McCracken 2012; Nazeer 2011). Medications may not be beneficial or side effects may not be tolerated in these individuals.

9.3.1 Atypical Antipsychotics

In addition to revealing benefit in aggressive behavior for children and adolescents with ASD, the United States NIHM RUPP ASD Network also demonstrated some reduction in RRBs with use of risperidone (McDougle et al. 2005). Aripiprazole has also shown an effect on RRBs as well (Marcus et al. 2009; Owen et al. 2009).

9.3.2 Selective Serotonin Reuptake Inhibitors (SSRIs)

The complexity of ASD is indicated by the failure of research studies to show RRBs reduction in patients placed on SSRIs despite the identified serotonin dysfunction found in ASD and despite the known improvement in obsessions as well as compulsions seen in other patients on SSRIs who have anxiety disorders (Kaplan and McCracken 2012). There are anectodal reports of improvement of RRBs in those with ASD on SSRIs and SSRIs are the most common type of psychopharmacologic agents prescribed for these patients (Mandell et al. 2008). One research study of fluoxetine (mean dose of 9.9 mg per day) demonstrated improvement in RRBs over placebo in 39 children aged 5 to 16 years of age (Hollander et al. 2005) while a larger trial of fluoxetine using 158 individuals aged 5 to 17 years of age did not demonstrate improvement (Weblink #3 n. d.).

A study of citalopram (using doses from 2.5 mg per day to a maximum dose of 20 mg per day) did not show reduction in RRBs in 149 patients aged 5 to 17 years of age; also one third developed serotonergic activation-type adverse effects that included insomnia, mood changes, and increased activity (King et al. 2009). An open label study using escitalopram revealed some benefit in RRB reduction (Owley et al. 2005). While some studies found that some ASD patients derive benefit from SSRIs with careful titration and monitoring (Kaplan and McCracken 2012), a RCT meta-analysis concluded the contrary (Williams et al. 2013). Some have suggested the use of mirtazapine which is a noradrenergic and specific serotonergic antidepressant (Nazeer 2011).

9.3.3 *Divalproex Sodium*

One study of 13 persons with ASD taking divalproex sodium over 8 weeks in a double-blind, placebo-controlled trial revealed significant lowering of RRBs in contrast to placebo (Hollander et al. 2006). Though encouraging, more confirmatory research is needed to consider or recommend this medication for management of RRBs in these patients.

9.4 Hyperactivity

Hyperactivity is a common symptom seen in children with ASD and research notes that between one-fourth and three fourths of these pediatric patients can be diagnosed with co-morbid attention deficit hyperactivity disorder (ADHD) (McCracken 2010; Murray 2010; Antshel et al. 2013). While the presence of hyperactive symptoms was long noted in ASD, previous DSM criteria did not allow for coding comorbid ADHD even when patients met full criteria, a dilemma that was resolved in DSM-5 (American Psychiatric Association 2013). ADHD is a neurobehavioral disorder with abnormalities in various neurotransmitter systems, particularly the dopaminergic and noradrenergic (Greydanus et al. 2013). At least 7 genes are involved in ADHD: DRD4, DRD5, DAT, DBH, 5-HTT, HTR1B, and SNAP25 (Greydanus et al. 2013). Table 9.10 lists medications that have been utilized to control various features of ADHD (Greydanus et al. 2003, 2007). The combination of ADHD and ASD represents a challenging clinical conundrum in which adverse effects to medications may be heightened and response to pharmacologic agents may be suboptimal (Nazeer 2011).

9.4.1 *Stimulants*

Hundreds of randomized, controlled, research studies have revealed that stimulant medications can improve the concentration ability of 75–95% of children, adolescents and adults with ADD/ADHD (Greydanus et al. 2003,2007). Surveys suggested that approximately 6–8% of those between 5 and 15 years of age in the United States take stimulant medication for ADHD (Greydanus et al. 2003). Stimulants

Table 9.10 Medications to manage hyperactivity and ADHD in children and adolescents with autism spectrum disorder

Stimulants (methylphenidate [MPH] and amphetamine)
Atomoxetine
Atypical Antipsychotics
Alpha-2 agonists (clonidine and guanfacine)

(i.e., methylphenidate [MPH] and amphetamines) are the third most commonly used class of pharmacologic agents used in management of children with ASD and MPH has been the most commonly prescribed stimulant agent in this situation (Mandell et al. 2008).

MPH is a schedule II medication first introduced in 1957. Its beneficial effect on attention dysfunction is related to an increase in extracellular dopamine in the central nervous system (CNS) due to selective binding of the presynaptic dopamine transporter in the striatal and prefrontal areas of the CNS; there is also a blockade of the norepinephrine transporter in the CNS (Greydanus et al. 2003, 2007).

Stimulants are more effective in managing children with ADHD alone than when it presents in co-morbidity with ASD. Some studies noted that stimulants are not effective in children with ASD and some controversy exists in this regard (Kaplan and McCracken 2012). However, the NIMH RUPP ASD Network conducted the MPH RCT study in 195 patients aged 5–13 years with ASD who were placed on MPH (immediate release) (RUPP 2005b). Three MPH doses were studied: 0.15, 0.25 and 0.5 mg/kg; reduction of hyperactivity and impulsiveness were noted in these children on these doses that are lower than typically used for non-ASD children with ADHD (RUPP 2005b; Paykina and Greenhill 2008).

Side effects of stimulants are noted in Table 9.11 and in this RUPP study, 18% had the MPH stopped because of irritability (RUPP 2005b). Table 9.12 notes contraindications to stimulants.

9.4.2 Atomoxetine

Atomoxetine is a non-stimulant medication that is a norepinephrine reuptake inhibitor without primary affects on dopamine levels. It was released in January of 2003 and is available as 10, 18, 25, 40 and 60 mg capsules. Atomoxetine is FDA-approved for children (6 years and up), adolescents, and adults with ADHD (Greydanus et al. 2003). Table 9.13 lists side effects of this medication. In 2005, the FDA issued a black box warning for atomoxetine regarding an increased risk for suicidal thinking in children and adolescents and asked that a Patient Medication Guide (MediGuide) be provided to patients prescribed this medication to alert consumers to this potential risk (Greydanus et al. 2003).Children and adolescents placed on this medication should be carefully observed for such features as agitation, irritability, suicidal thinking or behaviors, and unusual changes in behavior especially during the initial few months of starting the drug or at times of dosage change (increase or decrease).

Atomoxetine has been infrequently researched with respect to children with ASD. In one study of 16 children aged 5 to 15 years who were treated with doses of 1.2 to 1.4 mg/kg/day (not exceeding 100 mg/day) over 6 weeks, there was evidence of improvement in hyperactivity over placebo (Arnold et al. 2006). This study, though concluding the benefit was similar to that seen with MPH in the RUPP study, also allowed the addition of other psychotropics (RUPP 2005b; Arnold et al. 2006). In

Table 9.11 Potential side effects of Methylphenidate[a]

Headache[a]
Abdominal pain[a]
Jitteriness[a]
Irritability (including moodiness)[a]
Dizziness
Anorexia[a]
Insomnia (delayed onset of sleep)[a]
Social withdrawal[a]
Weight loss (due to decreased appetite)[a]
Nausea
Dry mouth
Constipation
Increase in heart rate, blood pressure and palpitations
"Unmasking" of Tourette's syndrome
Appearance of being "dazed or drugged"; perseveration and withdrawal
Rebound phenomenon
Increased hyperactivity
Appearance of psychosis or psychotic features
Personality change
Tolerance
Skin rash (rare)

[a] Most Common

Table 9.12 Contraindications to stimulant use

Overt sensitivity to these medications
Psychosis
Hyperthyroidism
Drug dependence
Uncontrolled hypertension
Symptomatic cardiovascular disease
Glaucoma
Concomitant use with monoamine oxidase inhibitors

a more recent study of 88 patients 6–17 years of age with ADHD and ASD placed on 1.2 mg/kg/day of atomoxetine for 20 weeks, there were significant reductions in inattention and hyperactivity-impulsivity; adverse events were described as mild, and included nausea as well as fatigue, and tended to lessen over time (Harfterkamp et al. 2013). Studies are increasingly demonstrating reduction in ADHD symptoms in children with ASD following treatment with atomoxetine (Eugene 2012).

Table 9.13 Side effects of atomoxetine

Anorexia
Constipation
Dizziness
Dry mouth
Dyspepsia
Emesis
Fatigue
Heightened pulse and blood pressure
Mood swings (FDA Black Box warning on increased suicidal thinking in children and adolescents)
Nausea up to several weeks
Sedation or insomnia
Sexual dysfunction
Voiding difficulty

9.4.3 Alpha-2-Agonists

Clonidine and guanfacine are alpha$_2$-adrenergic agonists that have been used off label for ADHD for years until recent slow release formulations of each agent (Kapvay and Intuniv) were approved by the FDA to treat ADHD (Kaplan 2012). While there are ample randomized controlled data showing the benefits of alpha$_2$-agonists in ADHD management, research in those with ASD is more limited but encouraging (Jaselskis et al. 1992; Scahill et al. 2006). Table 9.14 notes potential side effects of alpha-2-agonists.

9.4.4 Atypical antipsychotics (AAs)

The limited research on the use of atypical antipsychotics in children with ASD suggests some atypicals (i.e., risperidone and aripiprazole) can result in significant reduction in hyperactivity in these patients (Owen et al. 2009; McCracken et al. 2002). Atypical antipsychotics may be particularly useful in those with aggression and stimulant-resistant hyperactivity (Nazeer 2011).

9.5 Depression and Anxiety

Though clinicians often observe symptoms of dysphoria and anxiety in children and adolescents with ASD, there remains limited research in use of antidepressants and anxiolytics in these pediatric patients. Research evidence for the benefit of

Table 9.14 Side effects of clonidine

Sedation (50%)
Dry mouth
Headache
Attention impairment
Decreased glucose tolerance
Depression
Dermatitis (from the patch)
Dizziness
Dysphoria
Fatigue
Inconsistent effects
Irritability
Itchy eyes
Postural hypotension
Potentiation of neuroleptic anticholinergic side effects
Rebound Phenomenon
Tolerance
Weight gain
Withdrawal effects (rebound tachycardia and severe hypertension from sudden clonidine cessation)
Worsening of pre-existing cardiac arrhythmias

pharmacology (i.e., SSRIs) in non-ASD pediatric patients with anxiety disorders in general is strong (Ipser et al. 2009). Research on adults with ASD has shown some benefit in reduction of anxiety from use of SSRIs (Fatemi et al. 1998; McDougle et al. 1996). Few studies in children and adolescents with ASD and anxiety have been done. Clinicians have little guidance in this population. Some are concerned with the potential of adverse effects from SSRIs in children with ASD that include increased hyperactivity, somatic complaints, and disinhibition (Fatemi et al. 1998; McDougle et al. 1996).

The benefits of antidepressants for depressed non-ASD patients is abundantly supported in the literature (Rush and Nirenmberg 2009). Recent safety concerns, however, led the FDA to mandate a black box warning regarding the appearance of suicidal ideas in children, adolescents, and young adults. This action fostered significant controversy (Kaplan 2013) as many were concerned about the risks of undertreatment. In addition to the dearth of research supporting the use of antidepressants in ASD (Williams et al. 2013), the potential for self-harm ideation requires that clinicians carefully evaluate the risk/benefit ratio on a case by case basis.

9.6 Potential Future Agents

When pharmacotherapy is initiated after failure of behavioral approaches, atypical antipsychotics are the current first-line pharmacologic agent for irritability and other bothersome symptoms of children and adolescents with ASD. These agents have limited effectiveness and severe side effect risks. For this reason, research is seeking medications with improved outcomes and improved safety (Politte and McDougle 2014). Novel lines of research, for example, are looking at the oxytocin system which is involved in social development in addition to agents that target cholinergic and glutamatergic receptors (Farmer et al. 2013; Tachibana et al. 2013; Anagnostou et al. 2012). Regarding depression, a recent trial of eleven adolescents with ASD using reboxetine (norepinephrine reuptake inhibitor) noted significant but modest reduction in depression and ADHD symptoms; ninety percent reported what was described as tolerable adverse effects (Golubchik et al. 2013).

Research is also reviewing the potential benefit of memantine and D-cycloserine for social impairment seen in ASD (Doyle and McDougle 2012). Memantine is the first in a new class of medications for Alzheimer's disease that acts on the glutamatergic system with a mechanism that blocks NMDA-type (N-methyl D-aspartate) glutamate receptors (Hosenbocus and Chahai 2013). Cyclosporine is an antibiotic used to treat infection with *Mycoplasma tuberculosis*; it is a partial agonist of the neuronal NMDA receptor for glutamate. Another antibiotic, minocycline, is being studied for benefit in those with ASD, because it easily crosses the blood brain barrier and has salutary effects on CNS neuroinflammation, microglial activation, and neuroprotection of diverse neurological conditions (Pardo et al. 2013).

Finally, a significant body of new findings in the pathophysiology of Fragile X Syndrome, a leading cause of autism, has resulted in the development of several promising candidates. Of interest are those affecting the gamma-aminobutyric acid (GABA) and glutamate pathways. Several receptor modulators have been identified among which are arbaclofen (GABA agonist), fenobam (mGluR5 antagonist) (Healy et al. 2011) and ganaxolone (GABA modulator) (Weblink #4 n. d.).

9.7 Conclusions

Though behavioral treatments are often the mainstay of management principles for children and adolescents with ASD, psychotropic therapy has become very common when psychosocial options fail. In a recent review of 2853 individuals with ASD aged 2 to 17 years, 27% were taking one or more psychotropic medication (s); in this survey, 15% were on one medication, 7.4% were on two, and 4.5% were taking three or more agents (Coury et al. 2012). Medications are mostly used to treat associated features such as sleep disorders, aggression, uncontrollable repetitive behaviors, depression, anxiety, and hyperactivity. The advantages of current agents

are limited by the significant risk of adverse effects. Therefore, more research is needed to identify the safest and most effective psychotropic medications in pediatric and adult patients with ASD.

References

American Psychiatric Association (2013) Diagnostic and statistical manual of mental disorders, 5th edn. American Psychiatric Association, Washington, DC

Anagnostou E, Soorya L, Chaplin W, Bartz J, Halpern D, Wasserman S, Wang AT, Pepa L, Tanel N, Kushki A, Hollander E (2012) Intranasal oxytocin versus placebo in the treatment of adults with autism spectrum disorders: a randomized controlled trial. Mol Autism 3:6–21

Anderson LT, Campbell M, Grega DM, Perry R, Small AM, Green WH (1984) Haloperidol in the treatment of infantile autism: effects on learning and behavioral symptoms. Am J Psychiatry 141:1195–1202

Anderson IM, Kaczmarska J, McGrew S (2008) Melatonin for insomnia in children with autism spectrum disorders. J Child Neurol 23:482–485

Arnold LE, Aman MG, Cook AM, Witwer AN, Hall KL, Thompson S, Ramadan Y (2006) Atomoxetine for hyperactivity in autism spectrum disorders: placebo-controlled crossover pilot trial. J Am Acad Child Adolesc Psychiatry 45:196–205

Antshel KM, Zhang-James Y, Faraone SV (2013) The comorbidity of ADHD and autism spectrum disorder. Expert Rev Neurother 13:1117–1128

Bullock TH, Bennett MVL, Johnston D, Josephson R, Marder E, Fields RD (2005) The neuron doctrine, redux. Science 310:791–793

Butler MG, Youngs EL, Roberts JL, Hellings JA (2012) Assessment and treatment in autism spectrum disorders: a focus on genetics and psychiatry. Autism Res Treat 2012:1–11

Bruni OMS (2007) Sleep in children with autism spectrum disorder. Sleep Med 9:64–70

Caccia S (2013) Safety and pharmacokinetics of atypical antipsychotics in children and adolescents. Paediatr Drugs 15:217–233

Campbell M, Anderson LT, Meier M, Cohen IL, Small AM, Samit C, Sachar EJ (1978) A comparison of haloperidol and behavior therapy and their interaction in autistic children. J Am Acad Child Psychiatry 17:640–655

Campbell M, Armenteros J, Malone RP, Adams PB, Eisenberg ZW, Overall JE (1997) Neuroleptic-related dyskinesias in autistic children: a prospective, longitudinal study. J Am Acad Child Assoc Psychiatry 36:835–843

Chhangani B, Greydanus DE, Patel DR, Feucht C (2011) Pharmacology of sleep disorders in children and adolescents. Pediatr Clin N Am 58:273–291

Cortesi F, Giannotti F, Ivanenko A, Johnson K (2010) Sleep in children with autism spectrum disorder. Sleep Med 11:659–664

Coury DL, Anagnostou E, Manning-Courtney P, Reynolds A, Cole L, McCoy R, Whitaker A, Perrin JM (2012) Use of psychotropic medication in children and adolescents with autism spectrum disorders. Pediatrics 130(Suppl 2):S69–76

Dove D, Warrant Z, McPheeters ML, Taylor JL, Sathe NA, Veenstra-VanderWeele J (2012) Medications for adolescents and young adults with autism spectrum disorders: a systematic review. Pediatrics 130:717–726

Doyle CA, McDougle CJ (2012) Pharmacologic treatments for the behavioral symptoms associated with autism spectrum disorders across the lifespan. Dialogues Clin Neurosci 14:263–279

Eugene AL (2012) Children with hyperactivity & ASD research treatment study (CHARTS) consortium. Atomoxetine reduces ADHD symptoms in children with autism spectum disorder. Evid Based Ment Health 15:96–100

Farmer C, Thurm A, Grant P (2013) Pharmacotherapy for the core symptoms in autistic disorder: current status of research. Drugs 73:303–314

Fatemi SH, Realmuto GM, Khan L, Thuras P (1998) Fluoxetine in treatment of adolescent patients with autism: a longitudinal open trial. J Autism Dev Disord 28:303–307

Golubchik P, Sever J, Weizman A (2013) Reboxetine treatment for autistic spectrum disorder of pediatric patients with depressive and inattentive/hyperactive symptoms: an open-label trial. Clin Neuropharmacol 36:37–41

Greydanus DE, Pratt HD, Sloane MA, Rappley MD (2003) Attention-deficit/hyperactivity disorder in children and adolescents: interventions for a complex costly clinical conundrum. Pediatr Clin North Am 50:1049–1092

Greydanus DE, Pratt HD, Patel DR (2007) Attention deficit hyperactivity disorder across the lifespan. Dis Mon 53:65–132

Greydanus DE, Calles JL Jr, Patel DR (2008) Pediatric and adolescent psychopharmacology. Cambridge University Press, Cambridge, pp 199–222

Greydanus DE, Kaplan G, Antshel KM (2013) Attention deficit hyperactivity disorder: neuropsychologic and pharmacologic considerations. Int J Child Adolesc Health 6:22–30

Guy W (1976) ECDEU assessment manual for psychopharmacology. Department of Health, Education, and Welfare, Public Health Service, Alcohol, Drug Abuse, and Mental Health Administration, National Institute of Mental Health, Psychopharmacology Research Branch, Division of Extramural Research Programs, Washington, DC, pp 534–537

Handen BL, Johnson CR, Lubetsky M (2000) Efficacy of methylphenidate among children with autism and symptoms of attention-deficit hyperactivity disorder. J Autism Dev Disord 30:245–255

Harfterkamp M, Buitelaar JK, Minderaa RB, van de Loo-Neus G, van der Gaag RJ, Hoekstra PJ (2013) Long-term treatment with atomoxetine for attention-deficit/hyperactivity disorder symptoms in children and adolescents with autism spectrum disorder: an open-label extension study. J Child Adolesc Psychopharmacol 23:194–199

Healy A, Rush R, Ocain F (2011) Fragile X syndrome: an update on developing treatment modalities. ACS Chem Neurosci 17:402–410

Hosenbocus S, Chahai R (2013) Memantine: a review of possible uses in child and adolescent psychiatry. J Can Acad Child Adolesc Psychiatry 22:166–171

Hollander E, Phillips A, Chaplin W, Zagursky K, Novotny S, Wasserman S, Iyengar R (2005) A placebo controlled crossover trial of liquid fluoxetine on repetitive behaviors in childhood and adolescent autism. Neuropsychopharmacology 30:582–589

Hollander E, Soorya L, Wasserman S, Esposito K, Chaplin W, Anagnostou E (2006) Divalproex sodium vs placebo in the treatment of repetitive behaviors in autism spectrum disorder. Int J Neuropsychopharmacol 9:209–213

Hollander E, Chaplin W, Soorya L, Wasserman S, Novotny S, Rusoff J, Feirsen N, Pepa L, Anagnostou E (2010) Divalproex sodium vs placebo for the treatment of irritability in children and adolescents with autism spectrum disorder Neuropsychopharmacology 35:990–998

Huerta M, Bishop Sl, Duncan A, Hus V, Lord C (2012) Application of DSM-5 criteria for autism spectrum disorder to three samples of children with DSM-IV diagnoses of pervasive developmental disorders. Am J Psychiatry 169:1056–1064

Ipser JC, Stein DJ, Hawkridge S, Hoppe L (2009) Pharmacotherapy for anxiety disorders in children and adolescents. Cochrane Database Syst Rev 8:CD005170

Jaselskis CA, Cook EH Jr, Fletcher KE, Leventhal BL (1992) Clonidine treatment of hyperactive and impulsive children with autistic disorder. J Clin Psychopharmacol 12:322–327

Kaplan G (2012) Pharmacotherapy of adolescent ADHD. In: Greydanus DR, Patel DR, Omar HA, Feucht C, Merrick J (eds) Adolescent medicine: pharmacotherapeutics in general, mental, and sexual health. De Cruyter, Boston, pp 201–226.

Kaplan G (2013) What is new in adolescent psychiatry? A literature review and clinical implications. Adolesc Med State Art Rev 24:29–42

Kaplan G, McCracken JT (2012) Psychopharmacology of autism spectrum disorders. Pediatr Clin N Am 59:175–187

King BH, Hollander E, Sikich L, McCracken JT, Scahill L, Bregman JD, Donnelly CL, Anagnostou E, Dukes K, Sullivan L, Hirtz D, Wagner A, Ritz L, STAART Psychopharmacology Network (2009) Lack of efficacy of citalopram in children with autism spectrum disorders and high levels of repetitive behavior: citalopram ineffective in children with autism. Arch Gen Psychiatry 66:563–590

Kotagal S, Broomall E (2012) Sleep in children with autism spectrum disorder. Pediatr Neurol 47:242–251

Malone RP, Delaney MA, Hyman SB, Cater JR (2007) Ziprasidone in adolescents with autism: an open-label pilot study. J Child Adolesc Psychopharmacol 17:779–790

Mandell D, Morales K, Marcus SC, Stahmer AC, Doshi J, Polsky DE (2008) Psychotropic medication use among medicaid-enrolled children with autism spectrum disorders. Pediatrics 121:e441–448

Marcus RN, Owen R, Kamen L, Manos G, McQuade RD, Carson WH, Aman MG (2009) A placebo-controlled, fixed-dose study of aripiprazole in children and adolescents with irritability associated with autistic disorder. J Am Acad Child Adolesc Psychiatry 48:1110–1119

Martin A, Koenig K, Scahill L, Bregman J (1999) Open-label quetiapine in the treatment of children and adolescents with autistic disorder. J Child Adolesc Psychopharmacol 9:99–107

McCracken JT (2010) Disruptive behaviors. In: Hollander E, Kolevzon A, Coyle JT (eds) Autism spectrum disorders. American Psychiatric Publishing, Washington, DC, pp 159–167.

McCracken JT, McGough J, Shah B, Cronin P, Hong D, Aman MG, Arnold LE, Lindsay R, Nash P, Hollway J, McDougle CJ, Posey D, Swiezy N, Kohn A, Scahill L, Martin A, Koenig K, Volkmar F, Carroll D, Lancor A, Tierney E, Ghuman J, Gonzalez NM, Grados M, Vitiello B, Ritz L, Davies M, Robinson J, McMahon D, Research Units on Pediatric Psychopharmacology Autism Network (2002) Risperidone in children with autism and serious behavioral problems. N Engl J Med 347:314–321

McDougle CJ, Naylor ST, Cohen DJ, Volkmar FR, Heninger GR, Price LH (1996) A double-blind, placebo-controlled study of fluvoxamine in adults with autistic disorder. Arch Gen Psychiatry 53:1001–1008

McDougle CJ, Scahill L, Aman MG, McCracken JT, Tierney E, Davies M, Arnold LE, Posey DJ, Martin A, Ghuman JK, Shah B, Chuang SZ, Swiezy NB, Gonzalez NM, Hollway J, Koenig K, McGough JJ, Ritz L, Vitiello B (2005) Risperidone for the core symptom domains of autism: results from the study by the autism network of the research units on pediatric psychopharmacology. Am J Psychiatry 162:1142–1148

Miano S, Ferri R (2010) Epidemiology and management of insomnia in children with autistic spectrum disorders. Paediatr Drugs 12:78–84

Murray MJ (2010) Attention-deficit/hyperactivity disorder in the context of autism spectrum disorders. Curr Psychiatry Rep 12:282–288

Nazeer A (2011) Psychopharmacology of autistic spectrum disorders in children and adolescents. Pediatr Clin No Am 58:85–97

Owen R, Sikich L, Marcus RN, Corey-Lisle P, Manos G, McQuade RD, Carson WH, Findling RL (2009) Aripiprazole in the treatment of irritability in children and adolescents with autistic disorder. Pediatrics 124:1533–1540

Owley T, Walton L, Salt J, Guter SJ Jr, Winnega M, Leventhal BL, Cook EH Jr (2005) An open-label trial of escitalopram in pervasive developmental disorders. J Am Acad Child Adolesc Psychiatry 44:343–348

Paavonen EJ, Vehkalahi K, Vanhala R (2008) Sleep in children with Asperger syndrome. J Autism Dev Disord 38:41–51

Pardo CA, Buckley A, Thurm A, Lee LC, Azhagiri A, Neville DM, Swedo SE (2013) A pilot open-label trial of minocycline in patients with autism and regressive features. J Neurodev Disord 5:9

Parikh MS, Kolevzon A, Hollander E (2008) Psychopharmacology of aggression in children and adolescents with autism: a critical review of efficacy and tolerability. J Child Adolesc Psychopharmacol 18:157–178

Paykina N, Greenhill L (2008) Attention deficit hyperactivity disorder. In: Findling RL (ed) Clinical manual of child and adolescent psychopharmacology. American Psychiatric Publishing, Washington, DC, pp 33–87

Politte LC, McDougle CJ (2014) Aytpical antipschotics in the treatment of children and adolescents with pervasive developmental disorders. Psychopharmacology (Berl) 231:1023–1036

Posey DJ, Stigler KA, Erickson CA, McDougle CJ (2008) Antipsychotics in the treatment of autism. J Clin Invest 118:6–14

Potenza MN, Holmes JP, Kanes SJ, McDougle CJ (1999) Olanzapine treatment of children, adolescents, and adults with pervasive developmental disorders: an open-label pilot study. J Clin Psychopharmacol 19:37–44

Quintana H, Birmaher B, Stedge D, Lennon S, Freed J, Bridge J, Greenhill L (1995) Use of methylphenidate in the treatment of children with autistic disorder. J Autism Dev Disord 25:283–294

Richdale A (1999) Sleep problems in autism: prevalence, cause, and intervention. Dev Med Child Neurol 41:60–66

Robinson SJ (2012) Childhood epilepsy and autism spectrum disorders: psychiatric problems, phenotypic expression, and anticonvulsants. Neuropsychol Rev 22:271–279

Roke Y, Buitelaar JK, Boot AM, Tenback D, van Harten PN (2012) Risk of hyperprolactinemia and sexual side effects in males 10–20 years old diagnosed with autism spectrum disorders or disruptive behavior disorder and treated with risperidone. J Child Adolesc Psychopharmacol 22:432–439

Research Units on Pediatric Psychopharmacology Autism Network (RUPP[a]) (2005a) Risperidone treatment of autistic disorder: longer term benefits and blinded discontinuation after 6 months. Am J Psychiatry 162:1361–1369

Research Units on Pediatric Psychopharmacology Autism Network (RUPP[b]) (2005b) Randomized, controlled, crossover trial of methylphenidate in pervasive developmental disorders wtih hyperactivity. Arch Gen Psychiatry 62:1266–1274

Rush J, Nirenmberg A (2009) Mood disorders: treatment of depression. In: Sadock B, Sadock V, Ruiz P (eds) Kaplan and Sadock's comprehensive textbook of psychiatry. Lippincott Williams & Wilkins, Philadelphia, pp 1734–1742

Scahill L, Aman MG, McDougle CJ, McCracken JT, Tierney E, Dziura J, Arnold LE, Posey D, Young C, Shah B, Ghuman J, Ritz L, Vitiello B (2006) A prospective open trial of guanfacine in children with pervasive developmental disorders. J Child Adolesc Psychopharmacol 16:589–598

Schubart JR, Camacho F, Leslie D (2013) Psychotropic medication trends among children and adolescents with autism spectrum disorder in the medicaid program. Autism 18:631–637

Sharma A, Shaw S (2012) Efficacy of risperidone in managing maladaptive behaviors for children with autism spectrum disorder: a meta-analysis. J Ped Health Care 26:291–299

Siegel M (2012) Psychopharmacology of autism spectrum disorder: evidence and practice. Child Adolesc Psychiatr Clin N Am 21:957–973

Silver WG, Rapin I (2012) Neurobiological basis of autism. Pediatr Clin N Am 59:45–61

Tachibana M, Kagitani-Shimono K, Mohri I, Yamamoto T, Sanefuji W, Nakamura A, Oishi M, Kimura T, Onaka T, Ozono K, Taniike M (2013) Long-term administration of intranasal oxytocin is a safe and promising therapy for early adolescent boys with autism spectrum disorder. J Child Adolesc Psychopharmacol 23:123–127

Taurines R, Schwenck C, Westerwald E, Sachse M, Siniatchkin M, Freitag C (2012) ADHD and autism: differential diagnosis or overlapping traits? A selective review. Atten Defic Hyperact Disord 4:115–139

Tchaconas A, Adesman A (2013) Autistic spectrum disorders: a pediatric overview and update. Curr Opin Pediatr 25:130–144

Tuchman RF (2013) Deconstructing autism spectrum disorders: clinical perspective. Rev Neurol 56:S3–12

Tordjman S, Najjar I, Bellissant E, Anderson GM, Barburoth M, Cohen D, Jaafari N, Schischmanoff O, Fagard R, Lagdas E, Kermarrec S, Ribardiere S, Botbol M, Fougerou C, Bronsard G,

Vernay-Leconte J (2013) Advances in the research of melatonin in autism spectrum disorders: literature review and new perspectives. Int J Mol Sci 14:20508–20542
Weblink #1 (2012) http://www.forbes.com/sites/emilywillingham/2012/10/11/new-dsm-5-criteria-for-autism-who-will-be-left-behind/
Weblink #2 (2012) http://www.nytimes.com/2012/01/20/health/research/new-Autism-definition-would-exclude-many-studysuggests.html?_pagewanted=all&module=Search&mabReward=-relbias%3Ar
Weblink #3 (2009) http://www.autismspeaks.org/about-us/press-releases/autism-speaks-announces-results-reported-study-fluoxetine-autism-sofia
Weblink #4 (2012) http://www.clinicaltrials.gov/ct2/show/NCT01725152?term=ganaxolone&rank=3
Williams K, Wheeler DM, Silove N, Hazell P (2013) Selective serotonin reuptake inhibitors (SSRIs) for autism spectrum disorders (ASD). Cochrane Database Syst Rev 8:CD004677
Young D, Nagarajan L, de Klerk N (2007) Sleep problems in Rett syndrome. Brain Dev 29:609–616

Chapter 10
Behavioral Therapies

V. Mark Durand

Abstract Intervention for the core symptoms and comorbid conditions associated with autism spectrum disorder (ASD) primarily involves behavioral and/or cognitive behavioral therapy. This chapter reviews the relevant research for the effectiveness of these approaches across several areas. It begins with a review of the rapidly growing research focused on early intervention for young children with ASD. Next, interventions for persons on the severe end of the autism spectrum are discussed. Specifically, studies focus either on symptomatic treatment (e.g., increasing social skills) or on packages of treatments that are designed to address the range of difficulties persons with ASD display. The chapter then describes the nascent research on persons on the milder end of the spectrum—especially work on social skills training. Finally, a growing research base on treating comorbid conditions (e.g., anxiety, sleep problems) is also briefly reviewed. A theme throughout the chapter is the need to individualize treatment and the special needs of the range of persons with ASD.

Keywords Autism spectrum disorder · Treatment · Comorbid conditions · Early behavioral intervention · Treatment packages

10.1 Behavioral Therapies

Persons with autism spectrum disorder (ASD) present with a unique array of challenges as well as strengths (Amaral et al. 2011). The therapeutic focus initially emphasizes interventions for the core problems surrounding social interactions and relationships as well as the difficulties that arise due to their restricted and repetitive patterns of behavior, interests, and activities (American Psychiatric Association 2013). In addition, common comorbid problems such as anxiety, depression, sleep disorders, and difficulties with executive functioning further complicate efforts to assist these individuals with becoming more independent. A number of behavioral and cognitive-behavioral approaches exist to treat these conditions (Durand 2014a). This chapter reviews the extant evidence base for these treatments. However, first

V. M. Durand (✉)
Department of Psychology, University of South Florida St. Petersburg, USF St. Petersburg, 140 Seventh Avenue South, St. Petersburg, FL, 33701, USA
e-mail: vdurand@mail.usf.edu

S. Hossein Fatemi (ed.), *The Molecular Basis of Autism,*
Contemporary Clinical Neuroscience, DOI 10.1007/978-1-4939-2190-4_10

discussed will be the range of presentations of ASD and the social nature of this disorder since this guides treatment development, implementation and outcomes.

10.2 The Range and Nature of ASD

DSM-5 significantly (and somewhat controversially) reorganized how ASD is diagnosed by combining previously separate disorders (i.e., autistic disorder, Asperger's disorder, childhood disintegrative disorder, and pervasive developmental disorder—not otherwise specified) under a single diagnostic entity (American Psychiatric Association 2013; Lord and Jones 2012; Skuse 2012). Full discussion of the rationale for this change and the scientific and political implications is beyond the scope of this chapter. However, it is important to highlight that a diagnosis of ASD by itself has poor predictive and treatment validity. In other words, the range of functioning of persons with ASD and the developmental trajectories of the disorder are quite diverse (e.g., Fein et al. 2013; Lord et al. 2012) and therefore requires a comprehensive assessment of strengths and needs for each person affected (Steiner et al. 2012). The addition of three levels of severity included in the *DSM-5* diagnosis of ASD (Level 1—"Requiring support," Level 2—"Requiring substantial support," and "Level 3—"Requiring very substantial support") attempts to highlight these differences (American Psychiatric Association 2013), however their somewhat subjective nature and limited specificity provide only partial assistance for treatment design. This chapter adopts the convention of referring to persons with "mild" ASD and "severe" ASD (Durand 2014a). This division maps roughly onto the previous distinction between persons who are able to converse with others but demonstrate difficulties with pragmatics (previously diagnosed with Asperger's disorder) and those individuals who have severe language and communication problems (previously diagnosed with autistic disorder, childhood disintegrative disorder or pervasive developmental disorder—not otherwise specified). Although it is recognized that persons with ASD manifest a broad spectrum of challenges, research on treatment typically focuses separately on these two distinct groups.

In addition to the range of symptoms influencing treatment choice and outcome, the nature of ASD directly impacts treatment design. Specifically, the disruption in typical social motivation displayed by persons with ASD provides interventionists with unique challenges. Those with ASD either lack interest in others or are impaired in their ability to navigate social interactions (Dawson 2008; Mundy 2011). Therefore, unlike those with other disorders where the motivation to enter into treatment is usually some combination of the desire to reduce personal distress and the wish to please significant others (Barlow and Durand 2014), this is often not the case among persons with ASD. At the more severe end of the autism spectrum, the lack of social motivation to participate in treatment is typically supplemented by more extrinsic motivation (e.g., the use of non-social reinforcers). Those on the mild end of this spectrum will sometimes express distress over either their social difficulties alone (e.g., not being able to make and keep friendships) or comorbid

difficulties that may be the result of these social problems (e.g., anxiety, depression). However, difficulties such as in impairments with theory of mind (i.e., not being able to "read" and understand what others might be thinking or feeling) complicates the ability to apply standard approaches to intervention. As a result, social skills training programs as well as cognitive-behavioral treatments for comorbid problems need to be significantly adapted for this group (Durand 2014a).

10.3 Early Developmental/Behavioral Intervention

Early intervention approaches are receiving increased attention, especially as the ability to diagnosis children with ASD improves to the point where it may be possible to identify these children as early as 6 months of age (e.g., Elsabbagh et al. 2012; Kim and Lord 2012; Veness et al. 2012). One of the first large scale studies reported on the effects of using intensive intervention using techniques from applied behavior analysis with very young children with ASD (younger than 3 1/2 years of age) (Lovaas 1987). This intervention—now referred to as Early and Intensive Behavioral Intervention (EIBI)—involved 40 h per week of educational efforts over the course of two years. During the first year, the focus of treatment was to reduce repetitive behaviors, teach the children to speak in words, increase compliance on tasks and imitate adults as well as establishing the beginnings of appropriate toy play. The second year involved teaching targeted expressive and early abstract language and interactive play with peers and was extended into the community to teach children to function within a preschool setting. In that study, it was suggested that almost half of the children "recovered" from their ASD—meaning that several years later teachers could not tell them apart from their students without ASD. Although criticized on methodological grounds, that study led the way for a growing database that points to the value of early and intensive intervention for some children with ASD (Peters-Scheffer et al. 2011; Reichow and Wolery 2009; Strain and Bovey 2011; Warren et al. 2011).

Reviews of successful early intensive behavioral intervention programs suggest a number of common features in each program including; (1) comprehensive curriculum focusing on imitation, language, toy play, social interaction, motor, and adaptive behavior, (2) sensitivity to developmental sequence, (3) supportive, empirically validated teaching strategies (applied behavior analysis), (4) behavioral strategies for reducing interfering behaviors, (5) involvement of parents, (6) gradual transition to more naturalistic environments, (7) highly trained staff, (8) supervisory and review mechanisms, (9) intensive delivery of treatment (25 h per week for at least 2 years), and (10) initiation by 2–4 years of age (Dawson and Osterling 1997; Green et al. 2002; National Research Council 2001). More recent work that blends naturalistic and developmentally-driven behavioral interventions suggests that some of these suggestions (such as the intensity of training) may need to be revised (e.g., Dawson et al. 2010; Strain and Bovey 2011).

Researchers are now examining the characteristics of children (for example, language ability, IQ) that may predict the best treatment outcomes. In addition, some emerging important work suggests that early behavioral intervention may alter the functioning of the developing brain in these children to be comparable to typically developing children (Dawson et al. 2012; Voos et al. 2013). For example, Dawson et al. (2012) assessed brain activity to objects versus faces in young children with ASD (age 48–77 months) both before and after involvement in a developmental behavioral intervention (the Early Start Denver Model [ESDM]) and compared their responses to a community based control group. Their main finding was greater cortical activation while viewing social stimuli (faces) among the ESDM treatment group and they also noted that this finding was associated with improved social behavior. As discussed later in the chapter, this type of intervention may help improve social motivation at a critical developmental period and may assist with learning an array of social skills. Overall, the treatment progress in areas such as language, cognitive abilities, and adaptive behavior seems to greatly improve for many children with ASD if intensive behavioral intervention is implemented early in life and with fidelity (Dawson and Burner 2011; Strain and Bovey 2011). These changes appear quite robust, and for many children appear to be maintained years after initial intervention (e.g., Kasari et al. 2012a).

Described next is the research on treatment for the problems displayed by older children and adults on the more severe end of the autism spectrum, followed by work with individuals on the less severe end of this spectrum. Treatment typically begins with teaching social and communication skills (Goldstein 2002; Kasari and Locke 2011; National Research Council 2001) and implementing interventions for disruptive or destructive behaviors (e.g., tantrums, aggression, self-injury) (Durand 2012). The goals of treatment are to increase the individual's ability to be more independent and improve the overall quality of life (Myers and Johnson 2007).

10.4 Social Communication Intervention for Severe ASD

Behavioral research targeting social communication skills in persons with more severe ASD dates back to the early 1960's. At that time problems with social communication were viewed primarily as skill deficits. In other words, it was thought that individuals with ASD did not appropriately interact with others because, for reasons as yet unknown, they did not learn the necessary skills. Remediation, therefore, would involve using behavioral interventions to teach these new skills (Hewett 1965; Lovaas and Smith 1989). Intervention approaches typically involve techniques developed from applied behavior analysis (ABA), which uses principles of learning theory to encourage new behaviors by manipulating antecedents and consequences (Matson et al. 2012; Smith 2001). Some of the earliest efforts in this area were documented by Ivar Lovaas and his colleagues at UCLA. For example, they used the basic behavioral procedures of shaping and discrimination training to teach nonspeaking children with ASD to imitate others verbally (Lovaas et al. 1966).

Once children could imitate, teaching speech became easier, and progress was made in teaching some of these children to use labels, plurals, sentences, and other more complex forms of language (Lovaas 1987). Initially these treatments were delivered primarily in highly structured settings with adult directed activities and artificial consequences (referred to as discrete trial training) (McEachin and Leaf 1999; Smith 2001). Recent modifications allow for a more naturalistic and developmentally driven context, with behaviors taught in typical settings, child directed activities and natural consequences. Growing evidence supports this approach for improving generalization and maintenance of learned skills (Schreibman and Ingersoll 2011).

Despite some successful outcomes using behavioral interventions to teach communication skills there are limitations with this approach. First, many children with ASD are not able to benefit from this training such that they gain functional speech skills (Carr 1979; Howlin 1981). Second, for those who do acquire speech it is often limited to speech that would lead to non-social outcomes (e.g., asking for soda). Socially oriented speech (e.g., saying "Hello" to another person) is less likely to be produced (Koegel et al. 2011; Wetherby and Prutting 1984). This observation is related to the limited interest in social interactions by this population. For example, the skills needed to engage in a social exchange (e.g., saying "Hi! How are you?") are unlikely to be maintained given that the outcome is an increase in social attention from another person—a consequence that may not be reinforcing to many persons with severe ASD.

Currently, initial efforts with young children focuses on improving social motivation by teaching imaginative play and joint or shared attention (defined as "spontaneous seeking to share experience, enjoyment, interests, or achievements with other people" Mundy 2011, p. 151). These skills are characteristically absent or impaired in young children with ASD and are seen as pivotal prerequisites necessary for later development of important social, communicative and cognitive skills (Charman et al. 1997; Koegel et al. 2001; Poon et al. 2012). For example, longitudinal research suggests that deficits in joint attention predict language delays years later (Mundy et al. 1990; Stone and Yoder 2001; Thurm et al. 2007). The neurobiology of these skills is just beginning to be explored. One study of adults with ASD, for example, found an atypical pattern of brain activation (including in the dorsal medial prefrontal cortex (dMPFC) and right posterior superior temporal sulcus (pSTS)) in these adults during a joint attention task as compared to neurotypical adults during the same activity (Redcay et al. 2013).

A number of studies now show that many young children with ASD can be taught these skills through naturalistic behavioral approaches (Kasari et al. 2012b; Lawton and Kasari 2012; Wong and Kasari 2012). In one study, 58 3–4 year olds with ASD received either joint attention intervention, symbolic play intervention or were in control group (Kasari et al. 2006). Intervention involved a combination of behavioral (e.g., prompting behaviors, reinforcement) and naturalistic procedures (e.g., using child interests to create learning opportunities) to encourage either joint attention or symbolic play. Sessions were conducted 30 min daily for 5–6 weeks. The results demonstrated the success of this limited intervention in improving these skills in young children with ASD (Kasari et al. 2006). Importantly, a follow-up of

these children demonstrated that their improved ability to engage in joint attention and symbolic play predicted their spoken language ability 5 years after the initial training (Kasari et al. 2012a). This finding is significant since having functional spoken language at age 5 is highly predictive of positive social and cognitive outcomes (Billstedt et al. 2005; DeMyer et al. 1973; Venter et al. 1992).

Although it is premature to say definitively why teaching joint attention and related skills improves later speech, it may be helpful to consider the distinction between viewing social communication problems as a skill deficit alone and considering these difficulties also as a problem of social motivation. By teaching joint attention and play skills to young children with ASD, its value may lie in pairing activities the child enjoys with the presence of and interaction with others (Mundy and Gomes 1998). If other people are viewed as positive, engaging in social interactions will result in desirable outcomes, encouraging more engagement with others (Dawson 2008; Koegel et al. 2011; Stavropoulos and Carver 2013). Increased social motivation may be the mediating factor in the role of improved joint attention leading to improved social communication skills (Paul et al. 2013).

Intervention for the communicative skills of older individuals with limited abilities focuses on teaching either spoken language or the use of augmentative communication strategies (e.g., pointing to a picture, using vocal output devices) (Ganz et al. 2012; Odom et al. 2010b; Prelock et al. 2011). Using graphic symbols (e.g., pictures of desired items or activities) to encourage communication has an emerging research base and are employed as visual aids for monitoring school and home schedules (e.g., visual supports); additionally graphic symbols assist students to make choices (e.g., pointing to one of a series of activity options), and to engage in general expressive communication (Wegner 2012). A variation of this approach teaches students to select one or more pictures/words and hand them to another person—called the picture exchange communication system (PECS) (Bondy and Frost 1994; Tincani and Devis 2011). The potential advantage of this approach is that the student can initiate communication with someone even if the other person's attention is focused elsewhere.

A related approach uses speech generating devices (SGDs) to assist non-verbal students to communicate with others (Ganz et al. 2012). A variety of devices are available that can be programmed to generate human speech when a picture or word is pressed by the student (e.g., "Help me."). These devices have both the advantage of being able to catch the attention of others as well as being understood by anyone. With advancing technologies and available software that can be used to easily program these devices (e.g., tablet computers), their use is increasing. Some research suggests that students with ASD may have idiosyncratic preferences for one system over another (e.g., PECS versus SGDs) (van der Meer et al. 2012), and therefore the selection of any of these non-speech approaches to teaching social communication should in part be guided by student choice.

In addition to relying on teachers to instruct students on these new communication skills, some work points to advantages for peer-mediated strategies (using same age typically developing peers as tutors or models) (Kasari et al. 2012b; Kashinath 2012; Locke et al. 2012) as well as employing parents and siblings as instructors, as well (Dawson and Burner 2011).

Many approaches to teaching social skills use a variety of techniques that exploit the tendency of persons with ASD to learn skills better through visual rather than verbal cues. For example, variations of video modeling (including video self-modeling) show students with ASD how to behave and interact in a variety of social communication situations (Bellini and Akullian 2007; Buggey 2012; Charlop-Christy et al. 2000; Nikopoulos and Keenan 2004). Visually-based stories that students can use as cues in social situations (called social narratives) are often useful when teaching appropriate behavior in these scenarios (e.g., how to wait in line in the cafeteria) (Odom et al. 2010b; Test et al. 2011). It has only been relatively recently that this work has been subjected to rigorous testing through larger N studies and randomized clinical trials and evaluated with meta-analyses. More work is needed to identify factors related to better outcomes when teaching social communication skills (e.g., presence of comorbid intellectual disability) (Hume and Odom 2011; Maglione et al. 2012; National Professional Development Center on Autism Spectrum Disorders 2011).

10.5 Intervention for Challenging Behavior for Severe ASD

In addition to the social communication and interaction difficulties characteristic of persons with ASD, they also display behaviors that are relatively rigid and/or unusual (Leekam et al. 2011). The repetitive behaviors of those with severe ASD such as stereotyped movements, repetitive manipulation of objects and some self-injurious behaviors still remain a challenge for intervention efforts and the data base for effective interventions remains limited (Boyd et al. 2012). The reason these behaviors may be more intractable is that they may involve internal processes related to sensory activities. There is research suggesting that some of these repetitive behaviors may later be used by these individuals to manipulate their environment (Durand and Carr 1987), thus serving a social communicative function (Durand 1990).

A significant evidence base exists for the treatment of challenging behaviors such as aggression, self-injury and other disruptive outbursts which are more frequent among persons with severe ASD (Carr 2011). As with interventions for social communicative behaviors, interventions for challenging behavior rely mainly on techniques derived from applied behavior analysis. The general strategy involves assessing the function of the behavior (called functional behavior assessment) (Beavers et al. 2013; Loman and Horner 2013) and using a variety of techniques to support appropriate behavior and teach alternative behaviors (Durand 2012; Vismara and Rogers 2010). In one randomized clinical trial, parents of children with developmental disabilities including ASD were taught how to; (1) assess the function of their child's severe behavior problem (including aggression, tantrums and self-injury), (2) plan for emergency situations, (3) appropriately use consequences (e.g., praise) and, (4) teach their child alternative responses that serve the same function as the challenging behavior [called functional communication training; Durand 1990, 2012; Durand et al.

2013]. This clinic-based intervention demonstrated that parents could successfully intervene with their child's severe behavior problems and reduce them in a meaningful way at home and in the community. A large number of small N studies and a growing number of larger demonstrations document the success of these procedures to significantly improve challenging behavior in multiple settings and across a variety of behavioral topographies (Carr 2011; Durand 2012; Vismara and Rogers 2010).

10.6 Comprehensive Intervention Programs

There are numerous comprehensive treatment programs being used with those having severe ASD and they typically are implemented either in schools or in special clinical settings (Howlin et al. 2009; Maglione et al. 2012; Odom et al. 2010a; Peters-Scheffer et al. 2011; Reichow and Wolery 2009; Rogers and Vismara 2008). Most of these programs also integrate parents or other caregivers into the program in order to extend teaching into the home and in the community. There is a general consensus that for treatment to be optimally successful it should be carried out for a minimum of 25 h per week and across all 12 months of the year (National Research Council 2001). This is just a general guideline and the specific interventions and their level of intensity are determined individually according to the needs of the person (Wilczynski et al. 2012).

The comprehensive programs with some empirical support can be broadly divided into three categories; (1) behavioral programs using applied behavior analysis techniques, (2) behavioral programs that integrate developmental considerations to guide treatment targets and (3) programs that have a relationship-based focus (Howlin et al. 2009; Maglione et al. 2012; Odom et al. 2010a; Reichow and Wolery 2009). In their review of 30 comprehensive program models, Odom and colleagues evaluated these models based on several criteria including thorough documentation of their procedures, data on the fidelity of implementation, outcome data and whether or not there were independent replications of the program (Odom et al. 2010a). Overall they found that most of the models had little evidence for their efficacy, and for those with published outcome data, most of the studies rated low on the quality of their data. It is important to note that research evaluating techniques and programs for students with ASD is evolving rapidly and several of these programs are currently being studied in recent and ongoing randomized clinical trials (e.g., Dawson et al. 2010).

10.7 Social Communication Intervention for Mild ASD

Individuals with mild ASD do not have the cognitive delays and communication skills difficulties often found in persons with severe ASD, and can—with support—perform well academically in school. However, their social difficulties and common

comorbid problems (e.g., ADHD, anxiety) complicate their interactions with peers and teachers and can lead to disruptive behavior problems. A number of different behaviorally-based programs exist to assist school-aged children with mild ASD improve skills such as appropriate social interaction, problem-solving, self-control, recognizing emotions in others, expanding their often narrow range of interests, and improving their understanding of non-literal idioms (e.g., understanding that the phrase "You are pulling my leg." is not meant literally) (Karkhaneh et al. 2010; Koning et al. 2013; Laugeson et al. 2012; Rao et al. 2008).

Although this research base is emerging, one RCT used a comprehensive package to improve social skills in this population (Thomeer et al. 2012). This program used a 5 week summer camp for intensive work on a variety of social skills including social interactions, face-emotion recognition, interest expansion, and interpretation of non-literal language. A total of 17 children ranging in age from 7 to 12 years were randomly assigned to the treatment condition in groups with one staff member for every two children. Five 70 min treatment sessions were conducted each day, 5 days per week. The sessions focused on a target skill and adapted the social skills curriculum called *Skillstreaming* (A. P. Goldstein and McGinnis 1997). They found significant improvements in measures of knowledge of target social skills and understanding of idioms. On the other hand, there was not a significant improvement in facial-emotion recognition (Thomeer et al. 2012). Other programs specifically teach children how to make and maintain quality friendships (e.g., the Program for the Evaluation and Enrichment of Relational Skills—PEERS; Laugeson et al. 2012; Laugeson et al. 2009). As yet, the literature in this area remains in a nascent state and awaits more evidence for the effectiveness for these types of programs to make meaningful changes in the lives of persons with mild ASD.

Although techniques used to teach social skills to individuals with mild ASD can be successful in improving their abilities to interact with others, it is important to return to their difficulties with empathy and "mind reading." Although they may be able to learn specific skills (for example, how to approach a stranger and ask for assistance, or not to stand too close to someone and how to make appropriate eye contact) there still remains the limitations in their abilities to feel and anticipate the emotions of others. This difficulty clearly puts them at a distinct disadvantage when trying to read the expressions and body language of those with whom they are trying to engage. Teaching social skills is difficult enough even among those without ASD. The addition of the difficulty in reciprocal social exchange adds an additional level of challenge (Durand 2014a).

10.8 Comorbid Conditions

In addition to the core symptoms of the disorder, a number of other difficulties are frequently found among persons with ASD; including ADHD, anxiety, depression, and sleep difficulties. It is estimated that approximately 70% of children with ASD have another diagnosable psychiatric disorder and that 40% meet the diagnostic

criteria for two or more other disorders (Simonoff et al. 2008). Unfortunately, for most of these disorders there is relatively little empirical evidence for effective treatment.

Behavioral treatment for the symptoms of ADHD observed in approximately 28% of children with ASD parallel that of classroom and home intervention for children with ADHD (Pfiffner et al. 2011; Volpe et al. 2012). Functional behavioral assessments are conducted to assess the functions of behavioral problems, alternative behaviors are encouraged (e.g., teaching a child to request assistance rather than getting out of his/her seat or having tantrums) and reinforcement programs are put in place to encourage improved behavior at home and at school.

Symptoms of anxiety are commonly reported among approximately 50–80% of persons with ASD (de Bruin et al. 2007; Leyfer et al. 2006; White et al. 2009). Treatments for these problems with anxiety emulate work with persons not having ASD (cognitive behavior therapy—CBT) (Szatmari and McConnell 2011). For example, one randomized clinical trial evaluated a modified CBT protocol on the anxiety symptoms of 20 children with mild ASD and approximately 50% of the children who participated in the group treatment program had clinically significant reductions in anxiety (Reaven et al. 2012). More work is needed to determine what treatments will be effective for the range of individuals with ASD and anxiety.

Feelings of depression are commonly reported by persons with mild ASD, with rates approximating 25–34% (Ghaziuddin et al. 2002; Mayes et al. 2011). Although there is some indication that teaching social skills may improve self-image and self-reported feelings of depression (e.g., Hedley and Young 2006), there is not yet a body of work pointing to established treatments in this area.

Perhaps the most extensive body of research on a comorbid disorder exists for problems surrounding sleep (Durand 2014b). Sleep problems are highly prevalent, with the reported range from 50–80% of children with ASD (Couturier et al. 2005; Krakowiak et al. 2008; Richdale and Schreck 2009). Until recently, research on the treatment of sleep problems in persons with ASD mainly included small N studies using single subject designs (Schreck 2001; Vriend et al. 2011). The treatments that appear most successful include variations of extinction, adjusting sleep schedules, sleep restriction and scheduled awakening (Durand 2014b). Newer work employing larger Ns and RCTs is confirming the potential positive outcomes that can be achieved with these treatments (e.g., Malow et al. 2014).

Overall, the research on the treatment of comorbid conditions among persons with ASD is in its infancy and awaits greater attention to these problems.

10.9 Conclusions

Clearly, the best outcomes to date for persons with ASD are being achieved through early behavioral/developmental intervention for very young children (Peters-Scheffer et al. 2011; Reichow and Wolery 2009; Warren et al. 2011). Especially encouraging is the potential for lasting change being observed in brain functioning following

this type of treatment (e.g., Dawson et al. 2012). Symptomatic treatment for older children and adult persons with both severe and mild forms of ASD suggests that important strides are being made in promoting social communication skills and decreasing challenging behavior, although long-term studies are needed to determine how these improvements translate into improved quality of life and independence (Durand 2014a). Finally, an emerging data base is being created to assist with important comorbid conditions. Overall, the prognosis for persons with ASD has improved over the last decade with our increasing knowledge about how to best support and intervene with these individuals.

References

Amaral DG, Dawson G, Geschwind D (2011) Autism spectrum disorders. Oxford University Press, New York

American Psychiatric Association (2013) Diagnostic and statistical manual of mental disorders, 5th edn. American Psychiatric Association, Washington, DC

Barlow DH, Durand VM (2014) Abnormal psychology: an integrative approach, 7th edn. Wadsworth/Cengage Learning, Belmont

Beavers GA, Iwata BA, Lerman DC (2013) Thirty years of research on the functional analysis of problem behavior. J Appl Behav Anal 46:1–21

Bellini S, Akullian J (2007) A meta-analysis of video modeling and video self-modeling interventions for children and adolescents with autism spectrum disorders. Except Child 73:264–287

Billstedt E, Gillberg C, Gillberg C (2005) Autism after adolescence: population-based 13-to 22-year follow-up study of 120 individuals with autism diagnosed in childhood. J Autism Dev Disord 35:351–360

Bondy AS, Frost LA (1994) The picture exchange communication system. Focus Autism Other Dev Disabil 9:1–19

Boyd B, McDonough S, Bodfish J (2012) Evidence-based behavioral interventions for repetitive behaviors in autism. J Autism Dev Disord 42:1236–1248

Buggey T (2012) Video modeling applications for persons with autism. In: Prelock PA, McCauley RJ (eds) Treatment of autism spectrum disorders: Evidence based-intervention strategies for communication and social interactions. Paul H. Brookes, Baltimore, pp 345–369

Carr EG (1979) Teaching autistic children to use sign language: Some research issues. J Autism Dev Disord 9:345–359

Carr EG (2011) Positive behavior support and problem behavior. In: Amaral DG, Dawson G, Geschwind D (eds) Autism spectrum disorders. Oxford University Press, New York, pp 1144–1155

Charlop-Christy MH, Le L, Freeman KA (2000) A comparison of video modeling with in vivo modeling for teaching children with autism. J Autism Dev Disord 30:537–552

Charman T, Swettenham J, Baron-Cohen S, Cox A, Baird G, Drew A (1997) Infants with autism: an investigation of empathy, pretend play, joint attention, and imitation. Dev Psychol 33:781–789

Couturier JL, Speechley KN, Steele M, Norman R, Stringer B, Nicolson R (2005) Parental perception of sleep problems in children of normal intelligence with pervasive developmental disorders: prevalence, severity, and pattern. J Am Acad Child Adolesc Psychiatry 44:815–822

Dawson G (2008) Early behavioral intervention, brain plasticity, and the prevention of autism spectrum disorder. Dev Psychopathol 20:775–803

Dawson G, Burner KB (2011) Behavioral interventions in children and adolescents with autism spectrum disorder: a review of recent findings. Curr Opin Pediatr 23:616–620

Dawson G, Osterling J (1997) Early intervention in autism: effectiveness and common elements of current approaches. In: Guralnick MJ (ed) The effectiveness of early intervention: second generation research. Paul H. Brookes, Baltimore, pp 307–326

Dawson G, Rogers S, Munson J, Smith M, Winter J, Greenson J, Donaldson A, Varley J (2010) Randomized, controlled trial of an intervention for toddlers with autism: the early start denver model. Pediatr 125:e17–23

Dawson G, Jones EJH, Merkle K, Venema K, Lowy R, Faja S, Kamara D, Murias M, Greenson J, Winter J, Smith M, Rogers SJ, Webb SJ (2012) Early behavioral intervention is associated with normalized brain activity in young children with autism. J Am Acad Child Adolesc Psychiatry 51:1150–1159

de Bruin EI, Ferdinand RF, Meester S, de Nijs PFA, Verheij F (2007) High rates of psychiatric comorbidity in PDD-NOS. J Autism Dev Disord 37:877–886

DeMyer MK, Barton S, DeMyer WE, Norton JA, Allen J, Steele R (1973) Prognosis in autism: a follow-up study. J Autism Child Schizophr 3:199–246

Durand VM (1990) Severe behavior problems: a functional communication training approach. Guilford Press, New York

Durand VM (2012) Functional communication training: treating challenging behavior. In: Prelock PA, McCauley RJ (eds) Treatment of autism spectrum disorders: evidence based-intervention strategies for communication and social interactions. Paul H. Brookes, Baltimore, pp 107–138

Durand VM (2014a) Autism spectrum disorder: a clinical guide for general practitioners. American Psychological Association, Washington, D.C

Durand VM (2014b). Sleep better!: a guide to improving sleep for children with special needs (revised edition). Paul H. Brookes, Baltimore

Durand VM, Carr EG (1987) Social influences on "self-stimulatory" behavior: analysis and treatment application. J Appl Behav Anal 20:119

Durand VM, Hieneman M, Clarke S, Wang M, Rinaldi M (2013) Positive family intervention for severe challenging behavior I: a multi-site randomized clinical trial. J Posit Behav Interv 15:133–143

Elsabbagh M, Mercure E, Hudry K, Chandler S, Pasco G, Charman T, Pickles A, Baron-Cohen S, Bolton P, Johnson MH; BASIS Team (2012) Infant neural sensitivity to dynamic eye gaze is associated with later emerging autism. Curr Biol 22:338–342

Fein D, Barton M, Eigsti IM, Kelley E, Naigles L, Schultz RT, Stevens M, Helt M, Orinstein A, Rosenthal M, Troyb E, Tyson K (2013) Optimal outcome in individuals with a history of autism. J Child Psychol Psychiatry 54:195–205

Ganz JB, Earles-Vollrath TL, Heath AK, Parker RI, Rispoli M, Duran JB (2012) A meta-analysis of single case research studies on aided augmentative and alternative communication systems with individuals with autism spectrum disorders. J Autism Dev Disord 42:60–74

Ghaziuddin M, Ghaziuddin N, Greden J (2002) Depression in persons with autism: Implications for research and clinical care. J Autism Dev Disord 32:299–306

Goldstein AP, McGinnis E (1997) Skillstreaming the adolescent: new strategies and perspectives for teaching prosocial skills, vol 1. Research Press, Champaign

Goldstein H (2002) Communication intervention for children with autism: a review of treatment efficacy. J Autism Dev Disord 32:373–396

Green G, Brennan LC, Fein D (2002) Intensive behavioral treatment for a toddler at high risk for autism. Behav Modif 26:69–102

Hedley D, Young R (2006) Social comparison processes and depressive symptoms in children and adolescents with Asperger syndrome. Autism 10:139–153

Hewett FM (1965) Teaching speech to an autistic child through operant conditioning. Am J Orthopsychiatry 35:927–936

Howlin PA (1981) The effectiveness of operant language training with autistic children. J Autism Dev Disord 11:89–105

Howlin PA, Magiati I, Charman T (2009) Systematic review of early intensive behavioral interventions for children with autism. Am J Intellect Dev Disabil 114:23–41

Hume K, Odom SL (2011) Best practice, policy, and future directions: behavioral and psychosocial interventions. In: Amaral DG, Dawson G, Geschwind D (eds) Autism spectrum disorders. Oxford University Press, New York, pp 1295–1308

Karkhaneh M, Clark B, Ospina MB, Seida JC, Smith V, Hartling L (2010) Social Stories™ to improve social skills in children with autism spectrum disorder. Autism 14:641–662

Kasari C, Locke J (2011) Social skills interventions for children with autism spectrum disorders. In: Amaral DG, Dawson G, Geschwind D (eds) Autism spectrum disorders. Oxford University Press, New York, pp 1156–1166

Kasari C, Freeman S, Paparella T (2006) Joint attention and symbolic play in young children with autism: a randomized controlled intervention study. J Child Psychol Psychiatry 47:611–620

Kasari C, Gulsrud A, Freeman S, Paparella T, Hellemann G (2012a) Longitudinal follow-up of children with autism receiving targeted interventions on joint attention and play. J Am Acad Child Adolesc Psychiatry 51:487–495

Kasari C, Rotheram-Fuller E, Locke J, Gulsrud A (2012b) Making the connection: randomized controlled trial of social skills at school for children with autism spectrum disorders. J Child Psychol Psychiatry 53:431–439

Kashinath SP (2012) Meta-analysis finds that peer-mediated and video-modeling social skills interventions are effective for children with autism, but caution is urged in interpreting the results. Evid Based Commun Assess Interv 6:58–62

Kim S, Lord C (2012) New autism diagnostic interview-revised algorithms for Toddlers and Young Preschoolers from 12 to 47 Months of Age. J Autism Dev Disord 42:82–93

Koegel LK, Fredeen RM, Koegel RL, Lin CE (2011) Relationships, independence, and communication in autism and Asperger's disorder. In: Amaral DG, Dawson G, Geschwind D (eds) Autism spectrum disorders. Oxford University Press, New York, pp 1095–1112

Koegel RL, Koegel LK, McNerney EK (2001) Pivotal areas in intervention for autism. J Clin Child Psychol 30:19–32

Koning C, Magill-Evans J, Volden J, Dick B (2013) Efficacy of cognitive behavior therapy-based social skills intervention for school-aged boys with autism spectrum disorders. Res Autism Spectr Disord 7:1282–1290

Krakowiak P, Goolin-Jones B, Hertz-Picciotto I, Croen LA, Hansen RL (2008) Sleep problems in children with autism spectrum disorders, developmental delays, and typical development: a population-based study. J Sleep Res 17:197–206

Laugeson EA, Frankel F, Mogil C, Dillon AR (2009) Parent-assisted social skills training to improve friendships in teens with autism spectrum disorders. J Autism Dev Disord 39:596–606

Laugeson EA, Frankel F, Gantman A, Dillon A, Mogil C (2012) Evidence-based social skills training for adolescents with autism spectrum disorders: the UCLA PEERS program. J Autism Dev Disord 42:1025–1036

Lawton K, Kasari C (2012) Teacher-implemented joint attention intervention: pilot randomized controlled study for preschoolers with autism. J Consult Clin Psychol 80:687

Leekam SR, Prior MR, Uljarevic M (2011) Restricted and repetitive behaviors in autism spectrum disorders: a review of research in the last decade. Psychol Bull 137:562

Leyfer OT, Folstein SE, Bacalman S, Davis NO, Dinh E, Morgan J, Tager-Flusberg H, Lainhart JE (2006) Comorbid psychiatric disorders in children with autism: interview development and rates of disorders. J Autism Dev Disord 36:849–861

Locke J, Rotheram-Fuller E, Kasari C (2012) Exploring the social impact of being a typical peer model for included children with autism spectrum disorder. J Autism Dev Disord 42:1895–1905

Loman SL, Horner RH (2013) Examining the efficacy of a basic functional behavioral assessment training package for school personnel. J Posit Behav Interv 20:1–13

Lord C, Jones RM (2012) Annual research review: re-thinking the classification of autism spectrum disorders. J Child Psychol Psychiatry 53:490–509

Lord C, Luyster R, Guthrie W, Pickles A (2012) Patterns of developmental trajectories in toddlers with autism spectrum disorder. J Consult Clin Psychol 80:477

Lovaas OI (1987) Behavioral treatment and normal educational and intellectual functioning in young autistic children. J Consult Clin Psychol 55:3–9

Lovaas OI, Smith T (1989) A comprehensive behavioral theory of autistic children: paradigm for research and treatment. J Behav Ther Exp Psychiatry 20:17–29
Lovaas OI, Berberich JP, Perloff BF, Schaeffer B (1966) Acquisition of imitative speech by schizophrenic children. Science 151:705–707
Maglione MA, Gans D, Das L, Timbie J, Kasari C, Technical Expert Panel, HRSA Autism Intervention Research—Behavioral Network (2012) Nonmedical interventions for children with ASD: recommended guidelines and further research needs. Pediatrics 130:S169–S178
Malow BA, Adkins KW, Reynolds A, Weiss SK, Loh A, Fawkes D, Katz T, Goldman SE, Madduri N, Hundley R, Clemons T (2014) Parent-based sleep education for children with autism spectrum disorders. J Autism Dev Disord 44:216–228
Matson JL, Turygin NC, Beighley J, Rieske R, Tureck K, Matson ML (2012) Applied behavior analysis in autism spectrum disorders: recent developments, strengths, and pitfalls. Res Autism Spectr Disord 6:144–150
Mayes SD, Calhoun SL, Murray MJ, Ahuja M, Smith LA (2011) Anxiety, depression, and irritability in children with autism relative to other neuropsychiatric disorders and typical development. Res Autism Spectr Disord 5:474–485
McEachin J, Leaf R (1999) A work in progress: the autism partnership curriculum for discrete trial teaching with autistic children. DRL Books, New York
Mundy P (2011) The social behavior of autism: a parallel and distributed information processing perspective. In: Amaral DG, Dawson G, Geschwind D (eds) Autism spectrum disorders. Oxford University Press, New York, pp 149–171
Mundy P, Gomes A (1998) Individual differences in joint attention skill development in the second year. Infant Behav Dev 21:469–482
Mundy P, Sigman M, Kasari C (1990) A longitudinal study of joint attention and language development in autistic children. J Autism Dev Disord 20:115–128
Myers SM, Johnson CP (2007) Management of children with autism spectrum disorders. Pediatrics 120:1162–1182
National Professional Development Center on Autism Spectrum Disorders (2011) Overlap between evidence-based practices identified by the National Professional Development Center on Autism Spectrum disorders and the National Standards Project. University of North Carolina at Chapel Hill: Frank Porter Graham Child Development Institute
National Research Council (2001) Educating children with autism. National Academy Press, Washington, DC
Nikopoulos CK, Keenan M (2004) Effects of video modeling on social initiations by children with autism. J Appl Behav Anal 37:93
Odom SL, Boyd BA, Hall LJ, Hume K (2010a) Evaluation of comprehensive treatment models for individuals with autism spectrum disorders. J Autism Dev Disord 40:425–436
Odom SL, Collet-Klingenberg L, Rogers SJ, Hatton DD (2010b) Evidence-based practices in interventions for children and youth with autism spectrum disorders. Prev Sch Fail: Altern Edu Child Youth 54:275–282
Paul R, Campbell D, Gilbert K, Tsiouri I (2013) Comparing spoken language treatments for minimally verbal preschoolers with autism spectrum disorders.. J Autism Dev Disord 43:418–431
Peters-Scheffer N, Didden R, Korzilius H, Sturmey P (2011) A meta-analytic study on the effectiveness of comprehensive ABA-based early intervention programs for children with autism spectrum disorders. Res Autism Spectr Disord 5:60–69
Pfiffner LJ, Kaiser NM, Burner C, Zalecki C, Rooney M, Setty P, McBurnett K (2011) From clinic to school: translating a collaborative school-home behavioral intervention for ADHD. School Ment Health 3:127–142
Poon K, Watson L, Baranek G, Poe MD (2012) To what extent do joint attention, imitation, and object play behaviors in infancy predict later communication and intellectual functioning in ASD? J Autism Dev Disord 42:1064–1074
Prelock PA, Paul R, Allen EM (2011) Evidence-based treatments in communication for children with autism spectrum disorders. In: Reichow B, Doehring P, Cicchetti DV, Volkmar FR (eds)

Evidence-based practices and treatments for children with autism. Springer US, New York, pp 93–169
Rao PA, Beidel D, Murray M (2008) Social skills interventions for children with Asperger's syndrome or high-functioning autism: a review and recommendations. J Autism Dev Disord 38:353–361
Reaven JA, Blakeley-Smith A, Culhane-Shelburne K, Hepburn S (2012) Group cognitive behavior therapy for children with high-functioning autism spectrum disorders and anxiety: a randomized trial. J Child Psychol Psychiatry 53:410–419
Redcay E, Dodell-Feder D, Mavros PL, Kleiner M, Pearrow MJ, Triantafyllou C, Gabrieli JD, Saxe R (2013) Atypical brain activation patterns during a face-to-face joint attention game in adults with autism spectrum disorder. Hum Brain Mapp 34:2511–2523
Reichow B, Wolery M (2009) Comprehensive synthesis of early intensive behavioral interventions for young children with autism based on the UCLA young autism project model. J Austism Dev Disord 39:23–41
Richdale AL, Schreck KA (2009) Sleep problems in autism spectrum disorders: prevalence, nature, & possible biopsychosocial aetiologies. Sleep Med Rev 13:403–411
Rogers SJ, Vismara LA (2008) Evidence-based comprehensive treatments for early autism. J Clin Child Adolesc Psychol 37:8–38
Schreck KA (2001) Behavioral treatments for sleep problems in autism: empirically supported or just universally accepted? Behav Interv 16:265–278
Schreibman L, Ingersoll B (2011) Naturalistic approaches to early behavioral intervention. In: Amaral DG, Dawson G, Geschwind D (eds) Autism spectrum disorders. Oxford University Press, New York, pp 1056–1067
Simonoff E, Pickles A, Charman T, Chandler S, Loucas T, Baird G (2008) Psychiatric disorders in children with autism spectrum disorders: prevalence, comorbidity, and associated factors in a population-derived sample. J Am Acad Child Adolesc Psychiatry 47:921–929
Skuse DH (2012) DSM-5's conceptualization of autistic disorders. J Am Acad Child Adolesc Psychiatry 51:344–346
Smith T (2001) Discrete trial training in the treatment of autism. Focus Autism Other Dev Disabil 16:86–92
Stavropoulos KKM, Carver LJ (2013) Research review: social motivation and oxytocin in autism–implications for joint attention development and intervention. J Child Psychol Psychiatry 54:603–618
Steiner AM, Goldsmith TR, Snow AV, Chawarska K (2012) Practitioner's guide to assessment of autism spectrum disorders in infants and toddlers. J Autism Dev Disord 42:1183–1196
Stone WL, Yoder PJ (2001) Predicting spoken language level in children with autism spectrum disorders. Autism 5:341–361
Strain PS, Bovey EH (2011) Randomized, controlled trial of the LEAP model of early intervention for young children with autism spectrum disorders. Topics Early Child Spec Educ 31:133–154
Szatmari P, McConnell B (2011) Anxiety and mood disorders in individuals with autism spectrum disorder. In: Amaral DG, Dawson G, Geschwind D (eds) Autism spectrum disorders. Oxford University Press, New York, pp 330–338
Test DW, Richter S, Knight V, Spooner F (2011) A comprehensive review and meta-analysis of the social stories literature. Focus Autism Other Dev Disabil 26:49–62
Thomeer ML, Lopata C, Volker MA, Toomey JA, Lee GK, Smerbeck AM, Rodgers JD, McDonald CA, Smith, RA (2012) Randomized clinical trial replication of a psychosocial treatment for children with high-functioning autism spectrum disorders. Psychol Sch 49:942–954
Thurm A, Lord C, Lee LC, Newschaffer CJ (2007) Predictors of language acquisition in preschool children with autism spectrum disorders. J Autism Dev Disord 37:1721–1734
Tincani M, Devis K (2011) Quantitative synthesis and component analysis of single-participant studies on the picture exchange communication system. Remedial Spec Educ 32:458–470
van der Meer L, Sutherland D, O'Reilly MF, Lancioni GE, Sigafoos J (2012) A further comparison of manual signing, picture exchange, and speech-generating devices as communication modes for children with autism spectrum disorders. Res Autism Spectr Disord 6:1247–1257

Veness C, Prior M, Bavin E, Eadie P, Cini E, Reilly S (2012) Early indicators of autism spectrum disorders at 12 and 24 months of age: a prospective, longitudinal comparative study. Autism 16:163–177

Venter A, Lord C, Schopler E (1992) A follow-up study of high-functioning autistic children. J Child Psychol Psychiatry 33:489–507

Vismara LA, Rogers SJ (2010) Behavioral treatments in autism spectrum disorder: what do we know? Annu Rev Clin Psychol 6:447–468

Volpe RJ, Young GI, Piana MG, Zaslofsky AF (2012) Integrating classwide early literacy intervention and behavioral supports a pilot investigation. J Posit Behav Interv 14:56–64

Voos AC, Pelphrey KA, Tirrell J, Bolling DZ, Vander Wyk B, Kaiser MD, McPartland JC, Volkmar FR, Ventola P (2013) Neural mechanisms of improvements in social motivation after pivotal response treatment: two case studies. J Autism Dev Disord 43:1–10

Vriend JL, Corkum PV, Moon EC, Smith IM (2011) Behavioral interventions for sleep problems in children with autism spectrum disorders: current findings and future directions. J Pediatr Psychol 36:1017–1029

Warren Z, McPheeters ML, Sathe N, Foss-Feig JH, Glasser A, Veenstra-VanderWeele J (2011) A systematic review of early intensive intervention for autism spectrum disorders. Pediatrics, 127:e1303–e1311

Wegner JR (2012) Augmentative and alternative communication strategies: manual signs, picture communication, and speech-generating devices. In: Prelock PA, McCauley RJ (eds) Treatment of autism spectrum disorders: evidence based-intervention strategies for communication and social interactions. Paul H. Brookes, Baltimore, pp 27–48

Wetherby AM, Prutting CA (1984) Profiles of communicative and cognitive-social abilities in autistic children. J Speech Hear Res 27:364–377

White SW, Oswald D, Ollendick T, Scahill L (2009) Anxiety in children and adolescents with autism spectrum disorders. Clin Psychol Rev 29:216–229

Wilczynski SM, Rue HC, Hunter M, Christian L (2012) Elementary behavioral intervention strategies: discrete trial instruction, differential reinforcement, and shaping. In: Prelock PA, McCauley RJ (eds) Treatment of autism spectrum disorders: evidence based-intervention strategies for communication and social interactions. Paul H. Brookes, Baltimore, pp 49–77

Wong C, Kasari C (2012) Play and joint attention of children with autism in the preschool special education classroom. J Autism Dev Disord 42:2152–2161

Part II
Basic Science Aspects of Autism

Chapter 11
Autism and Dopamine

Guy Mittleman and Charles D. Blaha

Abstract In this chapter we present data from two mutant mouse strains (lurcher and *Fmr1*) that share in common with patients diagnosed with an autism spectrum disorder, the characteristic of developmental cerebellar neuropathology involving Purkinje cells. Evidence is presented indicating that Purkinje cell number has a profound influence on behaviors that are commonly disrupted in autism spectrum disorders including hyperactivity, increased repetitive behavior, and deficits in executive function. Additional experiments are presented which indicate that these behavioral deficits stem from developmental loss of cerebellar output that occurs as a function of Purkinje cell loss. Loss or dysregulation of Purkinje cell output to the deep cerebellar nuclei such as the cerebellar dentate nucleus in turn results in alterations in the functionality of cerebellar projections via the thalamus and ventral tegmental area to the medial prefrontal cortex (mPFC). This loss of functionality prominently includes reductions in cerebellar-mediated mPFC dopamine release. The reduction in mPFC dopamine release is likely caused by coincident reductions in glutamate available for release from cerebellar projections to the thalamus and ventral tegmental area (VTA). This loss of functionality also includes a shift in the balance of influence of the cerebellum on the mPFC, away from the cerebellar circuitry projecting to the ventral tegmental area, towards cerebellar projections to the thalamus. All of these changes consistently occurred in both lurcher and *Fmr1* mutant mice. In addition to modulating mPFC dopamine, the possibility that the cerebellum may also influence dopamine dynamics in the caudate and nucleus accumbens is also considered.

Keywords Autism spectrum disorders · Cerebellum · Prefrontal cortex · Dopamine · Fragile X mice · Lurcher mice · Amperometry · Dentate nucleus · Thalamus · Ventral tegmental area

C. D. Blaha (✉)
Department of Psychology, University of Memphis, 400 Innovation Drive, Memphis, TN 38152, USA
e-mail: cblaha@memphis.edu

G. Mittleman
Department of Psychological Science, Ball State University, North Quad, room 104, Muncie, IN 47306, USA
e-mail: gmittleman@bsu.edu

S. Hossein Fatemi (ed.), *The Molecular Basis of Autism,*
Contemporary Clinical Neuroscience, DOI 10.1007/978-1-4939-2190-4_11

11.1 Autism Spectrum Disorders

Autism spectrum disorders (ASD) are a group of neurodevelopmental disorders that have traditionally included autism, Asperger's syndrome, Rett syndrome, childhood disintegrative disorder, and pervasive developmental disorder not otherwise specified (American Psychiatric Association 2000). With the arrival of the DSM-5, Asperger's syndrome, pervasive developmental disorder and childhood disintegrative disorder have been consolidated into the overarching category of ASD, while Rett syndrome has been removed from the diagnostic manual (Lauritsen 2013). Disorders within the ASD spectrum are characterized by deficits in social skills and communication, stereotyped and repetitive behavior, and a range of cognitive function deficits that are diagnosed on average by 4.8 years of life, although symptoms suggestive of autism have been observed as early as 6 months of age (Rogers and Dilalla 1990; American Psychiatric Association 2000; Centers for Disease Control 2007; Ozonoff et al. 2010; Elsabbagh et al. 2012; Wolff et al. 2012). Deficits in cognitive function most commonly observed in ASD patients include impairments in memory and attention as well as in executive function including planning, cognitive flexibility, rule acquisition, and abstract thinking (Ozonoff et al. 2007).

In addition to cognitive deficits, individuals with autism commonly exhibit motor deficits (reviewed in Gowen and Hamilton 2013) which begin in infancy (Brian et al. 2008; Provost et al. 2007) and continue through adolescence and into adulthood (Fournier et al. 2010; Van Waelvelde et al. 2010). Motor appear to be relatively prevalent in this patient group, having been observed in 21–100 % of individuals with autism, depending on the sample (Ghaziuddin et al. 1994; Green et al. 2002; Manjiviona and Prior 1995; Miyahara et al. 1997; Pan et al. 2009). Individuals with autism exhibit both gross and fine motor deficits including slow and repetitive hand and foot movements (Dowell et al. 2009), slow and inaccurate manual dexterity (Green et al. 2002), unstable balance (Freitag et al. 2007), and impaired gait (Jansiewicz et al. 2006). Interestingly, motor and cognitive deficits appear to be related in autism. Specifically, better motor skills predict better daily living skills (Jasmin et al. 2009) and deficits in motor control predict increased severity of autistic symptoms in adulthood (Sutera et al. 2007).

It should also be noted that individuals diagnosed with ASD also exhibit impairments in reward processing (reviewed in Dichter and Adolphs 2012). Learning studies indicate that this deficit appears to be specific to social as opposed to non-social rewards. For example, Lin et al. (2012) compared performance of individuals with and without autism on an instrumental reward learning task in which the reward was social (pictures of positive and negative faces) or non-social (winning or losing money). Individuals with autism exhibited a specific behavioral insensitivity to social rewards. In addition to learning studies, autonomic response studies also indicate that individuals with autism are impaired in social reward processing. For example, Sepeta et al. (2012) examined the pupillary response in children with and without autism and found that, in comparison to controls, children with autism exhibited decreased pupillary diameter when looking at happy faces. These authors interpreted these differential findings as indicative of reduced sensitivity to reward

value of social stimuli in children with autism. Gaze orientation studies in which children with autism fail to orient to naturally occurring social stimuli also provide evidence of impaired social reward processing in autism (Dawson et al. 1998; Klin et al. 2009). Neuroimaging studies indicate that differences in reward circuitry likely underlie the observed differential performance of controls and children with autism on behavioral tasks (Dichter et al. 2012a, b).

11.2 Cerebellar Modulation of the mPFC

As many of the cognitive deficits observed in ASD have been found to be related to frontal cortical function (see Clark et al. 2004; Dalley et al. 2004; Robbins 2005) and as autistic children exhibit low mPFC dopaminergic activity compared to age and gender matched controls when measured by positron emission tomography (Ernst et al. 1997), we began by exploring the hypothesis that disruption of cerebellar-mPFC projections could lead to a dysregulation of dopaminergic-mediated activity in mPFC, and thereby cortically-mediated behaviors. This hypothesis came about because cerebellar hypoplasia and reduced cerebellar Purkinje cell numbers are the most consistent neuropathologies linked to ASD (Bauman 1991; Courchesne et al. 1988, 1994; Courchesne 1997; Palmen et al. 2004; DiCicco-Bloom et al. 2006; Bolduc et al. 2011). Patients have numerous microanatomic abnormalities in the cerebellum (Bailey et al. 1998; Bauman and Kemper 2005; Vargas et al. 2005; Whitney et al. 2008, 2009; Fatemi et al. 2012) and smaller cerebellar vermal volume (Webb et al. 2009). Quantitative MRI of autistic children revealed structural abnormalities in the frontal lobe the extent of which correlated with the degree of cerebellar abnormality (Carper and Courchesne 2005).

In our initial studies we used lurcher mutant and wildtype (control) mice (gene symbol: *Lc*/+ and +/+, respectively). Lurcher mice were specifically selected because they lose all cerebellar Purkinje cells between the 2nd and 4th weeks of life (Wetts and Herrup 1982); a time that is analogous to the loss of cells in autistic individuals (Pickett and London 2005). The lurcher gene codes for the δ2-glutamate receptor that is expressed in cerebellar Purkinje cells (Wetts and Herrup 1982; Zuo et al. 1997). To determine if loss of cerebellar Purkinje cells affected functioning in the mPFC we used in vivo fixed potential amperometry to monitor evoked mPFC dopamine release (efflux) following electrical stimulation of the cerebellar Purkinje cell layer (PCL) and one of the deep cerebellar nuclei in receipt of Purkinje cell outflow, the dentate nucleus (DN).

Mice were anesthetized with urethane (1.5 g/kg, i.p.) and placed in a stereotaxic frame. Body temperature was maintained at 37 ± 0.5 °C with a temperature-regulated heating pad. Three holes were drilled through the animal's skull for an Ag/AgCl reference/auxiliary electrode (positioned at the surface of the cortex), a concentric bipolar stimulating electrode (125 μm outer and 25 μm inner pole diam.), and a dopamine recording electrode (carbon fiber 10 μm o.d. 250 μm length; Forster and Blaha 2003; Lee et al. 2006). A stimulating electrode (tip) was positioned in the PCL and in the cerebellar DN, contralateral to the dopamine recording electrode

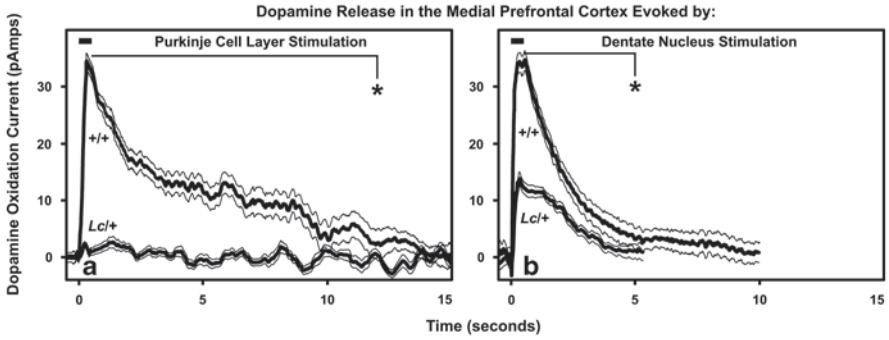


Fig. 11.1 Electrical stimulation (*black* bar, 25 pulses at 50 Hz, 200 μA) of the **a)** Purkinje cell layer (*PCL*) or **b)** dentate nucleus (*DN*) evokes a long lasting increase in medial prefrontal cortex dopamine oxidation current (*corresponding to release*) of wildtype (+/+) mice; an effect that is absent and markedly attenuated, respectively, in PCL and DN stimulated lurcher(Lc/+) mice. In (**a** and **b**) the *thick black lines* and outer *thin black lines* are the mean ± SEM, respectively ($n = 6$ and 10 for PCL and DN stimulation in +/+ mice, respectively, $n = 10$ and 10 for Lc/+ mice, respectively). * indicates significant difference from 0.5 to 12 s between +/+ and Lc/+ mice following PCL stimulation and significant difference from 0.5 to 5 s between +/+ and Lc/+ mice following DN stimulation. (Modified from Mittleman et al. (2008) with permission from Wiley)

positioned in the mPFC. Following implantation of all electrodes, a fixed positive potential (+0.8 V) was applied to the recording electrode and oxidation current monitored continuously (10K samples/sec) with an electrometer, filtered at 10 Hz low pass. Electrical stimulation of the PCL or DN (counterbalanced within individual +/+ and *Lc*/+ mice) consisted of 50 Hz trains of 25 cathodic monophasic pulses (200 μA intensity, 0.5 ms pulse duration) applied every 30 s over a 6 min period via an optical stimulus isolator and programmable pulse generator.

Figure 11.1 shows the results of this experiment (Mittleman et al. 2008). As expected, due to the developmental loss of Purkinje cells in *Lc*/+ mice, electrical stimulation of the PCL failed to evoke an increase in mPFC oxidation current. In comparison, stimulation of the PCL in +/+ mice evoked a significant increase in mPFC oxidation current that peaked within 0.4 s of stimulation and slowly declined thereafter to pre-stimulation levels within 11–12 s. Stimulation of the DN also resulted in significant genotype-related differences in that +/+ mice showed a much larger increase in mPFC dopamine oxidation current than *Lc*/+ animals. These results clearly indicated that functional Purkinje cell output through the DN exerts a modulatory influence on mPFC dopamine release and that this modulatory influence is lost or degraded by developmental loss of Purkinje cells.

11.3 Cerebellar-Cortical Pathways

On the basis of our initial neurochemical studies (Mittleman et al. 2008), we identified two potential neuronal circuits originating in the DN by which Purkinje cell activity in the cerebellum may modulate dopamine release in the mPFC (Fig. 11.2).

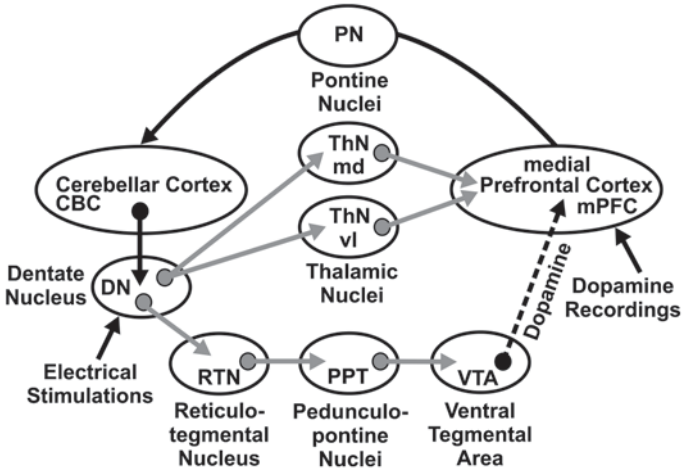


Fig. 11.2 Neuronal circuitry underlying cerebellar modulation of medial prefrontal cortex (*mPFC*) dopamine neurotransmission. Cerebellar modulation of dopamine release in the mPFC may occur via polysynaptic inputs from deep cerebellar nuclei, such as the dentate nucleus, to dopamine-containing cells in the ventral tegmental area or via a monosynaptic input to thalamic (*md, mediodorsal; vl, ventrolateral*) projections making close appositions with dopamine terminals in the mPFC. Glutamatergic pathways are shown as *gray arrows* and the dopaminergic pathway as a *dashed arrow*. *Black curved arrow* represents mPFC feedback to cerebellum via pontine nuclei. Nuclei abbreviations are shown in the *ovals*. (Modified from Mittleman et al. (2008) with permission from Wiley)

With the exception of the flocculonodular lobe which projects to vestibular nuclei in the brainstem, GABA-containing Purkinje cells in the cerebellar cortex project ipsilaterally to three deep cerebellar nuclei (dentate, interpositus, and fastigial) where they make on the order of 1000 contacts each with several types of nuclear cells (Teune et al. 1998, 2000; Llinas et al. 2004; Ruigrok 2011). Excitatory inputs entering the cerebellum as mossy fibers excite neurons in deep cerebellar nuclei via collaterals and then excite granule cells that stimulate Purkinje cells. The inhibitory output of Purkinje cells on deep cerebellar nuclei, in turn, modulates spatial and temporal patterns of activity of neurons within these nuclei, and therefore the output to various brain regions.

Deep cerebellar nuclei projections are generally very widespread and may be found throughout the entire brain stem, including the thalamus (Teune et al. 2000; Ruigrok 2011). With respect to the first cerebellar-mPFC circuit, the DN and the interpositus nucleus both have in common excitatory glutamatergic neurons that send contralateral projections to reticulo-tegmental nuclei of the pons (RTN) in a topographical manner (also known as the nucleus reticularis tegmenti pontis) (Angaut et al. 1985; Torigoe et al. 1986; Teune et al. 2000; Schwarz and Schmitz 1997). In addition to the RTN providing reciprocal excitatory glutamatergic projections back to the DN and interpositus nucleus (Cicirata et al. 2005; Mihailoff 1993; Gerrits and Voogd 1987), the RTN also sends rostral neuronal projections to pedunculopontine tegmental nuclei (PPT), which provide reciprocal projections back to the RTN

(Garcia-Rill et al. 2001; Reese et al. 1995; Vertes et al. 1986). Excitatory cholinergic and glutamatergic neurons, including neurons co-containing glutamate and acetylcholine, within the rostral and caudal aspects of the PPT project to dopamine-containing cells in the ventral tegmental area (VTA) and substantia nigra compacta (SNc) (Oakman et al. 1995, 1999; Lavoie and Parent 1994). Electrophysiological studies have shown that neurons in the RTN respond with prolonged spiking activity (up to 12 s) to stimulation of the PPT (Garcia-Rill et al. 2001). This sustained activity of RTN neurons provides reciprocally sustained activation of PPT glutamatergic/cholinergic afferents to dopaminergic cells in the VTA and SNc. These findings are consistent with those showing in rodents that a similar period of electrical stimulation of the PPT effectively enhances neurotransmission in both midbrain mesocortical and nigrostriatal dopaminergic systems (Blaha and Winn 1993; Blaha et al. 1996; Forster et al. 2002; Forster and Blaha 2003). Dopaminergic neurons in the VTA comprising the mesocortical dopaminergic system project mainly to limbic associative cortices such as anterior cingulate, insular, piriform, perirhinal, and entorhinal cortices, including the mPFC (Björklund and Lindvall 1984; Berger et al. 1988, 1991; Gaspar et al. 1989). Thus, it is noteworthy that cortical dopamine neurotransmission, particularly in the mPFC, has been shown to be associated with autism (Ernst et al. 1997), schizophrenia (Bennett 1998; Laruell et al. 2003), emotional processing (Morrow et al. 1999), and a variety of cognitive functions including attention, working memory, and planning (Aalto et al. 2005; Gamo et al. 2010; Jackson and Moghaddam 2004; Rose et al. 2010).

It is also worth noting that, in addition to a DN-RTN-PPT-VTA circuit mediating mPFC dopamine release, injections of the anterograde tracer biocytin into deep cerebellar nuclei of squirrel monkeys reveal fine collaterals from superior cerebellar peduncle fibers terminating onto dendrites and cell bodies in the PPT that contain choline acetyltransferase (Hazrati and Parent 1992). As well, Perciavalle et al. (1989) have shown in the rat that the DN, and to a lesser extent the interpositus nucleus, may provide direct contralateral projections to the VTA and dorsal region of the SNc, midbrain regions containing a high density of dopaminergic cells. Thus, it is possible that more direct circuits, such as DN-PPT-VTA or DN-VTA, may participate in modulating mesocortical dopaminergic cell activity.

A second cerebellar-mPFC neuronal circuit that may mediate dopamine release in the mPFC involves deep cerebellar nuclei glutamatergic projections to thalamic nuclei that in turn project to the cortex. Middleton and Strick (1997, 2001) have shown in cebus monkeys that cerebellar projections to prefrontal, oculomotor, and skeletomotor areas of cortex via contralateral thalamic relay nuclei are derived from topographically distinct regions of the ventral DN comprising 60 % of the volume of this cerebellar nuclei. In particular, using retrograde labelling, these investigators demonstrated that the DN to mPFC projections, via mediodorsal and ventrolateral thalamic nuclei (ThN md and ThN vl, respectively), target exclusively prefrontal cortex (PFC) areas 9 and 46-dorsal, while primarily avoiding ventral PFC areas 12 and 46-ventral. Interestingly, the majority of labelled neurons (90–100 %) were found in the DN, and only a small number (0–6 %) were located in the interpositus and fastigial nuclei. Similar findings of near exclusive projections to thalamic

nuclei from DN, compared to fastigial and interpositus nuclei, have been reported in cynomolgus monkeys (Erickson et al. 2004).

In the rodent there are four structural divisions of the mPFC: the medial agranular (AGm), anterior cingulate (AC), prelimbic (PL), and infralimbic (IL) cortices. The dorsal mPFC (AGm and AC) receive mainly sensorimotor input, whereas ventral mPFC (PL and IL) receive primarily limbic input. Thus, integration of information from the thalamus, including other subcortical structures, in the dorsal mPFC is thought to mediate goal directed actions. In contrast, the PL region of the ventral mPFC is positioned to serve a direct role in cognitive functions homologous to dorsolateral PFC of primates, whereas IL region of the ventral mPFC appears to represent a visceromotor center homologous to the orbitomedial PFC of primates (Hoover and Vertes 2007). In this regard, a number of tract tracing studies in rats have shown that thalamic nuclei, primarily ThN md compared to ThN vl, send glutamate-containing projections (Pirot et al. 1994; Pinto et al. 2003) predominantly to the limbic PL region of the ventral mPFC, compared to sensorimotor AGm or AC regions of the dorsal mPFC (Condé et al. 1990; Hoover and Vertes 2007). The PFC then projects back to the cerebellum via pontine nuclei completing a cerebellum-cortical- cerebellum loop (Kelly and Strick 2003; Middleton and Strick 2000).

At the cellular level, in rodents, primates, and humans, VTA dopaminergic afferents provide dense input to the deep layers V–VI in the mPFC, while the density of dopamine innervation in the superficial layers I–III varies across species (relatively sparser in rodents). Dopamine terminals in the mPFC form symmetric synapses on dendritic spines and shafts of pyramidal neurons in layers V–VI. Many of the postsynaptic spines innervated by dopamine terminals also receive asymmetric excitatory amino acid (glutamate) terminals forming a synaptic "triad" whereby pre- and postsynaptic modulation between dopamine and excitatory afferents to pyramidal cells, including GABAergic interneurons, may occur (Goldman-Rakic et al. 1989; Verney et al. 1990; Smiley and Goldman-Rakic et al. 1989; Carr and Sesack 1996; Paul et al. 2013). Indeed, morphological studies have shown that glutamate-containing terminals are in close apposition to dopamine-containing terminals in the mPFC (Pinto et al. 2003; Del Arco and Mora 2005), suggesting that glutamate exerts a local modulation of mPFC dopamine release. In support of this notion, reverse microdialysis of glutamate agonists and antagonists into the PFC has been shown to increase and decrease mesocortical dopaminergic transmission by activation and inhibition of ionotropic glutamatergic receptors in the PFC (Feenstra et al. 1995; Jedema and Moghaddam 1994). For example, antagonism of AMPA glutamate receptors in the PFC profoundly reduces dopamine release in the PFC suggesting that basal output of mesocortical dopamine is under tonic excitatory control from glutamatergic input to the PFC (Takahata and Moghaddam 1998). Although unlikely, an additional mechanism by which the cerebellum mediates dopamine release in the mPFC may involve thalamic activation of descending mPFC glutamatergic projections to the PPT and/or VTA that, in turn, stimulate mesocortical dopaminergic neurons to release dopamine in the mPFC (Overton and Clark 1997; Del Arco and Mora 2009).

11.4 Dopamine Physiological Dynamics in the mPFC

As noted above, the mPFC receives dense dopaminergic projections from the VTA that are essential for optimal cognitive function. A number of studies have suggested that dopamine inputs and GABAergic interneuron interactions with cortical pyramidal neurons serve to fine-tune recurrent excitation in mPFC networks that may ultimately underlie working memory and executive function (Sawaguchi and Goldman-Rakic 1991; Rao et al. 2000; Constantinidis et al. 2002). Electrophysiological studies in rat brain slices have shown that bath applied dopamine evokes a complex, temporally biphasic effect on inhibitory synaptic responses in the mPFC, as exhibited by an initial suppression, followed by a long-lasting facilitation of evoked IPSCs (Seamans et al. 2001a; Seamans and Yang 2004; Trantham-Davidson et al. 2004). The initial suppression of GABA interneuron IPSCs in the mPFC is thought to occur via activation of postsynaptic D2 receptors. The longer latency enhancement of IPSCs has been shown to be mediated by D1 receptors that increase the excitability of presynaptic GABAergic interneurons that ultimately modulate postsynaptic activity of cortical pyramidal cells (Trantham-Davidson et al. 2004). A similar D2-mediated initial suppression followed by a D1-mediated long-lasting facilitation has also been observed for excitability of cortical pyramidal cells and thus may be a common feature of dopaminergic modulation of mPFC neurons (Yang and Seamans 1996; Gulledge and Jaffe 1998; Gorelova and Yang 2000; Henze et al. 2000). In addition, D2 agonists have been shown to reduce NMDA glutamate receptor EPSCs, whereas D1 agonists increase them in mPFC neurons (Zheng et al. 1999; Seamans et al. 2001b).

Yang and co-workers have termed the D2-mediated initial suppression as the D2-dominated network dynamic “state 1” (Seamans et al. 2001a; Seamans and Yang 2004) and have suggested that state 1 may be important in situations requiring response flexibility, during which many options for action must be held in memory and compared. Synaptically located D2 receptors may respond first to the high phasic dopamine levels evoked by a particularly novel or salient stimulus. This would create a state 1 (transient) dynamic before dopamine diffuses into the extrasynaptic space, activating D1 receptors to initiate a state 2 (long-lasting) dynamic (Seamans and Yang 2004). Thus, the initial D2-mediated transient attenuation of IPSCs may function to reset PFC networks so that the robustness-enhancing effects of D1 receptor activation are reversed momentarily, allowing new information easy access to working memory buffers and the establishment of new goal-state representations. Under these conditions, state 1 would favor weak activation of a widespread cortical area, whereas state 2 would favor heightened “tuned” activation of a smaller and more focused cortical region (Durstewitz et al. 2000; Seamans et al. 2001a; Seamans and Yang 2004).

As such, dopamine does not provide PFC networks with specific information but rather sets them into one of two processing states. In either state, working memory buffers remain able to retain and use information transiently. Glutamatergic and GABAergic systems appear to be critical for the actual generation of persistent

activity patterns (Seamans et al. 2003). Dopamine only biases the expression of this activity, making glutamate- and GABA-mediated persistent activity more robust (state 2) or weaker yet more receptive to subsequent inputs (state 1). In this way dopamine is a true neuromodulator, acting on glutamate and GABA systems, biasing the way they process and maintain information in working memory, although providing no information itself to be held in working memory.

11.5 Developmental Loss of Cerebellar Purkinje Cells Produces Behavioral Deficits Similar to Those Common to ASD

Our initial neurochemical results indicating a dysregulation of cerebellar mediated dopamine transmission in the mPFC of lurcher mutant mice (Mittleman et al. 2008) provided a potential mechanism whereby developmental cerebellar damage and accompanying alterations in cerebellar-mPFC neuronal circuitry could lead to some of the behavioral deficits typically observed in ASD (Nieoullon 2002). In order to further investigate the hypothesis that developmental cerebellar damage could result in behavioral deficits we specifically targeted two behaviors in which patients with ASD have prominent defects; repetitive behavior and executive function. Specifically, it has been reported that repetitive and restrictive behaviors are a major behavioral component of ASD (e.g. Hutt and Hutt 1968; Bodfish et al. 2000; Militerni et al. 2002). In autism, repetitive behavior may function to control behavior-environment relations such as access to reinforcers, or to gain access to or eliminate particular types of stimulation such as hyperarousal (Ridley 1994; Kennedy et al. 2000). Hyperactivity often accompanies repetitive behavior in developmental disorders with approximately 30% of ASD children comorbid for attention deficit/hyperactivity disorder (ADHD; Simonoff et al. 2008). Thus, the common goal of the behavioral experiments was to determine if mice showed deficits similar to those observed in ASD, and more importantly to determine if these deficits related to Purkinje cell number.

In order to explore the relationship between repetitive behavior and cerebellar Purkinje cell number we used chimeric mice (*Lc*/+↔+/+) with varying proportions of cells from the wildtype +/+ and heterozygous *Lc*/+ genotypes (Goldowitz et al. 1992). Depending on incorporation of the wildtype lineage, 50% of the chimeric mice would be expected to be truly chimeric, or of a mixed genotype (*Lc*/+↔+/+). Thus total cerebellar Purkinje cell numbers in chimeric animals would be expected to vary, while very few Purkinje cells (<200) would be expected in *Lc*/+ mice. In contrast, +/+ mice would be expected to have a full complement of Purkinje cells (>150,000). Total numbers of cerebellar Purkinje cells were determined at the conclusion of the experiment by histological analysis of cerebellar Purkinje cell counts (Martin et al. 2010).

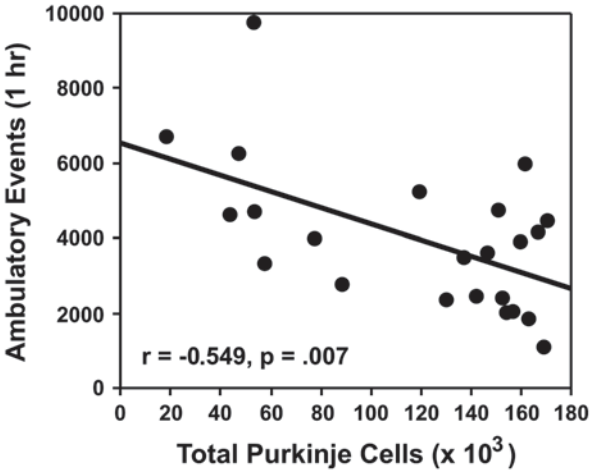


Fig. 11.3 Open Field Monitoring. Scatterplot showing the relationship between total cerebellar Purkinje cell number and one measure of ambulation during open-field exploration. Total Purkinje cell number was negatively related to ambulatory events ($r = -0.549$, $df = 21$, $p = 0.007$) as determined by Pearson correlations. (Modified from Martin et al. (2010) with permission from Wiley)

Chimeric mice were tested in two behavioral paradigms involving unconditioned ambulatory activity during exposure to a novel open field, and conditioned lever pressing in progressive ratio (PR) responding. The PR task is a commonly used lever-press task that programs a systematic increase in the value of the fixed-ratio (FR) requirement following reinforcer delivery. Subjects are free to lever-press as frequently or infrequently as they choose, but must make progressively more lever-presses to obtain successive reinforcers. In this case, subjects were reinforced for lever-pressing under an arithmetically increasing FR 5 schedule of reinforcement (i.e. 5, 10, 15, 20, etc lever-presses were necessary to obtain reinforcement), using a procedure similar to that described by Hodos and Kalman (1963). An experimental session terminated when the subject failed to lever-press for 5 consecutive minutes. The last ratio completed (reinforced) is known as the subject's *breakpoint* and is typically followed by a pause in responding known as the *post-reinforcer pause* (PRP). As repetitive behavior can take any form and occur in virtually any environment (Kennedy et al. 2000), we reasoned that this task would allow us to explore the role of cerebellar pathology in repetitive behaviors similar to those observed in autism.

Figure 11.3 shows the amount of ambulatory activity that occurred during a 1 h exposure to a novel open field as a function of Purkinje cell numbers in chimeric mice. A significant negative relationship between Purkinje cell numbers and ambulation was observed in that chimeric mice with low numbers of Purkinje cells were progressively more active than mice with higher numbers of these cells.

Figure 11.4a and b describes the relationship between Purkinje cells and repetitive lever-pressing in the PR task in *Lc*/+↔+/+ chimeras. Within the group of chimeric mice breakpoint was significantly and negatively correlated with the number of Purkinje cells in the cerebellum. We believe that the increases in lever-pressing that resulted in higher breakpoints in chimeric mice represent a repetitive and stereotyped behavior. As defined by Ridley (1994), stereotypy, in contrast to perse-

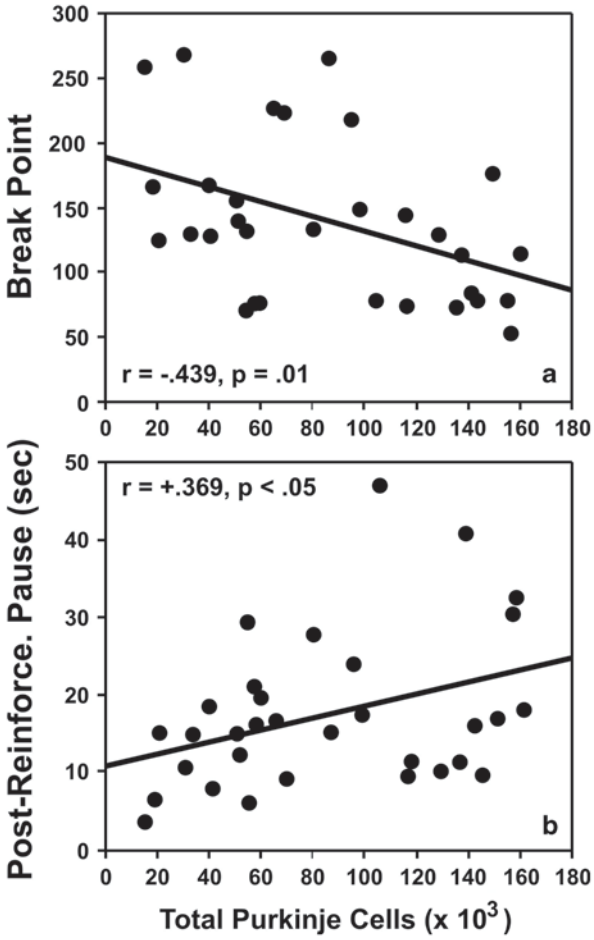


Fig. 11.4 Scatterplots showing the relationships between total cerebellar Purkinje cell number and both breakpoint and post-reinforcement pause. **a** Total Purkinje cell number was negatively related to breakpoint as revealed by Pearson correlations ($r = -0.439$, d.f. $= 31$, $p < 0.01$). Breakpoint declined as total Purkinje cell number decreased. **b** Total Purkinje cell number was positively related to post-reinforcement pause ($r = 0.369$, d.f.$= 31$, $p < 0.05$) in that mice with lower numbers of Purkinje cells had shorter post-reinforcement pauses. (Modified from Martin et al. (2010) with permission from Wiley)

veration, refers to "the excessive production of one type of motor act, or mental state, which necessarily results in repetition." In our case, Purkinje cell deficient mice were trained in a repetitive task to associate lever pressing with delivery of a primary reward. The significant positive relationship between Purkinje cell number and post-reinforcement-pause (Fig. 11.4b) demonstrates the increased focus of these mice on lever-pressing, as those chimeras with the fewest Purkinje cells, and therefore the highest breakpoints, paused for the shortest amount of time following reinforcement. This experiment supported the hypothesis that developmental cerebellar damage could result in behavioral deficits, specifically by demonstrating similar relationships between cerebellar Purkinje cell number, hyperactivity and repetitive behavior.

In the next experiment, we investigated the possibility that Purkinje cell number was also related to the degree of deficits in executive function. Executive function is an umbrella term for the group of closely linked, high level cognitive skills which

enable the effective execution of goal-directed behaviors (Hughes et al. 1994; Pennington and Ozonoff 1996). These skills, which include working memory, inhibition, and behavioral flexibility, have consistently been shown to be dependent on the PFC in humans, non-human primates, and rodents (Dalley et al. 2004; Robbins and Arnsten 2009; Robbins and Roberts 2007). Cognitive theories of autism suggest that fundamental deficits in executive function may underlie the clinically significant symptoms of this disorder (Pennington and Ozonoff 1996; Hill 2004).

In this study, we assessed the impact of variable developmental cerebellar Purkinje cell loss on behavioral flexibility using a chimeric mouse model (*Lc*/+ ↔ +/+). Specifically we investigated the acquisition and serial reversals of a conditional visual discrimination task, one type of reversal learning task used to assess behavioral flexibility in rodents. Reversal learning tasks measure behavioral flexibility by assessing the ability of the subject to adapt its behavior following a reversal of stimulus-reward or stimulus-response contingencies, and have been shown to depend heavily on the PFC (Clark et al. 2004; Ragozzino 2007). Two stages are involved in reversal learning; inhibition of a previously rewarded strategy and acquisition of a new strategy (Mackintosh 1974). Normal animals and humans typically show improvement in reversal learning across reversals. Thus, as perseverative responses decline, a faster rate of switching to the new response occurs. Essentially the subject learns that if the current response is incorrect, the other response must be correct. In this experiment, mice initially were trained in a simultaneous (simple) visual discrimination using procedures of Jones et al. (1991) of the type "respond where the light is" (cue light = S+) or "respond where the light is not" (cue light = S−). This task can be learned simply by approaching cues predictive of reward and thus requires no response-rule learning. Once the criterion for acquisition was met, mice were then tested on a series of reversals of the discrimination contingency (e.g. if they learned, "respond where the light is," they then must learn, "respond where the light is not"). The acquisition of reversals of stimulus-reward reversals is captured in two types of errors. Errors committed during sessions in which performance was below chance levels (≤40 % correct) were classified as *Perseverative* errors, suggesting that during these sessions mice continued to respond according to the response rules of the prior stage. Errors committed during sessions in which performance was above chance levels (41–85 % correct) were classified as *Learning* errors, suggesting that mice had shifted away from the use of the response rules of the prior stage.

All mice learned the initial visual discrimination and were then tested over 4 stimulus-reward reversals. Figure 11.5 shows the relationship between learning errors and Purkinje cell numbers in chimeric mice during reversals 3 and 4. Comparison of Purkinje cell number and performance in individual mice revealed that chimeras with fewer Purkinje cells committed more Learning errors than those with higher numbers of these cells. These data suggest that developmental cerebellar Purkinje cell loss can affect higher level cognitive processes which have previously been shown to be mediated by the mPFC, are disrupted in patients bearing cerebellar lesions (Bellebaum and Daum 2007), and are commonly deficient in ASD (Hill 2004; Pennington and Ozonoff 1996).

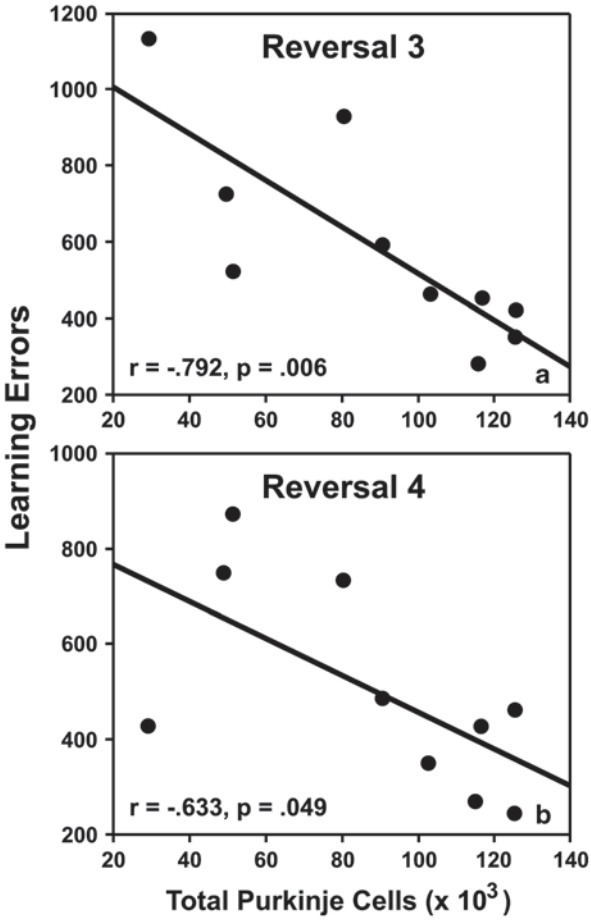


Fig. 11.5 In chimeric mice, there was a significant negative relationship between Purkinje cells and learning errors (those committed while session performance was between 41 % and 85 % correct) during reversals 3 (*top* panel) and 4 (*bottom* panel). (Modified from Dickson et al. (2010) with permission from Elsevier)

11.6 Individual Pathway Contributions to mPFC Dopamine Release in Lurcher Wildtype and Lc/+ Mutant Mice

As our behavioral experiments, together with the neurochemical results of Mittleman et al. (2008), strongly suggested that mPFC function was altered in direct relationship to the number of cerebellar Purkinje cells, and that Purkinje cell loss could disrupt dopamine transmission in the mPFC, we next investigated the relative contribution of each of the two candidate neuronal circuits in modulating mPFC dopamine release (Rogers et al. 2011). We again used in vivo fixed potential amperometry in combination with carbon-fiber microelectrodes (as in Mittleman et al. 2008) to monitor mPFC dopamine release evoked by DN electrical stimulation (100 cathodic monophasic pulses, 400 μA intensity, 0.5 ms pulse duration) at 50 Hz

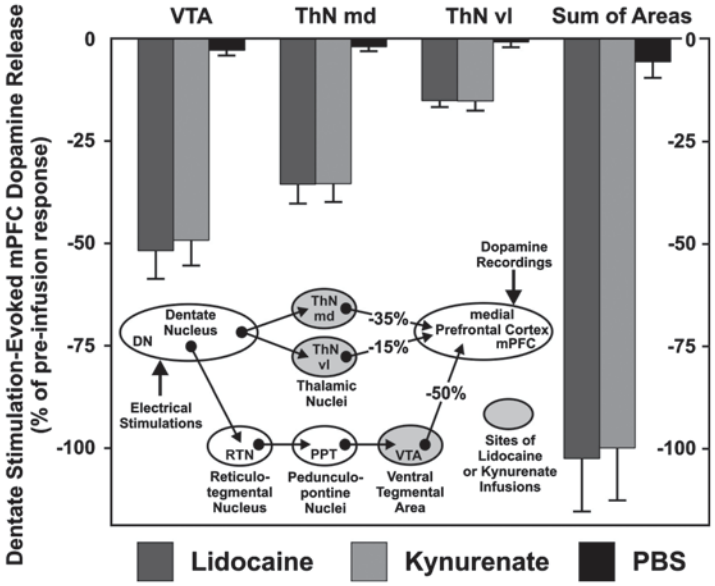


Fig. 11.6 Average percent decrease in dentate nucleus (*DN*) stimulation-evoked dopamine responses following lidocaine, kynurenate, or phosphate buffered saline (*PBS, control*) infusions into the ventral tegmental area (*VTA*), mediodorsal thalamus (*ThN md*), and the ventrolateral thalamus (*ThN vl*), as well as the summed average percent decrease of each drug infused across sites. Infusions of kynurenate or lidocaine into the VTA, ThN md, and ThN vl of separate groups of mice decreased the dopamine response by approximately 50, 35, and 15%, respectively while phosphate buffered saline (*PBS, control*) had no significant effect in any group. (Modified from Rogers et al. (2011) with permission from Wiley)

every 60 s before and during individual microinfusions of the local anesthetic lidocaine, the ionotropic glutamate receptor antagonist kynurenate, and the control (drug vehicle) solution phosphate buffered saline (PBS) into the ThN md, ThN vl, or VTA of urethane anesthetized lurcher wildtype (+/+) mice.

As shown in Fig. 11.6, the DN to mPFC pathway via dopaminergic neuronal cell bodies in the VTA was decreased by either lidocaine or kynurenate by ~50%, while the DN to mPFC via thalamic nuclei was also decreased similarly by either lidocaine or kynurenate by ~50% (35% in the ThN md and 16% in the ThN vl). Thus, the sum of the percent decreases occurring in each of these two pathways accounted for 100% of DN stimulation-evoked dopamine release recorded in the mPFC. PBS infusions resulted in relatively minimal site-specific average percent decreases of 2.8%±1.3%, 2.0%±1.1% and 0.8%±1.3% for VTA, ThN md, and ThN vl, respectively. The results of this study, therefore showed that in lurcher wildtype mice, the pathway through the thalamus (ThN md and ThN vl) and the pathway through the VTA each contribute 50% of the modulatory influence on mPFC dopamine neurotransmission. Additionally, reductions in dopamine release following lidocaine or kynurenate infusions were not significantly different which indicates that neuronal cells in the VTA and ThN were activated primarily if not entirely by glutamatergic inputs.

As these results indicated an equal contribution of the thalamic and VTA pathways to mPFC dopamine release, and those of Mittleman et al. (2008) showed that mPFC dopamine release was reduced in lurcher (*Lc*/+) mutant mice, the next experiment investigated the relative contributions of these pathways in *Lc*/+ mice (Rogers et al. 2013a). The same methods as Rogers et al. (2011) were used. Figure 11.7 (top panel) shows that *Lc*/+ mice exhibit a significant reduction in DN stimulation evoked dopamine release in the mPFC. Figure 11.7 (bottom panel) also shows that in *Lc*/+ mice there was a functional reorganization of the VTA and thalamic

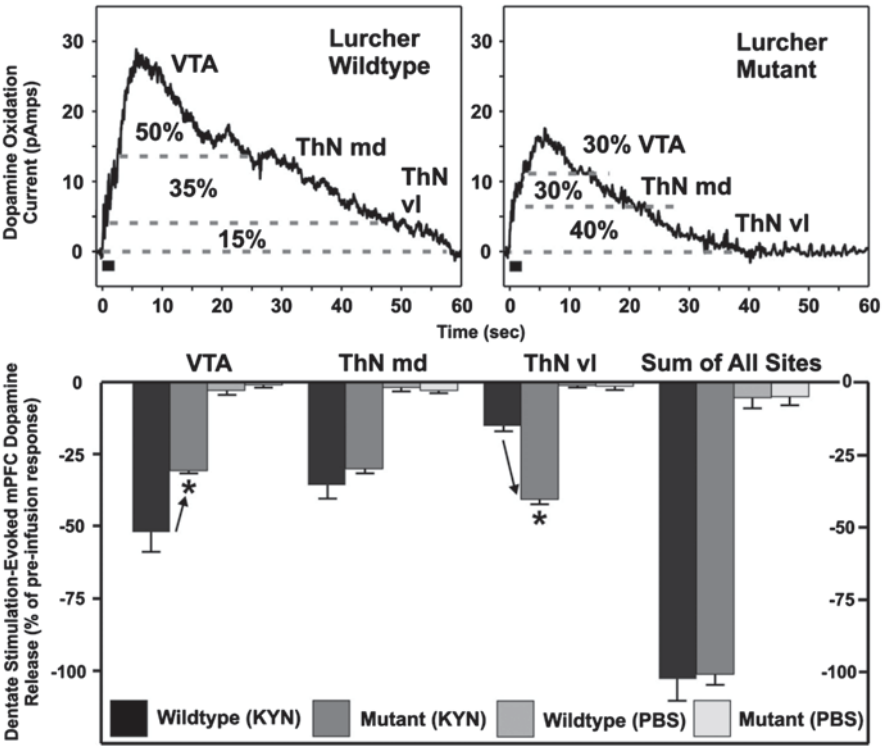


Fig. 11.7. Individual examples of medial prefrontal cortex dopamine release responses (*black bar*, 100 pulses at 50 Hz) (*top* panel) and bar histograms (*bottom* panel) showing the mean ± SEM percent decrease in dentate nucleus stimulation-evoked dopamine responses following kynurenate infusion into the ventral tegmental area (*VTA*), mediodorsal thalamus (*ThN md*), or the ventrolateral thalamus (*ThN vl*), as well as the summed average percent decrease of each drug infused sites in lurcher mutant vs. wildtype mice. In lurcher wildtype mice infusions of kynurenate into the VTA reduced dopamine responses by ~50%, while kynurenate infusions into the thalamus (md and vl combined) also reduced the dopamine response by a total of ~50% (*md* =~35% and *vl* =~15%). In contrast, the reduction in the dopamine response following kynurenate into the VTA in lurcher mutant mice (~30%) was significantly less, indicating a reduction in the modulatory strength of this pathway. This reduction in strength in mutant mice was coupled to an increase in signal strength of the pathway through the thalamus, specifically the ThN vl. Thus, kynurenate infused into this nucleus reduced dopamine responses by ~15% in wildtype mice compared to ~40% in mutant mice. (Modified from Rogers et al. (2013a) with permission from Springer)

pathways mediating cerebellar modulation of mPFC dopamine release. Following electrical stimulation of the DN, inactivation of the VTA pathway by intra-VTA lidocaine or kynurenate infusions decreased dopamine release by 50% in wildtype and 30% in *Lc*/+ mutant mice. Intra-ThN vl infusions of either drug decreased dopamine release by 15% in wildtype and 40% in mutant mice, while dopamine release remained relatively unchanged following intra-ThN md drug infusions. These results indicated a shift in strength towards the thalamic vl projection, away from the VTA. These results were consistent with the idea that developmental loss of cerebellar Purkinje cells results in a reorganization of the neuronal pathways mediating mPFC dopamine release that ultimately contributes to a reduction in mPFC dopamine release.

11.7 Individual Pathway Contributions to mPFC Dopamine Release in *Fmr1* Wildtype and Mutant Mice

One limitation of these results is that, although lurcher mutant mice represent a relatively good and reasonably selective model of cerebellar Purkinje cell loss, there is no known human equivalent to this genetic condition (i.e. 100% Purkinje cell loss). This observation necessarily limits the applicability of this mouse model to ASD, although both share the common feature of cerebellar Purkinje cell loss. In order to overcome this limitation we replicated the previous experiment using *Fmr1* mutant and wildtype mice. Fragile X syndrome is one of the forms of ASD with a known monogenetic cause, the loss of function of the *Fmr1* gene. *Fmr1*-KO (knockout) mice have an *Fmr1*^{tm1Cgr} targeted mutation and are widely used as a mouse model of Fragile X syndrome (Goodrich-Hunsaker et al. 2011; Verkerk et al. 1991; Rogers et al. 2013b). These KO "mutant"mice display several behavioral deficits similar to those seen in ASD such as hyperactivity, perseverative behavior, reduced spatial learning abilities, memory deficits, and reduced fear conditioning (Bernardet and Crusio 2006; Ey et al. 2011; Olmos-Serrano and Corbin 2011). *Fmr1* mutant mice display cerebellar abnormalities such as elongated Purkinje cell spines and decreased volume of deep cerebellar nuclei, which may also be indicative of reduced cerebellar output (Koekkoek et al. 2005; Ellegood et al. 2010). Abnormalities also exist in the PFC of *Fmr1* mutant mice. For example, layer 5 pyramidal neurons display hyperconnectivity in the mPFC, and synapses between these neurons are not able to recover from long term depression as rapidly as the same neurons in control mice (Testa-Silva et al. 2011).

The electrical stimulation and neurochemical recording methods of Mittleman et al. (2008) and Rogers et al. (2011) were used. We initially compared the magnitude of dentate stimulation-evoked dopamine release in *Fmr1* mutant and wildtype mice. Figure 11.8 (top panel) shows that, like *Lc*/+ mice, *Fmr1* mutants showed a significant reduction in mPFC dopamine release evoked by stimulation of the DN. Figure 11.8 (bottom panel) also indicates that in *Fmr1* mutant mice there was a

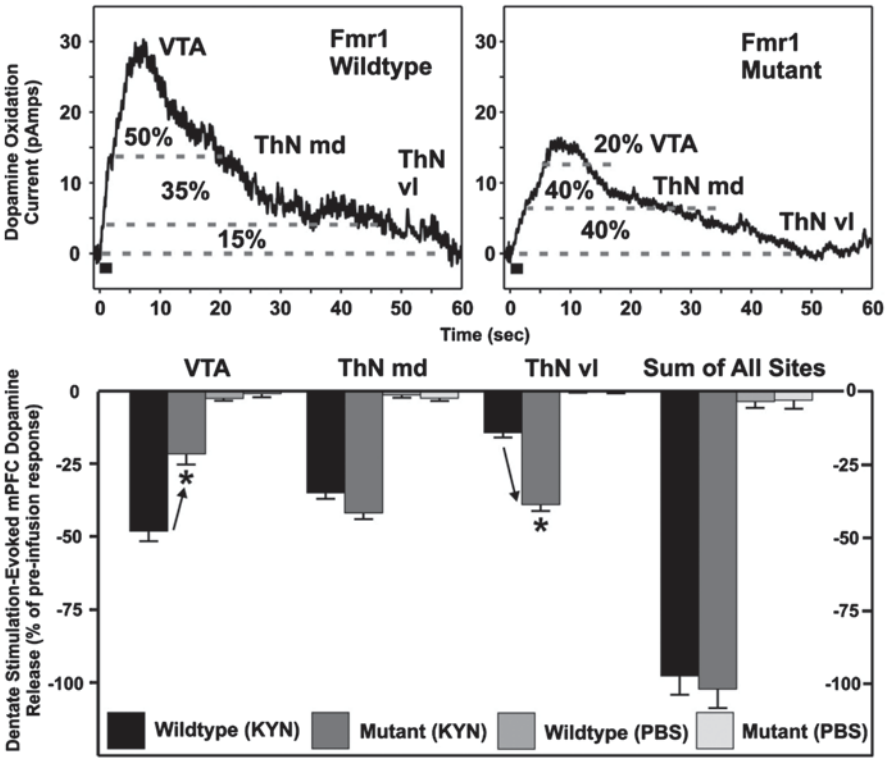


Fig. 11.8 Individual examples of medial prefrontal cortex dopamine release responses (*black bar*, 100 pulses at 50 Hz) (*top panel*) and bar histograms (*bottom panel*) showing the mean ± SEM percent decrease in dentate nucleus stimulation-evoked dopamine responses following kynurenate infusion into the ventral tegmental area (*VTA*), mediodorsal thalamus (*ThN md*), or the ventrolateral thalamus (*ThN vl*), as well as the summed average percent decrease of each drug infused sites in Fmr1 mutant vs. wildtype mice. In Fmr1 wildtype mice infusions of kynurenate into the VTA reduced dopamine responses by ~50%, while kynurenate infusions into the thalamus (*md and vl combined*) also reduced the dopamine response by a total of ~50% (*md* = ~35% and *vl* = ~15%). In contrast, the reduction in the dopamine response following kynurenate into the VTA in Fmr1 mutant mice (~20%) was significantly less, indicating a reduction in the modulatory strength of this pathway. This reduction in strength in mutant mice was coupled to an increase in signal strength of the pathway through the thalamus, specifically the ThN vl. Thus, kynurenate infused into this nucleus reduced dopamine responses by ~15% in wildtype mice compared to ~40% in mutant mice. Regardless of genotype (lurcher and Fmr1 mutant mice; see Fig. 11.8), kynurenate infused into the ThN md reduced the dopamine signal between 30 to 40%, respectively. (Modified from Rogers et al. (2013a) with permission from Springer)

functional reorganization of the VTA and thalamic pathways mediating cerebellar modulation of mPFC dopamine release that was very similar to that observed in *Lc*/+ mice (see Fig. 11.7). Inactivation of the VTA pathway by intra-VTA lidocaine or kynurenate infusions decreased dopamine release by 50% in wildtype and 20% in *Fmr1* mutant mice. Intra-ThN vl infusions of either drug decreased dopamine release by 15% in wildtype and 40% in *Fmr1* mutants, while evoked dopamine

release remained relatively unchanged following intra-ThN md drug infusions. As in *Lc*/+ mice these results indicated a shift in strength towards the thalamic vl projection, away from the VTA in *Fmr1* mutant mice. These results further indicated that the results observed in *Lc*/+ mice could be generalized to another mouse strain that exhibited developmental cerebellar neuropathology of a less severe nature. Both showed a similar reduction in mPFC evoked dopamine release suggesting a similar reorganization of the neuronal pathways mediating DN stimulation-evoked mPFC dopamine release (Rogers et al. 2011, 2013a). In this regard it should be additionally noted that *Fmr1* KO mice also show deficits in serial reversal learning that are similar to those observed in lurcher chimeras (Dickson et al. 2013).

11.8 Glutamate Dysfunction Drives the Reduction in mPFC Dopamine Release in Lurcher Mutant and Wildtype Mice

As we had previously observed that mPFC dopamine release evoked by stimulation of the DN was reduced in *Lc*/+ mutant mice in comparison to wildtype +/+ controls, and that efferents from the DN were likely predominantly glutamatergic (Rogers et al. 2011), we conducted an additional experiment in order to determine if reductions in the functional properties of glutamate were responsible for the reduced dopamine release. Thus, we evaluated glutamate release in RTP, PPT and VTA (VTA pathway) and ThN md and ThN vl (thalamic pathway) evoked by *localized* electrical stimulation using fixed potential amperometry in combination with glutamate enzyme-based recording probes in urethane anesthetized lurcher wildtype and *Lc*/+ mutant mice. Stimulations (100 monophasic 0.5 ms duration pulses at 100 Hz every 600 s) were applied to the ThN md, ThN vl, RTN, PPT, or VTA (at 800 μA) and glutamate release was monitored via a glutamate enzyme recording probe immediately adjacent to the stimulating electrode.

As shown in Fig. 11.9, results of this experiment showed that electrical stimulation of glutamatergic axons in the ThN md, ThN vl, RTN, and PPT of *Lc*/+ mice resulted in significantly less evoked glutamate release than in wildtype (control) mice (McKimm et al. 2014). Evoked glutamate release was equivalent in the VTA of both genotypes. As such, these results are consistent with the idea that both first and second order glutamatergic projections from the DN are abnormal in *Lc*/+ mutant mice possessing developmental cerebellar neuropathology that involves loss of cerebellar Purkinje cells (Caddy and Biscoe 1979; Zuo et al. 1997). It thus seems highly likely that it is this cerebellar neuropathology that drives the subsequent reduction in glutamate release observed in cerebellar efferents from the DN. As both pathways participate in the modulation of mPFC dopamine release, it appears reasonable that these reductions in glutamate transmission can account for the previously observed reduction in mPFC dopamine release elicited by electrical stimulation of the DN in *Lc*/+, compared to wildtype, mice (Mittleman et al. 2008; Rogers et al. 2013a).

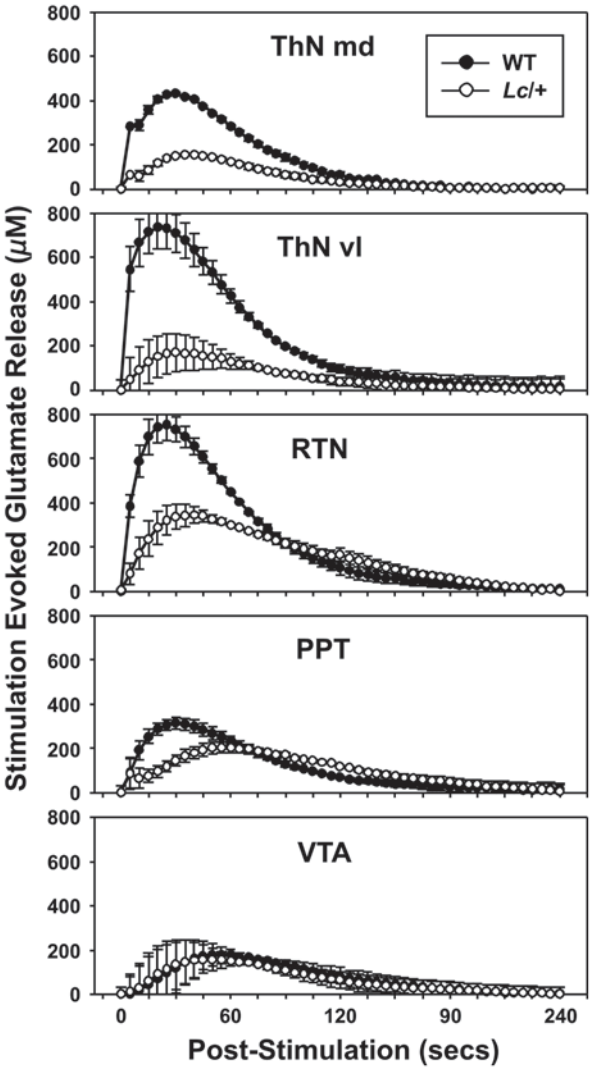


Fig. 11.9 The time-course of glutamate release evoked by electrical stimulation of the mediodorsal nucleus of the thalamus (*ThN md*), ventrolateral nucleus of the thalamus (*ThN vl*), reticulotegmental nucleus (*RTN*), pedunculopontine nuclei (*PPT*), or ventral tegmental area (*VTA*) in lucher (*Lc*/+) mutant mice or wildtype (*WT*) controls. A total of 3 stimulations (100 monophasic 0.5 ms duration pulses at 100 Hz every 600 s) were applied to the ThN md, ThN vl, RTN, PPT, or VTA (at 800 μA). Results averaged across the 3 stimulations are shown. *Vertical lines* indicate the standard error of the mean. (Modified from McKimm et al. with kind permission of Springer Science + Business Media (2013)) with kind permission of Springer Science + Business Media

11.9 Conclusions

In this series of experiments we have concentrated on understanding the neurophysiological bases of some of the most common behavioral symptoms of ASD, including hyperactivity, repetitive behavior and executive function. Considered together these results show that cerebellar Purkinje cell number in lurcher chimeras has a profound influence on these behaviors in that lower numbers of Purkinje cells are consistently associated with increased activity and repetitive behavior as well as deficits in executive function. It is likely that these behavioral deficits stem from

developmental loss of cerebellar output that occurs as a function of Purkinje cell loss. Loss or dysregulation of Purkinje cell output to the deep cerebellar nuclei such as the DN in turn results in alterations in the functionality of cerebellar pathways projecting to the mPFC. This loss of functionality prominently includes reductions in cerebellar mediated mPFC dopamine release. The reduction in mPFC dopamine release is likely caused by coincident reductions in glutamate available for release from cerebellar projections to the thalamus and VTA. This loss of functionality also includes a shift in the balance of influence of the cerebellum on the mPFC, away from the cerebellar circuitry projecting to the VTA, towards cerebellar projections to the thalamus. All of these changes consistently occurred in both lurcher and Fmr1 mutant mice indicating that multiple forms of developmental cerebellar pathology result in very similar neurophysiological and behavioral deficits.

11.10 Future Directions

As noted at the beginning of this chapter, some of the symptoms of ASD also include motoric disorders and deficits in social reward. Motoric and reward deficits in ASD strongly suggest the possibility of dysregulation in other midbrain dopaminergic systems. As reviewed by Dichter et al. (2012b) there is compelling evidence that the nigrostriatal dopamine system, originating in the SNc and projecting to the caudate nucleus (Bjorklund and Lindvall 1984), is altered in a mouse model of Fragile X syndrome. For example, using fast-scan cyclic voltammetry to measure electrically evoked dopamine release in striatal brain slices, Fulks et al. (2010) observed a decrease in both stimulated dopamine release and reuptake in *Fmr1* mutant mice. Interestingly, this functional dysregulation in dopamine transmission was correlated with alterations in repetitive stereotypic movements.

It has also been shown that disruptions in dopamine signalling in the mesoaccumbens dopaminergic system, originating in the VTA and projecting to limbic structures such as the nucleus accumbens (Bjorklund and Lindvall 1984), negatively impact reward processing that is important for social pair bonding behaviors (Curtis and Wang 2005). It is also possible that these deficits may extend beyond social reward to encompass other forms of reward. Experimental studies have linked the mesoaccumbens dopaminergic system and nucleus accumbens to learning about stimulus-reward relationships and the maintenance of learned behaviors (Berridge 2012). Utilizing fMRI, Dichter et al. (2012b) have shown in ASD patients a decrease in nucleus accumbens activation during monetary-reward anticipation, suggesting that these individuals are phenotypically characterized by reward-circuitry hypoactivation. Given our observations of a dysregulation in DN mediated mesocortical dopamine release in both lurcher and *Fmr1* mutant mice, it is not unreasonable to suggest that a similar form of dysregulation may extend to the nigrostriatal and mesoaccumbens dopaminergic systems.

We have recently investigated the possibility that, in addition to having a relatively profound modulatory influence on the mesocortical dopamine pathway,

cerebellar modulation could extend to the nigrostriatal and mesolimbic dopamine projections. Using the methods of Mittleman et al. (2008) we stimulated the DN and recorded evoked dopamine release in the medial-dorsal caudate and nucleus accumbens core in lucher wildtype mice. Figure 11.10 shows that stimulation of the DN resulted in a relatively prolonged increase in dopamine release that lasted for up to 14 s post-stimulation in both brain areas. This result is suggestive that cerebellar modulatory influences can extend beyond the mesocortical dopamine pathway. Given the well-known involvement of the caudate and nucleus accumbens in, re-

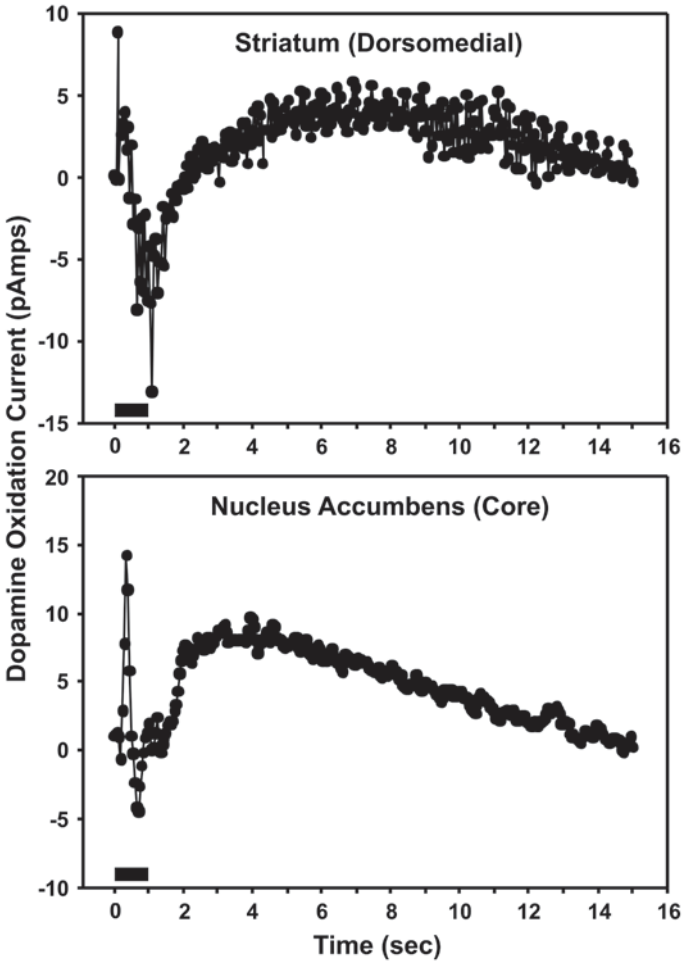


Fig. 11.10 Individual examples of dopamine release responses in the dorsomedial striatum (top panel) and nucleus accumbens core (bottom panel) following stimulation of the cerebellar dentate nucleus. Electrical stimulation (*black* bar: 800 μA intensity, 50 monophasic 0.5 ms duration pulses at 50 Hz) occurred at 0 s. The stimulation artefact occurred between 0 and 1 s. Thereafter, stimulation elicited a prolonged increase in dopamine release in both structures that lasted for up to 14 s post-stimulation

spectively, motor function and reward, these results certainly suggest that developmental cerebellar neuropathology could also result in deficits in these abilities. We believe this possibility is deserving of further investigation.

Acknowledgments This work was supported by a grant from the National Institute of Neurological Disorders and Stroke (R01 NS063009).

References

Aalto S, Brüch A, Laine M, Rinne J (2005) Frontal and temporal dopamine release during working memory and attention tasks in healthy humans: a positron emission tomography study using the high-affinity dopamine D2 receptor ligand [11C]FLB 457. J Neurosci 25:2471–2477

American Psychiatric Association (2000) Diagnostic and statistical manual of mental disorders-IV-TR, 4th edn. American Psychiatric Association, Washington

Angaut P, Cicirata F, Pantò MR (1985) An autoradiographic study of the cerebellopontine projections from the interposed and lateral cerebellar nuclei in the rat. J Hirnforsch 26:463–470

Bailey A, Luthert P, Dean A, Harding B, Janota I, Montgomery M, Rutter M, Lantos P (1998) A clinicopathological study of autism. Brain 121:889–890

Bauman ML (1991) Microscopic neuroanatomic abnormalities in autism. Pediatrics 87:791–796

Bauman ML, Kemper TL (2005) Neuroanatomic observations of the brain in autism: a review and future directions. Int J Dev Neurosci 23:183–187

Bellebaum C, Daum I (2007) Cerebellar involvement in executive control. Cerebellum 6:184–192

Bennett MR (1998) Monoaminergic synapses and schizophrenia: 45 years of neuroleptics. J Psychopharmacol 12:289–304

Berger B, Gasper P, Verney C (1991) Dopaminergic innervation of the cerebral cortex: unexpected differences between rodents and primates. Trends Neurosci 14:21–27

Berger B, Trottier S, Verney C, Gaspar P, Alvarez C (1988) Regional and laminar distribution of the dopamine and serotonin innervation in the Macaque cerebral cortex: a radioautographic study. J Comp Neurol 273:99–119

Bernardet M, Crusio WE (2006) Fmr1 KO mice as a possible model of autistic features. ScientificWorldJournal 6:1164–1176

Berridge KC (2012) From prediction error to incentive salience: mesolimbic computation of reward motivation. Eur J Neurosci 35:1124–1143

Bjorklund A, Lindvall O (1984) Dopamine-containing systems in the CNS. In: Bjorklund A, Hokfelt T (eds) Handbook of chemical neuroanatomy. Classical transmitters in the CNS, vol 2. Elsevier, Amsterdam, pp 55–122

Blaha CD, Winn P (1993) Modulation of dopamine efflux in the striatum following cholinergic stimulation of the substantia nigra in intact and pedunculopontine tegmental nucleus-lesioned rats. J Neurosci 13:1035–1044

Blaha CD, Allen LF, Das S, Inglis WL, Latimer MP, Vincent SR, Winn P (1996) Modulation of dopamine efflux in the nucleus accumbens after cholinergic stimulation of the ventral tegmental area in intact, pedunculopontine tegmental nucleus-lesioned, and laterodorsal tegmental nucleus-lesioned rats. J Neurosci 16:714–722

Bodfish JW, Symons FJ, Parker DE, Lewis MH (2000) Varieties of repetitive behavior in autism: comparisons to mental retardation. J Autism Dev Disord 30:237–243

Bolduc M, DuPlessis AJ, Sullivan N, Khwaja OS, Zhang X, Barnes K, Robertson RL, Limperopoulos C (2011) Spectrum of neurodevelopmental disabilities in children with cerebellar malformations. Dev Med Child Neurol 53:409–416

Brian J, Bryson SE, Garon N, Roberts W, Smith IM, Szatmari P, Zwaigenbaum L (2008) Clinical assessment of autism in high-risk 18-montholds. Autism 12:433–456

Caddy KW, Biscoe TJ (1979) Structural and quantitative studies on the normal C3H and Lurcher mutant mouse. Philos Trans R Soc Lond B Biol Sci 287:167–201
Carper RA, Courchesne E (2005) Localized enlargement of the frontal lobe in autism. Biol Psychiatry 57:126–133
Carr DB, Sesack SR (1996) Hippocampal afferents to the rat prefrontal cortex: synaptic targets and relation to dopamine terminals. J Comp Neurol 369:1–15
Centers for Disease Control and Prevention (2007) Prevalence of autism spectrum disorders-Autism and Developmental Disabilities Monitoring Network, 14 sites, United States, 2002. MMWR 56(No. SS-1):12–28
Cicirata F, Serapide MF, Parenti R, Pantò MR, Zappalà A, Nicotra A, Cicero D (2005) The basilar pontine nuclei and the nucleus reticularis tegmenti pontis subserve distinct cerebrocerebellar pathways. Prog Brain Res 148:259–282
Clark L, Cools R, Robbins TW (2004) The neuropsychology of ventral prefrontal cortex: decision-making and reversal learning. Brain Cogn 55:41–53
Condé F, Audinat E, Maire-Lepoivre E, Crépel F (1990) Afferent connections of the medial frontal cortex of the rat. A study using retrograde transport of fluorescent dyes. I. Thalamic afferents. Brain Res Bull 24:341–354
Constantinidis C, Williams GV, Goldman-Rakic PS (2002) A role for inhibition in shaping the temporal flow of information in prefrontal cortex. Nat Neurosci 5:175–180
Courchesne E (1997) Brainstem, cerebellar and limbic neuroanatomical abnormalities in autism. Curr Opin Neurobio 7:269–278
Courchesne E, Yeung-Courchesne R, Press GA, Hesselink JR, Jernigan TL (1988) Hypoplasia of cerebellar vermal lobules VI and VII in autism. N Engl J Med 318:1349–1354
Courchesne E, Saitoh O, Yeung-Corchesne R, Press GA, Lincoln AJ, Haas RH, Schreibman L (1994) Abnormality of cerebellar vermian lobules VI and VII in patients with infantile autism: Identification of hypoplastic and hyperplastic subgroups by MR imaging. Am J Roentgenology 162:123–130
Curtis JT, Wang Z (2005) Ventral tegmental area involvement in pair bonding in male prairie voles. Physiol Behav 86:338–346
Dalley JW, Cardinal RN, Robbins TW (2004) Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. Neurosci Biobehav Rev 28:771–784
Dawson G, Meltzoff AN, Osterling J, Rinaldi J, Brown E (1998) Children with autism fail to orient to naturally occurring social stimuli. J Autism Dev Disord 28:479–485
Del Arco A, Mora F (2005) Glutamate-dopamine in vivo interaction in the prefrontal cortex modulates the release of dopamine and acetylcholine in the nucleus accumbens of the awake rat. J Neural Transm 112:97–109
Del Arco A, Mora F (2009) Neurotransmitters and prefrontal cortex-limbic system interactions: implications for plasticity and psychiatric disorders. J Neural Transm 116:941–952
Dichter G, Adolphs R (2012) Reward processing in autism: a thematic series. J Neurodev Disord 4:20
Dichter GS, Felder JN, Green SR, Rittenberg AM, Sasson NJ, Bodfish JW (2012a) Reward circuitry function in autism spectrum disorders. Soc Cogn Affect Neurosci 7:160–172
Dichter GS, Damiano CA, Allen JA (2012b) Reward circuitry dysfunction in psychiatric and neurodevelopmental disorders and genetic syndromes: animal models and clinical findings. J Neurodev Disord 4:1–43
DiCicco-Bloom E, Lord C, Zwaigenbaum L, Courchesne E, Dager SR, Schmitz C et al (2006) The developmental neurobiology of autism spectrum disorder. J Neurosci 26:6897–6906
Dickson PE, Rogers TD, Del Mar N, Martin LA, Heck D, Blaha CD, Goldowitz D, Mittleman G (2010) Behavioral flexibility in a mouse model of developmental cerebellar Purkinje cell loss. Neurobio Learn Mem 94:220–228
Dickson PE, Corkill B, McKimm E, Miller MM, Calton MA, Goldowitz D, Blaha CD, Mittleman G (2013) Effects of stimulus salience on touchscreen serial reversal learning in a mouse model of fragile X syndrome. Beh Brain Res 252:126–135

Dowell LR, Mahone EM, Mostofsky SH (2009) Associations of postural knowledge and basic motor skill with dyspraxia in autism: implication for abnormalities in distributed connectivity and motor learning. Neuropsychology 23:563–570

Durstewitz D, Seamans JK, Sejnowski TJ (2000) Dopamine-mediated stabilization of delay-period activity in a network model of prefrontal cortex. J Neurophysiol 83:1733–1750

Ellegood J, Pacey LK, Hampson DR, Lerch JP, Henkelman RM (2010) Anatomical phenotyping in a mouse model of fragile X syndrome with magnetic resonance imaging. Neuroimage 53:1023–1029

Erickson SL, Melchitzky DS, Lewis DA (2004) Subcortical afferents to the lateral mediodorsal thalamus in cynomolgus monkeys. Neuroscience 129:675–690

Ernst M, Zametkin AJ, Matochik JA, Pascualvaca D, Cohen RM (1997) Low medial pre-frontal dopaminergic activity in autistic children. Lancet 350:638–639

Elsabbagh M, Mercure E, Hudry K, Chandler S, Pasco G, Charman T, BASIS Team (2012) Infant neural sensitivity to dynamic eye gaze is associated with later emerging autism. Curr Biol 22:338–342

Ey E, Leblond CS, Bourgeron T (2011) Behavioral profiles of mouse models for autism spectrum disorders. Autism Res 4:5–16

Fatemi SH, Aldinger KA, Ashwood P, Bauman ML, Blaha CD, Blatt GJ, Chauhan A, Chauhan V, Dager SR, Dickson PE, Estes AM, Goldowitz D, Heck DH, Kemper TL, King BH, Martin LA, Millen KJ, Mittleman G, Mosconi MW, Persico AM, Sweeney JA, Webb SJ, Welsh JP (2012) Consensus paper: pathological role of the cerebellum in autism. Cerebellum 11:777–807

Feenstra MG, van der Weij W, Botterblom MH (1995) Concentration-dependent dual action of locally applied N-methyl-D-aspartate on extracellular dopamine in the rat prefrontal cortex in vivo. Neurosci Lett 201:175–178

Fournier KA, Hass CJ, Naik SK, Lodha N, Cauraugh JH (2010) Motor coordination in autism spectrum disorders: a synthesis and meta-analysis. J Autism Dev Disord 40:1227–1240

Forster GL, Yeomans JS, Takeuchi J, Blaha CD (2002) M5 muscarinic receptors are required for prolonged accumbal dopamine release following electrical stimulation of the pons in mice. J Neurosci 22:RC190

Forster GL, Blaha CD (2003) Pedunculopontine tegmental stimulation evokes striatal dopamine efflux by activation of acetylcholine and glutamate receptors in the midbrain and pons of the rat. Eur J Neurosci 17:751–762

Freitag CM, Kleser C, Schneider M, von Gontard A (2007) Quantitative assessment of neuromotor function in adolescents with high functioning autism and Asperger Syndrome. J Autism Dev Disord 37:948–959

Fulks JL, O'Bryhim BE, Wenzel SK, Fowler SC, Vorontsova E, Pinkston JW, Ortiz AN, Johnson MA (2010) Dopamine release and uptake impairments and behavioral alterations observed in mice that model Fragile X mental retardation syndrome. ACS Chem Neurosci 1:679–690

Gamo NJ, Wang M, Arnsten AF (2010) Methylphenidate and atomoxetine enhance prefrontal function through α2-adrenergic and dopamine D1 receptors. J Am Acad Child Adolesc Psychiatry 49:1011–1023

Garcia-Rill E, Skinner RD, Miyazato H, Homma Y (2001) Pedunculopontine stimulation induces prolonged activation of pontine reticular neurons. Neuroscience 104:455–465

Gaspar P, Berger B, Febvret A, Vigny A, Henry J-P (1989) Catecholamine innervation of the human cerebral cortex as revealed by comparative immunohistochemistry of tyrosine hydroxylase and dopamine-beta-hydroxylase. J Comp Neurol 279:249–271

Gerrits NM, Voogd J (1987) The projection of the nucleus reticularis tegmenti pontis and adjacent regions of the pontine nuclei to the central cerebellar nuclei in the cat. J Comp Neurol 258:52–69

Ghaziuddin M, Butler E, Tsai L, Ghaziuddin N (1994) Is clumsiness a marker for Asperger syndrome? J Intellect Disabil Res 38(Pt 5):519–527

Goldman-Rakic PS, Leranth C, Williams SM, Mons N, Geffard M (1989) Dopamine synaptic complex with pyramidal neurons in primate cerebral cortex. Proc Nat Acad Sci U S A 86:9015–9019

Goldowitz D, Moran H, Wetts R (1992) Mouse chimeras in the study of genetic and structural determinants of behavior. In: Goldowitz D, Wahlsten D, Wimer RE (eds) Techniques for the genetic analysis of brain and behavior: focus on the mouse. Elsevier, Amsterdam, pp 271–290

Goodrich-Hunsaker NJ, Wong LM, McLennan Y, Tassone F, Harvey D, Rivera SM, Simon TJ (2011) Adult female Fragile X premutation carriers exhibit age- and CGG repeat length-related impairments on an attentionally based enumeration task. Front Hum Neurosci 5:63

Gowen E, Hamilton A (2013) Motor abilities in autism: a review using a computational context. J Autism Dev Disord 43:323–344

Gorelova NA, Yang CR (2000) Dopamine D1/D5 receptor activation modulates a persistent sodium current in rat prefrontal cortical neurons in vitro. J Neurophysiol 84:75–87

Green D, Baird G, Barnett AL, Henderson L, Huber J, Henderson SE (2002) The severity and nature of motor impairment in Asperger's syndrome: a comparison with specific developmental disorder of motor function. J Child Psychol Psychiatry 43:655–668

Gulledge AT, Jaffe DB (1998) Dopamine decreases the excitability of layer V pyramidal cells in the rat prefrontal cortex. J Neurosci 18:9139–9151

Hazrati LN, Parent A (1992) Projection from the deep cerebellar nuclei to the pedunculopontine nucleus in the squirrel monkey. Brain Res 585:267–271

Henze DA, Gonzalez-Burgos GR, Urban NN, Lewis DA, Barrionuevo G (2000) Dopamine increases excitability of pyramidal neurons in primate prefrontal cortex. J Neurophysiol 84:2799–2809

Hill EL (2004) Executive dysfunction in autism. Trends Cogn Sci 8:26–32

Hodos W, Kalman G (1963) Effects of increment size and reinforcer volume on progressive ratio performance. J Exp Anal Behav 6:387–392

Hoover WB, Vertes RP (2007) Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. Brain Struct Funct 212:149–179

Hughes C, Russell J, Robbins TW (1994) Evidence for executive dysfunction in autism. Neuropsychologia 32:477–492

Hutt SJ, Hutt C (1968) Stereotypy, arousal and autism. Hum Dev 11:277–286

Jackson ME, Moghaddam B (2004) Stimulus-specific plasticity of prefrontal cortex dopamine neurotransmission. J Neurochem 88:1327–1334

Jansiewicz EM, Goldberg MC, Newschaffer CJ, Denckla MB, Landa R, Mostofsky SH (2006) Motor signs distinguish children with high functioning autism and Asperger's syndrome from controls. J Autism Dev Disord 36:613–621

Jedema HP, Moghaddam B (1994) Glutamatergic control of dopamine release during stress in the rat prefrontal cortex. J Neurochem 63:785–788

Jasmin E, Couture M, McKinley P, Reid G, Fombonne E, Gisel E (2009) Sensori-motor and daily living skills of preschool children with autism spectrum disorders. J Autism Dev Disord 39:231–241

Jones GH, Marsden CA, Robbins TW (1991) Behavioural rigidity and rule-learning deficits following isolation-rearing in the rat: neurochemical correlates. Behav Brain Res 43:35–50

Kelly RM, Strick PL (2003) Cerebellar loops with motor cortex and prefrontal cortex of a nonhuman primate. J Neurosci 23:8432–8444

Kennedy CH, Meyer KA, Knowles T, Shukla S (2000) Analyzing the multiple functions of stereotypical behavior for students with autism: implications for assessment and treatment. J Appl Behav Anal 33:559–571

Klin A, Lin DJ, Gorrindo P, Ramsay G, Jones W (2009) Two-year-olds with autism orient to nonsocial contingencies rather than biological motion. Nature 459:257–261

Koekkoek SK, Yamaguchi K, Milojkovic BA, Dortland BR, Ruigrok TJ, Maex R, De Graaf W, Smit AE, VanderWerf F, Bakker CE, Willemsen R, Ikeda T, Kakizawa S, Onodera K, Nelson DL, Mientjes E, Joosten M, De Schutter E, Oostra BA, Ito M, De Zeeuw CI (2005) Deletion of FMR1 in Purkinje cells enhances parallel fiber LTD, enlarges spines, and attenuates cerebellar eyelid conditioning in Fragile X syndrome. Neuron 47:339–352

Laruell M, Kegeles L, Abi-Dargham A (2003) Glutamate, dopamine, and schizophrenia: from pathophysiology to treatment. Ann NY Acad Sci 103:138–158

Lauritsen MB (2013) Autism spectrum disorders. Eur Child Adolesc Psychiatry 22(Suppl. 1):S37–42
Lavoie B, Parent A (1994) Pedunculopontine nucleus in the squirrel monkey: distribution of cholinergic and monoaminergic neurons in the mesopontine tegmentum with evidence for the presence of glutamate in cholinergic neurons. J Comp Neurol 344:190–209
Lee KH, Blaha CD, Cooper S, Hitti FL, Leiter JC, Roberts DW, Kim U (2006) Dopamine efflux in the rat striatum evoked by electrical stimulation of the subthalamic nucleus: potential mechanism of action in Parkinson's disease. Eur J Neurosci 23:1005–1014
Lin A, Rangel A, Adolphs R (2012) Impaired learning of social compared to monetary rewards in autism. Front Neurosci 6:143
Llinas RR, Walton KD, Lang EJ (2004) Cerebellum. In: Shepherd GM (ed) The synaptic organization of the brain. Oxford University Press, New York (Chap. 7)
Mackintosh NJ (1974) The psychology of animal learning. Academic, London
Manjiviona J, Prior M (1995) Comparison of Asperger syndrome and high-functioning autistic children on a test of motor impairment. J Autism Dev Disord 25:23–39
Martin LA, Goldowitz D, Mittleman G (2010) Repetitive behavior and increased activity in mice with Purkinje cell loss: a model for understanding the role of cerebellar pathology in autism. Eur J Neurosci 31:544–555
McKimm EJ, Corkill B, Goldowitz D, Albritton LM, Homayouni R, Blaha CD, Mittleman G (2014) Glutamate dysfunction associated with developmental cerebellar damage: relevance to autism spectrum disorders. Cerebellum 13:346–353
Middleton FA, Strick PL (1997) Cerebellar output channels. Int Rev Neurobiol 41:61–82
Middleton FA, Strick PL (2000) Basal ganglia and cerebellar loops: motor and cognitive circuits. Brain Res Reviews 31:236–250
Middleton FA, Strick PL (2001) Cerebellar projections to the prefrontal cortex of the primate. J Neurosci 21:700–712
Mihailoff GA (1993) Cerebellar nuclear projections from the basilar pontine nuclei and nucleus reticularis tegmenti pontis as demonstrated with PHA-L tracing in the rat. J Comp Neurol 330:130–146
Militerni R, Bravaccio C, Falco C, Fico C, Palermo MT (2002) Repetitive behaviors in autistic disorder. Eur Child Adolesc Psychiatry 11:210–218
Mittleman G, Goldowitz D, Heck DH, Blaha CD (2008) Cerebellar modulation of frontal cortex dopamine efflux in mice: relevance to autism and schizophrenia. Synapse 62:544–550
Miyahara M, Tsujii M, Hori M, Nakanishi K, Kageyama H, Sugiyama T (1997) Brief report: motor incoordination in children with Asperger syndrome and learning disabilities. J Autism Dev Disord 27:595–603
Morrow BA, Elsworth JD, Rasmusson AM, Roth RH (1999) The role of mesoprefrontal dopamine neurons in the acquisition and expression of conditioned fear in the rat. Neuroscience 92:553–564
Nieoullon A (2002) Dopamine and the regulation of cognition and attention. Prog Neurobiol 67:53–83
Oakman SA, Faris PL, Kerr PE, Cozzari C, Hartman BK (1995) Distribution of pontomesencephalic cholinergic neurons projecting to substantia nigra differs significantly from those projecting to ventral tegmental area. J Neurosci 15:5859–5869
Oakman SA, Faris PL, Cozzari C, Hartman BK (1999) Characterization of the extent of pontomesencephalic cholinergic neurons' projections to the thalamus: comparison with projections to midbrain dopaminergic groups. Neuroscience 94:529–547
Olmos-Serrano JL, Corbin JG (2011) Amygdala regulation of fear and emotionality in fragile X syndrome. Dev Neurosci 33:365–378
Overton PG, Clark D (1997) Burst firing in midbrain dopaminergic neurons. Brain Res-Brain Res Rev 25:312–334
Ozonoff S, South M, Provencal S (2007) Executive functions in autism: theory and practice. In: Pérez JM, González PM, Comí MC et al (eds) New developments in Autism: the future is today. Asociación de Padres de Personas con Autismo, Philadelphia, pp 185–213

Ozonoff S, Iosif AM, Baquio F, Cook IC, Hill MM, Hutman T, Young GS (2010) A prospective study of the emergence of early behavioral signs of autism. J Am Acad Child Adolesc Psychiatry 49:256–266
Palmen SJ, van Engeland H, Hof PR, Schmitz C (2004) Neuropathological findings in autism. Brain 127:2572–2583
Pan CY, Tsai CL, Chu CH (2009) Fundamental movement skills in children diagnosed with autism spectrum disorders and attention deficit hyperactivity disorder. J Autism Dev Disord 39:1694–1705
Pennington BF, Ozonoff S (1996) Executive functions and developmental psychopathology. J Child Psychol Psychiatry 37:51–87
Paul K, Venkitaramani DV, Cox CL (2013) Dampened dopamine-mediated neuromodulation in prefrontal cortex of fragile X mice. J Physiol 591(Pt 4):1133–1143
Perciavalle V, Berretta S, Raffaele R (1989) Projections from the intracerebellar nuclei to the ventral midbrain tegmentum in the rat. Neuroscience 29:109–119
Pickett J, London E (2005) The neuropathology of autism: a review. J Neuropathol Exp Neurol 64:925–935
Pinto A, Jankowski M, Sesack SR (2003) Projections from the paraventricular nucleus of the thalamus to the rat prefrontal cortex and nucleus accumbens shell: ultrastructural characteristics and spatial relationships with dopamine afferents. J Comp Neurol 459:142–155
Pirot S, Jay TM, Glowinski J, Thierry AM (1994) Anatomical and electrophysiological evidence for an excitatory amino acid pathway from the thalamic mediodorsal nucleus to the prefrontal cortex in the rat. Eur J Neurosci 6:1225–1234
Provost B, Lopez BR, Heimerl S (2007) A comparison of motor delays in young children: autism spectrum disorder, developmental delay, and developmental concerns. J Autism 37:321–328
Rao SG, Williams GV, Goldman-Rakic PS (2000) Destruction and creation of spatial tuning by disinhibition: GABAA blockade of prefrontal cortical neurons engaged by working memory. J Neurosci 20:485–494
Ragozzino ME (2007) The contribution of the medial prefrontal cortex, orbitofrontal cortex, and dorsomedial striatum to behavioral flexibility. Ann NY Acad Sci 1121:355–375
Reese NB, Garcia-Rill E, Skinner RD (1995) The pedunculopontine nucleus-auditory input, arousal and pathophysiology. Prog Neurobiol 47:105–133
Ridley RM (1994) The psychology of perserverative and stereotyped behaviour. Prog Neurobiol 44:221–231
Robbins TW (2005) Chemistry of the mind: neurochemical modulation of prefrontal cortical function. J Comp Neurol 493:140–146
Robbins TW, Arnsten AF (2009) The neuropsychopharmacology of fronto-executive function: monoaminergic modulation. Annu Rev Neurosci 32:267–287
Robbins TW, Roberts AC (2007) Differential regulation of fronto-executive function by the monoamines and acetylcholine. Cereb Cortex 17(Suppl. 1):i151–i160
Rogers SJ, DiLalla DL (1990) Age of symptom onset in young children with pervasive developmental disorders. J Am Acad Child Adolesc Psychiatry 29:863–872
Rogers TD, Dickson PE, Heck DH, Goldowitz D, Mittleman G, Blaha CD (2011) Connecting the dots of the cerebro-cerebellar role in cognitive function: neuronal pathways for cerebellar modulation of dopamine release in the prefrontal cortex. Synapse 65:1204–1212
Rogers TD, Dickson PE, McKimm E, Heck DH, Goldowitz D, Blaha CD, Mittleman G (2013a) Reorganization of circuits underlying cerebellar modulation of prefrontal cortical dopamine in mouse models of autism spectrum disorder. Cerebellum 12:547–556
Rogers TD, McKimm E, Dickson PE, Goldowitz D, Blaha CD, Mittleman G (2013b) Is autism a disease of the cerebellum? An integration of clinical and pre-clinical research. Front Syst Neurosci 7:15
Rose J, Schiffer A-M, Dittrich L, Güntürkün O (2010) The roles of dopamine in maintenance and distractibility of attention in the "prefrontal cortex" of pigeons. Neuroscience 167:232–237
Ruigrok TJ (2011) Ins and outs of cerebellar modules. Cerebellum 10:464–474

Sawaguchi T, Goldman-Rakic PS (1991) D1 dopamine receptors in prefrontal cortex: involvement in working memory. Science 251:947–950

Schwarz C, Schmitz Y (1997) Projection from the cerebellar lateral nucleus to precerebellar nuclei in the mossy fiber pathway is glutamatergic: a study combining anterograde tracing with immunogold labeling in the rat. J Comp Neurol 381:320–334

Seamans JK, Yang CR (2004) The principal features and mechanisms of dopamine modulation in the prefrontal cortex. Prog Neurobiol 74:1–57

Seamans JK, Durstewitz D, Christie B, Stevens CF, Sejnowski TJ (2001b) Dopamine D1/D5 receptor modulation of excitatory synaptic inputs to layer V prefrontal cortex neurons. Proc Natl Acad Sci U S A 98:301–306

Seamans JK, Gorelova N, Durstewitz D, Yang CR (2001a) Bidirectional dopamine modulation of GABAergic inhibition in prefrontal cortical pyramidal neurons. J Neurosci 21:3628–3638

Seamans JK, Nogueira L, Lavin A (2003) Synaptic basis of persistent activity in prefrontal cortex in vivo and in organotypic cultures. Cereb Cortex 13:1242–1250

Sepeta L, Tsuchiya N, Davies MS, Sigman M, Bookheimer SY, Dapretto M (2012) Abnormal social reward processing in autism as indexed by pupillary responses to happy faces. J Neurodev Disord 4:17

Simonoff E, Pickles A, Charman T, Chandler S, Loucas T, Baird G (2008) Psychiatric disorders in children with autism spectrum disorders: prevalence, comorbidity, and associated factors in a population-derived sample. J Am Acad Child Adolesc Psychiatry 47:921–929

Smiley JF, Goldman-Rakic PS (1993) Heterogeneous targets of dopamine synapses in monkey prefrontal cortex demonstrated by serial section electron microscopy: a laminar analysis using the silver-enhanced diaminobenzidone sulfide (SEDS) immunolabeling technique. Cereb Cortex 3:223–238

Sutera S, Pandey J, Esser EL, Rosenthal MA, Wilson LB, Barton M et al (2007) Predictors of optimal outcome in toddlers diagnosed with autism spectrum disorders. J Autism Dev Disord 37:98–107

Takahata R, Moghaddam B (1998) Glutamatergic regulation of basal and stimulus-activated dopamine release in the prefrontal cortex. J Neurochem 71:1443–1449

Testa-Silva G, Loebel A, Giugliano M, de Kock CP, Mansvelder HD, Meredith RM (2011) Hyperconnectivity and slow synapses during early development of medial prefrontal cortex in a mouse model for mental retardation and autism. Cereb Cortex 22:1333–1342

Teune TM, van der Burg J, de Zeeuw CI, Voogd J, Ruigrok TJ (1998) Single Purkinje cell can innervate multiple classes of projection neurons in the cerebellar nuclei of the rat: a light microscopic and ultrastructural triple-tracer study in the rat. J Comp Neurol 392:164–178

Teune TM, van der Burg J, van der Moer J, Voogd J, Ruigrok TJ (2000) Topography of cerebellar nuclear projections to the brain stem in the rat. Prog Brain Res 124:141–172

Torigoe Y, Blanks RH, Precht W (1986) Anatomical studies on the nucleus reticularis tegmenti pontis in the pigmented rat. II. Subcortical afferents demonstrated by the retrograde transport of horseradish peroxidase. J Comp Neurol 243:88–105

Trantham-Davidson H, Neely LC, Lavin A, Seamans JK (2004) Mechanisms underlying differential D1 versus D2 dopamine receptor regulation of inhibition in prefrontal cortex. J Neurosci 24:10652–10659

Van Waelvelde H, Oostra A, Dewitte G, Van DBroeckC, Jongmans MJ (2010) Stability of motor problems in young children with or at risk of autism spectrum disorders, ADHD, and ordevelopmental coordination disorder. Dev Med Child Neurol 52:e174–e178

Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA (2005) Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann Neurol 57:67–81

Verkerk AJ, Pieretti M, Sutcliffe JS, Fu YH, Kuhl DP, Pizzuti A, Reiner O, Richards S, Victoria MF, Zhang FP et al (1991) Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell 65:905–914

Verney C, Alvarez C, Geffard M, Berger B (1990) Ultrastructural double labeling study of dopamine terminals and GABA-containing neurons in rat anteromedial cerebral cortex. Eur J Neurosci 2:295–298

Vertes RP, Martin GF, Waltzer R (1986) An autoradiographic analysis of ascending projections from the medullary reticular formation in the rat. Neuroscience 19:873–898

Webb SJ, Sparks BF, Friedman SD, Shaw DW, Giedd J, Dawson G, Dager SR (2009) Cerebellar vermal volumes and behavioral correlates in children with autism spectrum disorder. Psychiatry Res 172:61–67

Wetts R, Herrup K (1982) Interaction of granule, Purkinje, and inferior olivary neurons in lurcher chimeric mice. I. Qualitative studies. J Embryo Exp Morphol 68:87–98

Whitney ER, Kemper TL, Bauman ML, Rosene DL, Blatt GJ (2008) Cerebellar Purkinje cells are reduced in a subpopulation of autistic brains: a stereologic experiment using calbindin-D28k. Cerebellum 7:406–416

Whitney ER, Kemper TL, Rosene DL, Bauman ML, Blatt GJ (2009) Density of cerebellar basket and stellate cells in autism: evidence for a late developmental loss of Purkinje cells. J Neurosci Res 87:2245–2254

Wolff JJ, Gu H, Gerig G, Elison JT, Styner M, Gouttard S, Piven J (2012) Differences in white matter fiber tract development present from 6 to 24 months in infants with autism. Am J Psychiatry 169:589–600

Yang CR, Seamans JK (1996) Dopamine D1 receptor actions in layer V-VI rat prefrontal cortex neurons in vitro: modulation of dendriticsomatic signal integration. J Neurosci 16:1922–1935

Zheng P, Zhang XX, Bunney BS, Shi WX (1999) Opposite modulation of cortical N-methyl-D-aspartate receptor-mediated responses by low and high concentrations of dopamine. Neuroscience 91:527–535

Zuo J, De Jager PL, Takahashi KA, Jiang W, Linden DJ, Heintz N (1997) Neurodegeneration in Lurcher mice caused by mutation in δ2 glutamate receptor gene. Nature 388:769–773

Chapter 12
Autism and Glutamate

Maria L. Carlsson

Abstract In recent years there has been a growing interest in the role played by the excitatory neurotransmitter glutamate in autism spectrum disorders. The present chapter describes results from brain imaging and biochemical studies that directly or indirectly strengthen the notion of dysfunctional glutamatergic transmission in autism spectrum disorders. Of particular interest is the increasing number of studies showing white matter, including corpus callosum, abnormalities and deficient cortical "connectivity" in autism. Accumulating biochemical evidence also points to aberrations in glutamatergic signalling in autism. Several studies testing different agents that modulate glutamatergic transmission have been conducted but larger placebo-controlled studies are needed. An alternative possible avenue involves combined dopaminergic and serotonergic stabilization which would mediate indirect modulation of glutamatergic systems.

Keywords Autism · Glutamate · Connectivity · Corpus callosum

In recent years there has been a steadily growing interest in the role played by the excitatory neurotransmitter glutamate in autism spectrum disorders. Fifteen years ago I proposed that infantile autism may be a hypoglutamatergic disorder (Carlsson 1998)—I based my hypothesis on published results from neuroanatomical and neuroimaging studies that indicated aberrations in brain regions which are rich in glutamate neurons, and similarities between symptoms produced by glutamatergic N-methyl-D-aspartate (NMDA) antagonists in healthy subjects and those seen in autism. In this chapter I describe results from brain imaging and biochemical studies that directly or indirectly strengthen the notion of dysfunctional glutamatergic transmission in autism spectrum disorders. Of particular interest is the increasing number of studies showing white matter, including corpus callosum, abnormalities and deficient cortical "connectivity" in autism (see also Carlsson 2010).

M. L. Carlsson (✉)
Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Sahlgrenska Science Park, Medicinaregatan 8A, 41346, Gothenburg, Sweden
e-mail: maria.carlsson@psychiat.gu.se

S. Hossein Fatemi (ed.), *The Molecular Basis of Autism,*
Contemporary Clinical Neuroscience, DOI 10.1007/978-1-4939-2190-4_12

12.1 Corpus Callosum and Brain Connectivity

As the bulk of fibers running through the corpus callosum are glutamatergic (Conti and Manzoni 1994; Kumar and Huguenard 2001), studies on this structure may give an indication about the integrity of brain glutamatergic neurons. Indeed, the corpus callosum has been referred to as "a sensitive indicator of the cerebral cortical state" (Hayakawa et al. 1989). Several magnetic resonance imaging (MRI) studies have reported reductions in callosal size in persons with autism, be it the entire structure or on a regional level (Egaas et al. 1995; Piven et al. 1997; Hardan et al. 2000; Waiter et al. 2005; Vidal et al. 2006; Alexander et al. 2007; Just et al. 2007; Mason et al. 2008; Frazier and Hardan 2009). Assuming that callosal size reflects the number of callosal nerve fibers (Aboitiz et al. 1992; see however Lamantia and Rakic 1990), these results are in line with the notion of hypoglutamatergia in autism. While the corpus callosum is thus fairly consistently reported to be smaller in autism, the cerebral hemispheres, the cerebellum and the caudate nucleus are observed to be enlarged (Stanfield et al. 2008). Along with the brain enlargement in autism, especially recent studies have observed regional increases of gray matter, and white matter volume reductions (see Bonilha et al. 2008). It has been suggested that in autism there is an abundance of short connective fibers relative to long ones and increased local but reduced long-distance cortico-cortical activity (Courchesne and Pierce 2005; Just et al. 2007; Bonilha et al. 2008).

The majority of long fibers are considered to be glutamatergic while the short cortical fibers appear to be predominantly GABAergic (Jones 1986; Somogyi et al. 1998). Hence, the above MRI findings do not seem to be in line with recent hypotheses that autism is a disorder of deficient GABA activity coupled with excessive glutamate transmission (Rubenstein and Merzenich 2003). Also results from a number of PET studies in autism/Asperger's disorder showing decreased deoxyglucose metabolism in various brain regions innervated by glutamatergic neurons, e.g., the temporal lobes (Chugani et al. 1996), the anterior cingulate gyrus/medial frontal cortex, i.e., Brodmann areas 24 and 25 (Haznedar et al. 1997; Hazlett et al. 2004), ventral caudate, putamen, and thalamus (Haznedar et al. 2006); are difficult to reconcile with a general hyperglutamatergia, as excessive glutamate activity would rather be expected to produce the opposite result, since a major part of the glucose oxidation in the brain occurs in the nerve terminals of glutamate neurons (see Rothman et al. 2003). Since GABAergic neurons make a minor contribution (about 20 % in the cortex) to neuronal glucose oxidation (Patel et al. 2005) there is of course a theoretical possibility that the decreased metabolism observed is due solely to decreased GABAergic activity; this interpretation does not seem plausible though, in part because decreased activity of GABAergic interneurons—assuming that GABA in autism during ontogenesis undergoes a normal switch from excitatory to inhibitory transmitter (see Herlenius and Lagercrantz 2001)—ought to result in excessive glutamatergic neuronal activity, which in turn would cause enhanced metabolism. In the striatum, GABAergic interneurons are reported to constitute less than 5 % of the neurons (Partridge et al. 2009; Gittis and Kreitzer 2012). Taken together, results

from MRI and PET studies seem to support the notion of deficient glutamatergic transmission in at least parts of the brain in autism.

The growing number of reports of structural white matter deficits in autism (Barnea-Goraly et al. 2004; Keller et al. 2007; Alexander et al. 2007; Lee et al. 2007; Bonilha et al. 2008; for review see Travers et al. 2012) tally with functional MRI studies reporting reduced "functional connectivity" inter- as well as intrahemispherically. For instance, Just et al. (2004, 2007) found that high-functioning autistic individuals displayed a lower degree of synchronization in activation between different cortical areas, including between frontal and parietal areas compared to controls (for reviews see Philip et al. 2012; Dichter 2012). Furthermore, in subjects with autism, but not in controls, a positive correlation between the size of the anterior region of the corpus callosum and frontal-parietal functional connectivity has been observed, i.e., a smaller anterior corpus callosum is associated with reduced cortical connectivity (Just et al. 2007; Kana et al. 2006; Mason et al. 2008).

The above observations, in turn, fit nicely with our own neuropsychological study aimed at testing the efficiency of callosal information transfer in children and adolescents with autism spectrum disorders. The study included auditory, visual, and motor measures covering information transfer within, as well as across, modalities. We found evidence for aberrant interhemispheric, but also intrahemispheric, transfer of information, both of which seem to depend on the integrity of the corpus callosum. We concluded that our observations were in support of the hypothesis of autism being a hypoglutamatergic disorder (Nydén et al. 2004). Interesting in this context are also observations in individuals with developmental absence (agenesis), complete or partial, of the corpus callosum (for reviews and references see Paul et al. 2007; Paul 2011); these individuals display deficits that are typically found in persons with autism, e.g., impairments in abstract reasoning and generalization, and in the comprehension of idioms, proverbs, vocal prosody and narrative humour. Parents of affected individuals have reported that impaired social skills and poor personal insight are the factors that interfere most with the daily lives of their children. For instance, they have difficulties making conversation, taking all conversation literally, and are unable to take the perspective of others (see Paul et al. 2007; Badaruddin et al. 2007).

12.2 Biochemistry

12.2.1 Plasma Glutamine/Glutamate Levels

There are several studies reporting alterations of plasma levels of glutamate and the glutamate precursor/metabolite, glutamine in autism. Moreno-Fuenmayor et al. (1996) observed a tendency for enhanced plasma glutamate levels in children with autism, whereas the glutamine levels were significantly decreased, and there appeared to be a negative correlation between glutamate and glutamine levels. Also

Zavala et al. (2001) found that plasma levels of glutamine were lower in patients with autism. Similarly, increased plasma glutamate and lowered glutamine levels have been found in patients with autism and Asperger's syndrome (Aldred et al. 2003), as well as in patients with high-functioning autism (Shimmura et al. 2011). Shinohe et al. (2006), finally, reported that serum levels of glutamate were significantly higher in young adult patients with autism than in controls, whereas the glutamine levels did not differ.

As glutamate and glutamine are actively transported out from the CNS (Hawkins et al. 2006), plasma levels may reflect CSF levels. Indeed, a positive correlation between CSF and blood concentrations of glutamate has been observed (Alfredsson et al. 1988). Thus, if the peripheral phenomena described above mirror an altered glutamine-glutamate balance in the CNS, this may suggest a disturbed glutamine-glutamate cycling and hence support the notion of impaired glutamatergic neurotransmission. Platelet levels of glutamine and glutamate have also been measured and were found to be lower in children with autism than in controls (Rolf et al. 1993). It should be added that the alterations in glutamate and glutamine in the studies cited here were often accompanied by group differences in other amino acids.

12.2.2 Brain Glutamate Biochemistry

In a proton magnetic resonance spectroscopic imaging (^{1}H-MRS) study conducted in 26 boys with autism and 29 male comparison subjects, lower levels of gray matter N-acetylaspartate and glutamate + glutamine were found in the autism group in most cerebral lobes and in the cerebellum, suggesting widespread decreased neuronal viability and perturbation of glutamatergic transmission (DeVito et al. 2007). In another ^{1}H-MRS study Bernardi et al. (2011) observed lower glutamate + glutamine concentrations in the right anterior cingulate cortex of 14 high-functioning medication-free adults with autism spectrum disorder compared to a healthy control group of 14 age- and IQ-matched subjects. Lower glutamate + glutamine levels in the frontal lobes were observed in a group of 12 children that was compared to a control group of 16 children (Kubas et al. 2012).

Page et al. (2006), on the other hand, studying 25 adults with autistic spectrum disorders and 21 control subjects, observed a higher concentration of glutamate + glutamine in the amygdala-hippocampal region in the autism group. Bejjani et al. (2012) reported elevated levels of glutamate + glutamine in pregenual anterior cingulate cortex in children with autism spectrum disorder (two studies consisting of altogether 34 patients and 26 controls). Joshi et al. (2013), finally, observed significantly higher glutamate levels in the anterior cingulate cortex of seven adolescents with autistic disorder compared to seven control subjects, and a trend for lower glutamate in the right medial temporal lobe.

Glutamate is synthesized from glutamine by glutaminase in brain neurons. In a post-mortem study Shimmura et al. (2013) found that the protein levels of kidney-type glutaminase, which converts glutamate from glutamine, were lower in subjects with autism than in controls in the anterior cingulate cortex.

In a postmortem investigation, Purcell et al. (2001) reported decreased AMPA-type glutamate receptor density in the cerebellum in autism. In other postmortem studies, elevations of metabotropic glutamate receptor 5 (mGluR5) protein in children with autism have been observed in the superior frontal cortex (Fatemi and Folsom 2011) and in the cerebellar vermis (Fatemi et al. 2011). In a postmortem study in fragile X patients, finally, mGluR5 protein expression was found to be increased in the prefrontal cortex (Lohith et al. 2013).

Taken together, there is accumulating biochemical evidence pointing to aberrations in glutamatergic signalling in autism.

12.3 Genetics

An increasing number of genetic studies support the involvement of glutamate system in autism. In a postmortem brain study, Purcell et al. (2001) reported increased expression of glutamate transporter genes in autism. Other studies have implicated the involvement of the ionotropic glutamate receptor 6 (Jamain et al. 2002; Shuang et al. 2004) and the metabotropic glutamate receptor 8 (Serajee et al. 2003) genes in autism. Chung et al. (2007), reported an association between the D-amino acid oxidase gene and autism spectrum disorders. D-amino acid oxidase catabolises D-serine, an agonist of the NMDA receptor-associated glycine site, which impacts glutamatergic transmission.

For a recent overview of further genes potentially associated with glutamatergic signalling in autism, see Choudhury et al. 2012.

12.4 Fever

Since case reports and anecdotal evidence have suggested a beneficial effect of fever on autistic symptoms, Curran et al. (2007) conducted a prospective study in children with autism spectrum disorders during and after an episode of fever. Febrile patients were found to display fewer aberrant behaviours than afebrile patients on the Aberrant Behaviour Checklist subscales of hyperactivity, stereotypy, and inappropriate speech. This is interesting in view of the hypoglutamatergic hypothesis of autism, since a relationship between fever and increased glutamate release has been observed in a number of studies (Monda et al. 1998; Shuaib et al. 1996).

12.5 Clinical Trials with Glutamatergic Agents

Early clinical trials with glutamatergic agents have been conducted in autism and include low-moderate affinity un-competitive NMDA antagonists, a partial glycine agonist, an AMPA receptor modulator and glutamate release inhibiting agents.

The moderate affinity un-competitive NMDA antagonist memantine has been tested in a few open trials. In an 8-week, open-label study in 12 children with pervasive developmental disorders (PDDs), the majority with autistic disorder, no significant differences were found from baseline on measures of expressive or receptive language or nonverbal IQ, with the exception of an improvement on a memory test, Children's Memory Scale Dot Learning Subtest. There were also significant improvements on a number of Aberrant Behavior Checklist (ABC) subscales, filled out weekly by parents/caretakers, such as hyperactivity, lethargy and irritability. However, ratings by the clinician on the Clinical Global Impression (CGI) severity scale did not show any significant improvement (Owley et al. 2006). A further study consisting of 18 patients with PDD (of whom 13 had a diagnosis of autistic disorder) treated with open-label memantine for a mean duration of 19.3 weeks (range 1.5–56 weeks) was reviewed retrospectively. 72% of the patients received stable doses of concomitant medications. 61% of the patients were judged responders to memantine based on a rating of "much improved" or "very much improved" on the CGI-Improvement subscale (CGI-I). Primarily, social withdrawal and inattention seemed to be improved (Erickson et al. 2007). In a third open-label study, add-on therapy with memantine was offered to 151 patients with autism (69.7%) or Pervasive Developmental Disorder Not Otherwise Specified. CGI-I ratings showed significant improvements in language function and social behaviour (Chez et al. 2007). In a 10-week, double-blind, placebo-controlled add-on study, children with autism received risperidone in combination with memantine ($n=20$) or placebo ($n=20$); the group that received memantine was reported to have a greater reduction in ABC-C subscale scores for irritability, stereotypic behaviour and hyperactivity (Ghaleiha et al. 2013).

The mechanism behind any beneficial effect of memantine in autism remains unclear. In the context of Alzheimer's disease it has been suggested that the reported cognition enhancing effects of memantine are due to several reasons enumerated below: 1) reinforced AMPA-receptor-mediated transmission (Müller et al. 1995); 2) a preferential blockade of NMDA receptors pertaining to inhibitory GABAergic interneurons, resulting in subsequent disinhibition of glutamatergic neurons (Dimpfel 1995); 3) an improved "signal to noise" ratio in glutamatergic systems (Parsons et al. 2007) and/or 4) a muscarinic agonist effect (Drever et al. 2007; see Johnson and Kotermanski 2006). More recently, there was a report from a mouse model, suggesting that a stimulatory effect of memantine on excitatory synapse formation could also contribute to the therapeutic effects of memantine (Wei et al. 2012).

The low affinity uncompetitive NMDA receptor antagonist amantadine was tested in a double-blind, placebo-controlled 4-week study in 39 children and adolescents with autism. Parents' ratings (The Aberrant Behavior Checklist-Community Version) of irritability and hyperactivity were not significantly different for the placebo versus the amantadine group but there were statistically significant improvements in clinician-rated hyperactivity and inappropriate speech (King et al. 2001).

Following encouraging case reports with the antitussive low-affinity, uncompetitive NMDA receptor antagonist dextromethorphan in autism, this agent was tested in a double-blind, placebo-controlled study in eight children with autism spectrum

disorder and varying degrees of mental retardation. There were no significant effects found at the group level, leading the authors to conclude that dextromethorphan is not a generally effective treatment for autism (Woodard et al. 2007).

In a pilot study on ten drug-free children with autistic disorder, D-cycloserine, a partial agonist of the NMDA receptor-associated glycine site, was reported to improve measures on the CGI-Severity scale and the ABC Social Withdrawal subscale (Posey et al. 2004).

The positive modulator of AMPA-sensitive glutamate receptors, piracetam, was tested as add-on therapy to risperidone in a 10-week, double-blind, placebo-controlled study in 40 children (piracetam + risperidone: $n=20$; placebo + risperidone: $n=20$) with autistic disorder and was reported to improve total score on the Aberrant Behavior Checklist-Community (ABC-C) Rating Scale (Akhondzadeh et al. 2008).

The glutamate release inhibiting agent lamotrigine was not found to be superior to placebo in a double-blind, 18-week study in 28 children with autistic disorder who were drug-free for at least 1 month before entering the trial (Belsito et al. 2001). On the other hand, a recent case report with the glutamate release inhibiting agent riluzole, used as add-on therapy, described beneficial effects on repetitive behaviors and irritability in three individuals (one adolescent and two young adults) with autistic disorder (Wink et al. 2011).

Two small clinical trials (one open and one placebo-controlled) with mGluR5 antagonists showed encouraging results in patients with fragile X syndrome (Berry-Kravis et al. 2009; Jacquemont et al. 2011). However, there are at present no published reports with this type of agents in autism spectrum disorder.

Large-scale double-blind, placebo-controlled studies are needed to consolidate the positive reports on some of the glutamatergic agents described above. In addition, it might be worthwhile testing in autism some compounds that have shown promising results in schizophrenia, including D-serine, an agonist of the NMDA receptor-associated glycine site (Tsai et al. 1998; Heresco-Levy et al. 2005), the glycine transporter 1 inhibitor sarcosine (Tsai et al. 2004) and a metabotropic glutamate 2/3 (mGlu2/3) receptor agonist like LY2140023 (Patil et al. 2007).

An observation that seems to speak against the notion of deficient glutamatergic transmission in autism, at least as far as NMDA receptors are concerned, is the fact that while chronic use of an NMDA antagonist like phencyclidine can produce paranoid delusions and auditory hallucinations, these symptoms are not typically seen in autism. However, this may have to do with the time course of the disease. For example, autism is clinically evident from birth or at the latest at 3 years of age. There is often a severe gating defect, so that incoming stimuli of various modalities are grossly reinforced or distorted. To protect the brain from this sensory overload it is necessary for the child with autism to more or less completely shut out the outside world. A probable prerequisite for the build-up of paranoid delusions is that a non-autistic individual engages in social interactions and develops relationships with other people, but in autism a lack of social interest and interaction is manifested from a very early age, presumably partly due to the problems with sensory overload. Just as this self-imposed social isolation could explain why paranoid delusions of-

ten do not develop in autism, it could also explain the lack of auditory hallucinations (e.g., commanding voices) in this disorder.

12.6 Monoamine Stabilizers as Modulators of Glutamatergic Transmission

It is believed that positive and negative corticostriatothalamocortical feedback loops exert stimulatory and inhibitory control on psychomotor functions, and an optimal balance between the activity levels in these two circuits are thought to be important for motor and mental health. Included in these circuits are corticostriatal and thalamocortical glutamate neurons. Since these systems are modulated by midbrain dopamine neurons, it might be possible to improve a disturbed activity in glutamate neurons by treatment with dopaminergic agents. The first generation antipsychotics cause severe hypodopaminergia and resultant extrapyramidal side effects, anhedonia and impaired cognition. The second generation antipsychotics are less likely to cause extrapyramidal side effects but in general still cause other mental side effects. A new principle for modulation of dopaminergic transmission is via stabilization of dopamine tone, which means enhancing transmission in hypodopaminergic states and dampening transmission in hyperdopaminergic states (see Carlsson et al. 2004).

The dopamine stabilizers OSU6162 and ACR16 have shown efficacy in early clinical trials of three different disorders where both glutamate and dopamine are in all likelihood involved, i.e., schizophrenia, Huntington's disease and L-DOPA-induced dyskinesias in Parkinson's disease (Tedroff et al. 1999; Gefvert et al. 2000, unpublished data; Lundberg et al. 2002; Information from Neurosearch A/S, Denmark, published in an International Offering Circular September 22, 2006; see Carlsson and Carlsson 2006). In a more recent phase III trial in Huntington's disease, improvements in motor symptoms were observed (de Yebenes et al. 2011). In two small studies in schizophrenia, OSU6162 has been shown to reduce PANSS (positive and negative syndrome scale for schizophrenia) scores, with indications of effects on both positive and negative symptoms (Gefvert et al. 2000 and unpublished observations). Schizophrenic patients were described as more sociable following OSU6162 treatment (O. Gefvert, personal communication). Hence, it might be worthwhile to test these dopamine stabilizers also in autism spectrum disorders.

(−)-OSU6162, which has a stabilizing effect on rodent behaviour (Rung et al. 2008), behaves in vivo as a pure antagonist on a binding site of the D2 receptors, which is identical with the ordinary (orthosteric) binding site for dopamine itself, although with preference for presynaptic autoreceptors and with low affinity for postsynaptic receptors. In addition, (−)-OSU6162 has been proposed to act on an additional (allosteric) binding site on the dopamine D2 receptor, which leads to a stimulation of the receptor (Lahti et al. 2007; Rung et al. 2008).

More recent research has shown that (−)-OSU6162 additionally exerts a stabilizing effect on serotonergic neuronal circuits, acting as a partial 5-hydroxytryptamine 2A receptor (5-HT2A) agonist (Carlsson et al. 2011; Burstein et al. 2011).

Interestingly, stimulation of excitatory 5-HT2A receptors has been observed to induce either inhibition or activation of cortical glutamate neurons in a region-specific manner, depending on whether the location of the 5-HT2A receptor is on the pyramidal neuron itself or on a GABAergic interneuron (Marek and Aghajanian 1994; Aghajanian and Marek 1997). In both cases, by virtue of its partial agonism on 5-HT2A receptors, OSU6162 has the potential for a stabilization of glutamatergic transmission.

12.7 Concluding Remarks

The evidence suggesting that autism spectrum disorders involve dysfunctional brain glutamate systems is strengthened by an increasing number of publications. Several studies testing different agents that modulate glutamatergic transmission have been conducted but larger placebo-controlled studies are needed. An alternative possible avenue involves combined dopaminergic and serotonergic stabilization which would mediate indirect modulation of glutamatergic systems.

Acknowledgments This work was supported by Västra Götalandsregionen, Sweden.

References

Aboitiz F, Scheibel AB, Fisher RS, Zaidel E (1992) Fiber composition of the human corpus callosum. Brain Res 598:143–153

Aghajanian GK, Marek GJ (1997) Serotonin induces excitatory postsynaptic potentials in apical dendrites of neocortical pyramidal cells. Neuropharmacology 36:589–599

Akhondzadeh S, Tajdar H, Mohammadi MR, Mohammadi M, Nouroozinejad GH, Shabstari OL, Ghelichnia HA (2008) A double-blind placebo controlled trial of piracetam added to risperidone in patients with autistic disorder. Child Psychiatry Hum Dev 39:237–245

Aldred S, Moore KM, Fitzgerald M, Waring RH (2003) Plasma amino acid levels in children with autism and their families. J Autism Dev Disord 33:93–97

Alexander AL, Lee JE, Lazar M, Boudos R, DuBray MB, Oakes TR, Miller JN, Lu J, Jeong EK, McMahon WM, Bigler ED, Lainhart JE (2007) Diffusion tensor imaging of the corpus callosum in autism. Neuroimage 34:61–73

Alfredsson G, Wiesel FA, Tylec A (1988) Relationships between glutamate and monoamine metabolites in cerebrospinal fluid and serum in healthy volunteers. Biol Psychiatry 23:689–697

Badaruddin DH, Andrews GL, Bölte S, Schilmoeller KJ, Schilmoeller G, Paul LK, Brown WS (2007) Social and behavioral problems of children with agenesis of the corpus callosum. Child Psychiatry Hum Dev 38:287–302

Barnea-Goraly N, Kwon H, Menon V, Eliez S, Lotspeich L, Reiss AL (2004) White matter structure in autism: preliminary evidence from diffusion tensor imaging. Biol Psychiatry 55:323–326

Bejjani A, O'Neill J, Kim JA, Frew AJ, Yee VW, Ly R, Kitchen C, Salamon N, McCracken JT, Toga AW, Alger JR, Levitt JG (2012) Elevated glutamatergic compounds in pregenual anterior cingulate in pediatric autism spectrum disorder demonstrated by 1H MRS and 1H MRSI. PLoS One 7:e38786

Belsito KM, Law PA, Kirk KS, Landa RJ, Zimmerman AW (2001) Lamotrigine therapy for autistic disorder: a randomized, double-blind, placebo-controlled trial. J Autism Dev Disord 31:175–181

Bernardi S, Anagnostou E, Shen J, Kolevzon A, Buxbaum JD, Hollander E, Hof PR, Fan J (2011) In vivo 1H-magnetic resonance spectroscopy study of the attentional networks in autism. Brain Res 1380:198–205

Berry-Kravis E, Hessl D, Coffey S, Hervey C, Schneider A, Yuhas J, Hutchison J, Snape M, Tranfaglia M, Nguyen DV, Hagerman R (2009) A pilot open label, single dose trial of fenobam in adults with fragile X syndrome. J Med Genet 46:266–271

Bonilha L, Cendes F, Rorden C, Eckert M, Dalgalarrondo P, Li LM, Steiner CE (2008) Gray and white matter imbalance—typical structural abnormality underlying classic autism? Brain Dev 30:396–401

Burstein ES, Carlsson ML, Owens M, Ma JN, Schiffer HH, Carlsson A, Hacksell U. (2011) II. In vitro evidence that (–)-OSU6162 and (+)-OSU6162 produce their behavioral effects through 5-HT2A serotonin and D2 dopamine receptors. J Neural Transm 118:1523–1533

Carlsson ML (1998) Hypothesis: is infantile autism a hypoglutamatergic disorder? Relevance of glutamate—serotonin interactions for pharmacotherapy. J Neural Transm 105:525–535

Carlsson ML (2010) The glutamate system in Autism. In: Hollander E, Kolevzon A, Coyle JT (eds) Textbook of autism spectrum disorders, 1st edn. American Psychiatric Publishing, Arlington, pp 327–334

Carlsson A, Carlsson ML (2006) A dopaminergic deficit hypothesis of schizophrenia: the path to discovery. Dialogues Clin Neurosci 8:137–142

Carlsson ML, Carlsson A, Nilsson M (2004) Schizophrenia: from dopamine to glutamate and back. Curr Med Chem 11:267–277

Carlsson ML, Burstein ES, Kloberg A, Hansson S, Schedwin A, Nilsson M, Rung JP, Carlsson A (2011) I. In vivo evidence for partial agonist effects of (–)-OSU6162 and (+)-OSU6162 on 5-HT2A serotonin receptors. J Neural Transm 118:1511–1522

Chez MG, Burton Q, Dowling T, Chang M, Khanna P, Kramer C (2007) Memantine as adjunctive therapy in children diagnosed with autistic spectrum disorders: an observation of initial clinical response and maintenance tolerability. J Child Neurology 22:574–579

Choudhury PR, Lahiri S, Rajamma U (2012) Glutamate mediated signaling in the pathophysiology of autism spectrum disorders. Pharmacol Biochem Behav 2100:841–849

Chugani HT, Da Silva E, Chugani DC (1996) Infantile spasms. III. Prognostic implications of bitemporal hypometabolism on positron emission tomography. Ann Neurol 39:643–649

Chung S, Hong JP, Yoo HK (2007) Association of the DAO and DAOA gene polymorphisms with autism spectrum disorders in boys in Korea: a preliminary study. Psychiatry Res 153:179–182

Conti F, Manzoni T (1994) The neurotransmitters and postsynaptic actions of callosally projecting neurons. Behav Brain Res 64:37–53

Courchesne E, Pierce K (2005) Why the frontal cortex in autism might be talking only to itself: local over-connectivity but long-distance disconnection. Curr Opin Neurobiol 15:225–230

Curran LK, Newschaffer CJ, Lee LC, Crawford SO, Johnston MV, Zimmerman AW (2007) Behaviors associated with fever in children with autism spectrum disorders. Pediatrics 120:e1386–1392

DeVito TJ, Drost DJ, Neufeld RW (2007) Evidence for cortical dysfunction in autism: a proton magnetic resonance spectroscopic imaging study. Biol Psychiatry 61:465–473

de Yebenes JG, Landwehrmeyer B, Squitieri F, Reilmann R, Rosser A, Barker RA, Saft C, Magnet MK, Sword A, Rembratt A, Tedroff J, MermaiHD study investigators (2011) Pridopidine for the treatment of motor function in patients with Huntington's disease (MermaiHD): a phase 3, randomised, double-blind, placebo-controlled trial. Lancet Neurol 10:1049–1057

Dichter GS (2012) Functional magnetic resonance imaging of autism spectrum disorders. Dialogues Clin Neurosci 14:319–330

Dimpfel W (1995) Effects of memantine on synaptic transmission in the hippocampus in vitro. Arzneimittelforschung 45:1–5

Drever BD, Anderson WGL, Johnson H, O'Callaghan M, Seo S, Choi DY, Riedel G, Platt B (2007) Memantine acts as a cholinergic stimulant in the mouse hippocampus. J Alzheimers Dis 12:319–333

Egaas B, Courchesne E, Saitoh O (1995) Reduced size of the corpus callosum in autism. Arch Neurol 52:794–801

Erickson CA, Posey DJ, Stigler KA, Mullett J, Katschke AR, McDougle CJ (2007) A retrospective study of memantine in children and adolescents with pervasive developmental disorders. Psychopharmacology 191:141–147

Fatemi SH, Folsom TD (2011) Dysregulation of fragile X mental retardation protein and metabotropic glutamate receptor 5 in superior frontal cortex of individuals with autism: a postmortem brain study. Mol Autism 2:6

Fatemi SH, Folsom TD, Kneeland RE, Liesch SB (2011) Metabotropic glutamate receptor 5 upregulation in children with autism is associated with underexpression of both Fragile X mental retardation protein and GABAA receptor beta 3 in adults with autism. Anat Rec (Hoboken) 294:1635–1645

Frazier TW, Hardan AY (2009) A meta-analysis of the corpus callosum in autism. Biol Psychiatry 66: 935–941

Gefvert O, Lindström LH, Dahlbäck O, Sonesson C, Waters N, Carlsson A, Tedroff J (2000) (–)-OSU6162 induces a rapid onset of antipsychotic effect after a single dose. A doubleblind placebo-controlled pilot study. In: von Knorring L (ed) Scandinavian society for psychopharmacology, 41st annual meeting. Nord J Psychiat, Copenhagen, pp 93–94

Ghaleiha A, Asadabadi M, Mohammadi MR, Shahei M, Tabrizi M, Hajiaghaee R, Hassanzadeh E, Akhondzadeh S (2013) Memantine as adjunctive treatment to risperidone in children with autistic disorder: a randomized, double-blind, placebo-controlled trial. Int J Neuropsychopharmacol 16:783–789

Gittis AH, Kreitzer AC (2012) Striatal microcircuitry and movement disorders. Trends Neurosci 35:557–564

Hardan AY, Minshew NJ, Keshavan MS (2000) Corpus callosum size in autism. Neurology 55:1033–1036

Hawkins RA, O'Kane RL, Simpson IA, Viña JR (2006) Structure of the blood-brain barrier and its role in the transport of amino acids. J Nutr 136:218S–226S

Hayakawa K, Konishi Y, Matsuda T, Kuriyama M, Konishi K, Yamashita K, Okumura R, Hamanaka D (1989) Development and aging of brain midline structures: assessment with MR imaging. Radiology 172:171–177

Hazlett EA, Buchsbaum MS, Hsieh P, Haznedar MM, Platholi J, LiCalzi EM, Cartwright C, Hollander E (2004) Regional glucose metabolism within cortical Brodmann areas in healthy individuals and autistic patients. Neuropsychobiology 49:115–125

Haznedar MM, Buchsbaum MS, Metzger M, Solimando A, Spiegel-Cohen J, Hollander E (1997) Anterior cingulate gyrus volume and glucose metabolism in autistic disorder. Am J Psychiatry 154:1047–1050

Haznedar MM, Buchsbaum MS, Hazlett EA, LiCalzi EM, Cartwright C, Hollander E (2006) Volumetric analysis and three-dimensional glucose metabolic mapping of the striatum and thalamus in patients with autism spectrum disorders. Am J Psychiatry 163:1252–1263

Heresco-Levy U, Javitt DC, Ebstein R, Vass A, Lichtenberg P, Bar G, Catinari S, Ermilov M (2005) D-serine efficacy as add-on pharmacotherapy to risperidone and olanzapine for treatment-refractory schizophrenia. Biol Psychiatry 57:577–585

Herlenius E, Lagercrantz H (2001) Neurotransmitters and neuromodulators during early human development. Early Hum Dev 65:21–37

Jacquemont S, Curie A, des Portes V, Torrioli MG, Berry-Kravis E, Hagerman RJ, Ramos FJ, Cornish K, He Y, Paulding C, Neri G, Chen F, Hadjikhani N, Martinet D, Meyer J, Beckmann JS, Delange K, Brun A, Bussy G, Gasparini F, Hilse T, Floesser A, Branson J, Bilbe G, Johns D, Gomez-Mancilla B (2011) Epigenetic modification of the FMR1 gene in fragile X syndrome is associated with differential response to the mGluR5 antagonist AFQ056. Sci Transl Med 3:64ra1

Jamain S, Betancur C, Quach H, Philippe A, Fellous M, Giros B, Gillberg C, Leboyer M, Bourgeron T, Paris Autism Research International Sibpair (PARIS) Study (2002) Linkage and association of the glutamate receptor 6 gene with autism. Mol Psychiatry 7:302–310

Johnson JW, Kotermanski SE (2006) Mechanism of action of memantine. Curr Opin Pharmacol 6:61–67

Jones EG (1986) Neurotransmitters in the cerebral cortex. J Neurosurg 65:135–153

Joshi G, Biederman J, Wozniak J, Goldin RL, Crowley D, Furtak S, Lukas SE, Gönenç A (2013) Magnetic resonance spectroscopy study of the glutamatergic system in adolescent males with high-functioning autistic disorder: a pilot study at 4T. Eur Arch Psychiatry Clin Neurosci 263:379–384

Just MA, Cherkassky V L, Keller TA, Minshew NJ (2004) Cortical activation and synchronization during sentence comprehension in highfunctioning autism: evidence of underconnectivity. Brain 127:1811–1821

Just MA, Cherkassky1 VL, Keller TA, Kana RK, Minshew NJ (2007) Functional and anatomical cortical underconnectivity in autism: evidence from an fMRI study of an executive function task and corpus callosum morphometry. Cerebral Cortex 17:951–961

Kana RK, Keller TA, Cherkassky, VL, Minshew NJ, Just MA (2006) Sentence comprehension in autism: Thinking in pictures with decreased functional connectivity. Brain 129:2484–2493

Keller TA, Kana RK, Just MA (2007) A developmental study of the structural integrity of white matter in autism. Neuroreport 18:23–27

King BH, Wright DM, Handen BL, Sikich L, Zimmerman AW, McMahon W, Cantwell E, Davanzo PA, Dourish CT, Dykens EM, Hooper SR, Jaselskis CA, Leventhal BL, Levitt J, Lord C, Lubetsky MJ, Myers SM, Ozonoff S, Shah BG, Snape M, Shernoff EW, Williamson K, Cook EH Jr (2001) Double-blind, placebo-controlled study of amantadine hydrochloride in the treatment of children with autistic disorder. J Am Acad Child Adolesc Psychiatry 40:658–665

Kubas B, Kulak W, Sobaniec W, Tarasow E, Lebkowska U, Walecki J (2012) Metabolite alterations in autistic children: a 1H MR spectroscopy study. Adv Med Sci 57:152–156

Kumar SS, Huguenard JR (2001) Properties of excitatory synaptic connections mediated by the corpus callosum in the developing rat neocortex. J Neurophysiol 86:2973–2985

Lahti RA, Tamminga CA, Carlsson A (2007) Stimulating and inhibitory effects of the dopamine "stabilizer" (–)-OSU6162 on dopamine D(2) receptor function in vitro. J Neural Transm 114:1143–1146

Lamantia AS, Rakic P (1990) Cytological and quantitative characteristics of four cerebral commissures in the rhesus monkey. J Comp Neurol 291:520–537

Lee JE, Bigler ED, Alexander AL, Lazar M, DuBray MB, Chung MK, Johnson M, Morgan J, Miller JN, McMahon WM, Lu J, Jeong EK, Lainhart JE (2007) Diffusion tensor imaging of white matter in the superior temporal gyrus and temporal stem in autism. Neurosci Lett 424:127–132

Lohith TG, Osterweil EK, Fujita M, Jenko KJ, Bear MF, Innis RB (2013) Is metabotropic glutamate receptor 5 upregulated in prefrontal cortex in fragile X syndrome? Mol Autism 4:15

Lundberg T, Tedroff J, Waters N, Sonesson C, Carlsson A, Hagström P, Lindström H, Gefvert O (2002) Safety and early clinical experience with (–)-OSU6162, a dopamine stabilizer with antipsychotic properties. In: SCNP 43rd annual and 2nd mediterranean meeting, Juan-les-Pins, France. Nord J Psychiatry 56:24

Marek GJ, Aghajanian GK (1994) Excitation of interneurons in piriform cortex by 5-hydroxytryptamine (5-HT): blockade by MDL 100,907, a highly selective 5-HT2A antagonist. Eur J Pharmacol 259:137–141

Mason RA, Williams DL, Kana RA, Minshew N, Just MA (2008) Theory of mind disruption and recruitment of the right hemisphere during narrative comprehension in autism. Neuropsychologia 46:269–280

Monda M, Viggiano A, Sullo A, De Luca V (1998) Aspartic and glutamic acids increase in the frontal cortex during prostaglandin E 1 hyperthermia. Neuroscience 83:1239–1243

Moreno-Fuenmayor H, Borjas L, Arrieta A, Valera V, Socorro-Candanoza L (1996) Plasma excitatory amino acids in autism. Invest Clin. 37:113–128

Müller WE, Mutschler E, Riederer P (1995) Noncompetitive NMDA receptor antagonists with fast open-channel blocking kinetics and strong voltage-dependency as potential therapeutic agents for Alzheimer's dementia. Pharmacopsychiatry 28:113–124

Nydén A, Carlsson M, Carlsson A, Gillberg C (2004) Interhemispheric transfer in high-functioning children and adolescents with autism spectrum disorders: a controlled pilot study. Dev Med Child Neurol 46:448–454

Owley T, Salt J, Guter S, Grieve A, Walton L, Ayuyao N, Leventhal BL, Cook EH Jr (2006) A prospective, open-label trial of memantine in the treatment of cognitive, behavioral, and memory dysfunction in pervasive developmental disorders. J Child Adolescent Psychopharmacol 16:517–524

Page LA, Daly, E, Schmitz N, Simmons A, Toal F, Deeley Q, Ambery F, McAlonan GM, Murphy KC, Murphy DG (2006) In vivo 1H-Magnetic resonance spectroscopy study of amygdala-hippocampal and parietal regions in autism. Am J Psychiatry 163:2189–2192

Parsons CG, Stöffler A, Danysz W (2007) Memantine: a NMDA receptor antagonist that improves memory by restoration of homeostasis in the glutamatergic system—too little activation is bad, too much is even worse. Neuropharmacology 53:699–723

Partridge JG, Janssen MJ, Chou DY, Abe K, Zukowska Z, Vicini S (2009) Excitatory and inhibitory synapses in neuropeptide Y-expressing striatal interneurons. J Neurophysiol 102:3038–3045

Patel AB, de Graaf RA, Mason GF, Rothman DL, Shulman RG, Behar KL (2005) The contribution of GABA to glutamate/glutamine cycling and energy metabolism in the rat cortex in vivo. Proc Natl Acad Sci U S A 102:5588–5593

Patil ST, Zhang L, Martenyi F, Lowe SL, Jackson KA, Andreev BV, Avedisova AS, Bardenstein LM, Gurovich IY, Morozova MA, Mosolov SN, Neznanov NG, Reznik AM, Smulevich AB, Tochilov VA, Johnson BG, Monn JA, Schoepp DD (2007) Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized Phase 2 clinical trial. Nat Med 13:1102–1107

Paul LK (2011) Developmental malformation of the corpus callosum: a review of typical callosal development and examples of developmental disorders with callosal involvement. J Neurodev Disord 3:3–27

Paul LK, Brown WS, Adolphs R, Tyszka JM, Richards LJ, Mukherjee P, Sherr EH (2007) Agenesis of the corpus callosum: genetic, developmental and functional aspects of connectivity. Nat Rev Neurosci 8:287–299

Philip RC, Dauvermann MR, Whalley HC, Baynham K, Lawrie SM, Stanfield AC (2012) A systematic review and meta-analysis of the fMRI investigation of autism spectrum disorders. Neurosci Biobehav Rev 36:901–942

Piven J, Bailey J, Ranson B J, Arndt S (1997) An MRI study of the corpus callosum in autism. Am J Psychiatry 154:1051–1056

Posey DJ, Kem DL, Swiezy NB, Sweeten TL, Wiegand RE, McDougle CJ (2004) A pilot study of D-cycloserine in subjects with autistic disorder. Am J Psychiatry 161:2115–2117

Purcell AE, Jeon OH, Zimmerman AW, Blue ME, Pevsner J (2001) Postmortem brain abnormalities of the glutamate neurotransmitter system in autism. Neurology 57:1618–1628

Rolf LH, Haarmann FY, Grotemeyer KH, Kehrer H (1993) Serotonin and amino acid content in platelets of autistic children. Acta Psychiatr Scand 87:312–316

Rothman DL, Behar KL, Hyder F, Shulman RG (2003) In vivo NMR studies of the glutamate neurotransmitter flux and neuroenergetics: implications for brain function. Annu Rev Physiol 65:401–427

Rubenstein JL, Merzenich MM (2003) Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav 2:255–267

Rung JP, Rung E, Helgeson L, Johansson AM, Svensson K, Carlsson A, Carlsson ML (2008) Effects of (–)-OSU6162 and ACR16 on motor activity in rats, indicating a unique mechanism of dopaminergic stabilization. J Neural Transm 115:899–908

Serajee FJ, Zhong H, Nabi R, Huq AH (2003) The metabotropic glutamate receptor 8 gene at 7q31: partial duplication and possible association with autism. J Med Genet 40:e42

Shimmura C, Suda S, Tsuchiya KJ, Hashimoto K, Ohno K, Matsuzaki H, Iwata K, Matsumoto K, Wakuda T, Kameno Y, Suzuki K, Tsujii M, Nakamura K, Takei N, Mori N (2011) Alteration of plasma glutamate and glutamine levels in children with high-functioning autism. PLoS One 6:e25340

Shimmura C, Suzuki K, Iwata Y, Tsuchiya KJ, Ohno K, Matsuzaki H, Iwata K, Kameno Y, Takahashi T, Wakuda T, Nakamura K, Hashimoto K, Mori N (2013) Enzymes in the glutamate-glutamine cycle in the anterior cingulate cortex in postmortem brain of subjects with autism. Mol Autism 4:6

Shinohe A, Hashimoto K, Nakamura, Tsujii M, Iwata Y, Tsuchiya KJ, Sekine Y, Suda S, Suzuki K, Sugihara G, Matsuzaki H, Minabe Y, Sugiyama T, Kawai M, Iyo M, Takei N, Mori N (2006) Increased serum levels of glutamate in adult patients with autism. Prog Neuropsychopharmacol Biol Psychiatry 30:1472–1477

Shuaib A, Kanthan R, Goplen G, Griebel R, el-Azzouni H, Miyashita H, Liu L, Hogan T (1996) In-vivo microdialysis study of extracellular glutamate response to temperature variance in subarachnoid hemorrhage. Acta Neurochir Suppl 67:53–58

Shuang M, Liu J, Jia MX, Yang JZ, Wu SP, Gong XH, Ling YS, Ruan Y, Yang XL, Zhang D (2004) Family-based association study between autism and glutamate receptor 6 gene in Chinese Han trios. Am J Med Genet B Neuropsychiatr Genet 131:48–50

Somogyi P, Tamás G, Lujan R, Buhl EH (1998) Salient features of synaptic organisation in the cerebral cortex. Brain Res Rev 26:113–135

Stanfield AC, McIntosh AM, Spencer MD, Philip R, Gaur S, Lawrie SM (2008) Towards a neuroanatomy of autism: a systematic review and meta-analysis of structural magnetic resonance imaging studies. Eur Psychiatry 23:289–299

Tedroff J, Ekesbo A, Sonesson C, Waters N, Carlsson A (1999) Long-lasting improvement following (–)-OSU6162 in a patient with Huntington's disease. Neurology 53:1605–1606

Travers BG, Adluru N, Ennis C, Tromp do PM, Destiche D, Doran S, Bigler ED, Lange N, Lainhart JE, Alexander AL (2012) Diffusion tensor imaging in autism spectrum disorder: a review. Autism Res 5:289–313

Tsai G, Yang P, Chung LC (1998) D-serine added to antipsychotics for the treatment of schizophrenia. Biol Psychiatry 44:1081–1089

Tsai G, Lane HY, Yang P, Chong MY, Lange N (2004) Glycine transporter I inhibitor, N-methylglycine (sarcosine), added to antipsychotics for the treatment of schizophrenia. Biol Psychiatry 55:452–456

Vidal CN, Nicholson R, DeVito TJ, Hayashi KM, Geaga JA, Drost DJ, Williamson PC, Rajakumar N, Sui Y, Dutton RA, Toga AW, Thompson PM (2006) Mapping corpus callosum deficits in autism: an index of aberrant cortical connectivity. Biol Psychiatry 60:218–225

Waiter GD, Williams JHG, Murray AD, Gilchrist A, Perrett DI, Whiten A (2005) Structural white matter deficits in high-functioning individuals with autistic spectrum disorder: a voxel-based investigation. NeuroImage 24:455–461

Wei H, Dobkin C, Sheikh AM, Malik M, Brown WT, Li X (2012) The therapeutic effect of memantine through the stimulation of synapse formation and dendritic spine maturation in autism and fragile X syndrome. PLoS One 7:e36981

Wink LK, Erickson CA, Stigler KA, McDougle CJ (2011) Riluzole in autistic disorder. J Child Adolesc Psychopharmacol 21:375–379

Woodard C, Groden J, Goodwin M, Bodfish J (2007) A placebo double-blind pilot study of dextromethorphan for problematic behaviors in children with autism. Autism 11:29–41

Zavala M, Castejón HV, Ortega PA, Castejón OJ, Marcano de Hidalgo A, Montiel N (2001) Imbalance of plasma amino acids in patients with autism and subjects with attention deficit/hyperactivity disorder. Rev Neurol 33:401–408

Chapter 13

Serotonin in Autism Spectrum Disorder: Insights from Human Studies and Animal Models

Natalia Benza and Diane C. Chugani

Abstract Increased blood serotonin in people with autism was first reported over 50 years ago, and this biogenic amine has remained a focus for the understanding, risk and treatment of Autism Spectrum Disorders (ASD). There is growing evidence that serotonergic transmission is altered by disparate genetic and environmental risk factors for ASD. This review will focus on recent developments regarding serotonin in ASD. Recent studies include epidemiology studies linking ASD with conditions that alter prenatal serotonin in the fetus, altered serotonin in diverse genetic and environmental animal models, and human pathology and molecular and functional brain imaging studies.

Keywords Serotonin · Serotonin transporter · Tryptophan hydroxylase · Monoamine oxidase · Serotonin receptors

Increased blood serotonin is the earliest biomarker reported for ASD (Schain and Freedman 1961). This biogenic amine has remained a focus for the understanding, risk and treatment of ASD. Current evidence now suggests that autism is likely to be caused by dysfunction of many genes (Zhao et al. 2007). Given that many different genes can cause autism, it is striking that 30–50 % of autistic individuals show elevated serotonin in the blood (Schain and Freedman 1961; Hoshino et al. 1984; Anderson et al. 1987; Cook et al. 1990). Furthermore, there is growing evidence that serotonergic transmission is altered by disparate genetic and environmental risk factors for ASD. This review will focus on recent developments on serotonin in autism. Recent studies include epidemiologic studies linking ASD with conditions that alter prenatal serotonin in the fetus, altered serotonin in diverse genetic and environmental animal models, and human pathology and molecular and functional brain imaging studies.

D. C. Chugani (✉) · N. Benza
Department of Pediatrics, Wayne State University School of Medicine, Detroit, MI, USA
e-mail: dchugani@pet.wayne.edu

D. C. Chugani
Division of Clinical Pharmacology and Toxicology, Children's Hospital of Michigan, 3901 Beaubien Blvd., Room 3N47, Detroit, MI 48201, USA

S. Hossein Fatemi (ed.), *The Molecular Basis of Autism,*
Contemporary Clinical Neuroscience, DOI 10.1007/978-1-4939-2190-4_13

13.1 Overview of the Serotonin Pathway

Serotonin is synthesized from the precursor tryptophan, an essential amino acid constituting one percent of the total amino acid pool (Hamon et al. 1981). The majority of tryptophan in blood is bound to plasma protein, with only free plasma tryptophan being available for transport into the brain. Tryptophan is transported into the brain via the large neutral amino acid carrier (LAT1) where it competes for transport with the other large neutral amino acids (Pardridge et al. 1977; Smith et al. 1987). The first and rate-limiting step of serotonin synthesis is the formation of 5-hydroxytryptophan catalyzed by the enzyme tryptophan hydroxylase (TPH). Tryptophan hydroxylase is only 50% saturated with tryptophan, resulting in the dependence of brain serotonin levels on the plasma concentration of free tryptophan as well as the plasma levels of the other large neutral amino acids (Fernstrom and Wurtman 1971). This dependence of free plasma tryptophan on brain serotonin levels has been exploited to study the effects of tryptophan depletion on brain function in adults with ASD (Daly et al. 2012, see below). 5-hydroxytryptophan is further metabolized by aromatic amino acid decarboxylase (AADC) to form serotonin (Christenson et al. 1972). Serotonin is metabolized by the enzyme monoamine oxidase A (MAOA) to produce its major metabolite 5-hydorxyindoleacetic acid (5HIAA) (for review see Bortolato et al. 2008).

The serotonin transporter (5HTT or SERT) mediates uptake of serotonin following release at the synapse and at other sites such as the platelet. The gene for the serotonin transporter has 2 forms, the short and the long form, that are common in the general population. The short variant of the 5HTT gene has 30–40% lower mRNA expression leading to 50% lower serotonin uptake than transporters produced by the long form (Lesch et al. 1996). To date, fifteen receptors for serotonin have been identified, and these are expressed in the brain and throughout the body where they regulate numerous processes (for review see Berger et al. 2009). In addition to receptor-mediated mechanisms, serotonin has been recently reported to signal through a process called serotonylation (for review see, Walther et al. 2011). Serotonylation involves the covalent linkage of serotonin to proteins mediated by the enzyme transglutaminase. Linkage of serotonin to GTPases has been reported to increase alpha-granule exocytosis in platelets (Walther et al. 2003).

There are 2 genes encoding tryptophan hydroxylase, TPH1 and TPH2 (Sakowski et al. 2006). TPH2 is found in the brain and in the gastrointestinal nervous system (Zhang et al. 2004), while TPH1 is located in the pineal gland and throughout the rest of the body. Many current references suggest that the majority of the serotonin synthesized in the body is generated by the enterochromaffin cells in the gastrointestinal tract (Gershon and Tack 2007). However, tryptophan hydroxylase is located at many peripheral sites, and there have been no studies comparing the amount of serotonin produced at all of these sites to that synthesized in the gastrointestinal tract. There is much evidence that TPH1 is ubiquitously distributed and that serotonin is involved in many different functions throughout the body. Many of these functions might be related to different aspects of autism. In addition to the

gastrointestinal tract and the central nervous system, serotonin is synthesized in bronchial epithelium, taste papillae, thyroid parafollicular cells, ovaries, thymus, pancreas, breast, skin and arteries (Eddahibi et al. 2006; Ortiz-Alvarado et al. 2006; Matsuda et al. 2004; Stull et al. 2007; Slominski et al. 2002, Ni et al. 2008). TPH and AADC mRNAs are expressed in the heart and serotonin is produced and released in cardiomyocytes (Ikeda et al. 2005). TPH1 mRNA and TPH protein are expressed in trigeminal neurons and regulated during the estrous cycle (Berman et al. 2006). Serotonin plays an important role in mammary gland function. TPH mRNA is elevated during pregnancy and lactation and serotonin is present in mammary epithelium and in milk (Matsuda et al. 2004). Elevated plasma serotonin in autism might also be affected by changes in storage and degradation. Plasma serotonin is taken up by platelets through serotonin transporters (Ni and Watts 2006), and is cleared by liver and lung endothelium (Nocito et al. 2007). Finally, serotonin and tryptophan are very tightly regulated at the fetal/maternal interface in the placenta. The serotonin transporter is highly expressed in the brush border membrane of the human placenta and may mediate transport of serotonin from the maternal circulation to the developing fetus (Balkovetz et al. 1989). Tryptophan is transported competitively at the placenta via the large neutral amino-acid carrier (LAT1) (Kudo and Boyd 2002). The placenta expresses TPH2 resulting in serotonin production (Correa et al. 2009). In addition, tryptophan in the placenta is catalyzed along the kynurenine pathway by tryptophan 2,3-dioxygenase and indoleamine 2,3-dioxygenase (IDO), both of which are expressed in the placenta and have a vital role in the prevention of allogeneic rejection of the fetus (Munn et al. 1998) and regulation of feto-placental blood flow in late gestation (Ligam et al. 2005). Finally, Bonnin et al. (2011) demonstrated that serotonin in the forebrain during the early fetal period in the rat has its origin in the placenta. Studies in mice deficient in peripheral serotonin synthesis have shown that maternal serotonin production is crucial for normal fetal neurogenesis and development (Cote et al. 2007).

13.2 Elevated Blood Serotonin in Autism

As mentioned above, elevated serotonin in ASD is a long-standing (Schain and Freedman 1961) and replicated finding (Hoshino et al. 1984; Anderson et al. 1987; Cook et al. 1990), and extended with the recognition that blood serotonin is also elevated in the first degree relatives of autistic individuals (Leventhal et al. 1990; Piven et al. 1991; Cook et al. 1994; Leboyer et al. 1999). The mechanism for the elevated platelet serotonin may be due to increased exposure to serotonin or altered handling of serotonin, as recently examined by Anderson et al. (2012). Based on the lack of difference in the serotonin concentration in platelet poor plasma in samples from an ASD group, an ASD subgroup with hyperserotonemia and a control group, these authors suggest that hyperserotonemia may be more related to platelet handling of serotonin than exposure to serotonin. Mechanisms they discussed for further study included study of the transporter, the granular transporter and dense

granule storage mechanisms, serotonin release processes and platelet activation. In this regard, Veenstra-VanderWeele et al. (2012) developed a mouse model based upon the SERT Ala56 variant that is over-transmitted to ASD probands. The SERT Ala56 mouse model showed hyperserotonemia, consistent with a role of the transporter in elevated blood serotonin. However, this mechanism does not explain most cases of hyperserotonemia, as mutations in SERT are not found in the majority of cases of ASD.

13.3 Serotonin and Human Brain Pathology in Autism

Given the long standing recognition of altered serotonin in the blood in those with ASD, it is surprising that studies of serotonergic markers in postmortem brains from persons with ASD have not been examined until recently. Two reports from the same group have reported increased serotonin transporter immunoreactivity in human postmortem brains from individuals with autism aged 2–29 years (Azmitia et al. 2011a, b). Increased SERT immunoreactivity was demonstrated for the principle ascending fiber bundles of the medial and lateral forebrain bundles, Ansa lenticularis and stria terminalis, and in the innervation density of the globus pallidus, amygdala, and in the piriform, superior temporal and parahippocampal cortices. In addition, in cases over 8 years of age, the presence of thick, heavily stained dystrophic axons were observed. In contrast, Oblak et al. (2013) reported significant reduction in the density of SERT binding in the deep layers of the fusiform gyrus and normal binding levels in the superficial layers of the fusiform gyrus, as well as in both layers of the posterior cingulate cortex. The same investigators reported significant reductions in 5-HT1A receptor-binding density in superficial and deep layers of the posterior cingulate cortex and fusiform gyrus and reduced density of 5-HT2A receptors in superficial layers of the posterior cingulate cortex and fusiform gyrus. Differences in the results between the immunocytochemistry studies and the binding studies for SERT may be due to the use of different brain regions, differences in age or use of different investigational techniques. However, molecular imaging studies have reported SERT binding in both children and adults with ASD, including some of the same brain regions that showed an increase SERT immunoreactivity (see below).

13.4 Molecular Imaging of Serotonin Precursor, Transporter and Receptor Studies

In the earliest molecular imaging studies of serotonin in ASD, Chugani et al. (1997) applied alpha[C-11]methyl-L-tryptophan (AMT) as a PET tracer in children with autism. AMT, which was developed as a tracer for serotonin synthesis with PET (Diksic et al. 1990), is an analogue of tryptophan, the precursor for serotonin

synthesis. Two types of serotonergic abnormality were found in children with autism (Chugani et al. 1997, 1999; Chandana et al. 2005). The first was altered whole brain serotonin synthesis capacity in autistic children compared to age matched nonautistic children. Serotonin synthesis capacity was greater than 200% of adult values until the age of 5 years followed by a decline to adult values in non-autistic children. In contrast, serotonin synthesis capacity in autistic children increased gradually between the ages of 2 years and 15 years to values 1.5 times the adult normal values (Chugani et al. 1999). These data suggested that developmental regulation of brain serotonin synthesis capacity postnally in humans, was disrupted in children with autism. Secondly, focal symmetries of AMT uptake in frontal cortex, thalamus and cerebellum were visualized in children with autism, suggesting a role of the dentato-thalamo-cortical pathway in autism (Chugani et al. 1997). Subsequently, the same group measured brain serotonin synthesis in a large group of autistic children ($n = 117$) with AMT PET and related these data to handedness and language function (Chandana et al. 2005). Autistic children with left cortical AMT decreases showed a higher prevalence of severe language impairment, whereas those with right cortical decreases showed a higher prevalence of left and mixed handedness. These results suggest that global as well as focal abnormally asymmetric development in the serotonergic system could lead to miswiring of the neural circuits specifying hemispheric specialization.

Decreased serotonin transporter binding has been reported in both children and adults with autism. Makkonen et al. (2008) using the SPECT tracer [^{123}I] nor-beta-CIT labeling both the dopamine and serotonin transporter, reported reduced serotonin transporter binding capacity in medial frontal cortex, midbrain and temporal lobes. Similarly, Nakamura et al. (2010) reported decreased serotonin transporter binding throughout the brain in adults with autism (20 men, 18–26 years) using [^{11}C]McN-5652 imaged with PET. Furthermore, the reduction in binding in anterior and posterior cingulate cortices was correlated with impairment in social cognition, while the reduction in serotonin transporter binding in the thalamus was correlated with repetitive and/or obsessive behavior. In contrast, Girgis et al. (2011) reported no significant difference in brain serotonin transporter binding, measured with [^{11}C] DASB and PET, in a group of 8 adults with Asperger's Disorder (mean age 29.7 years) and 8 healthy control subjects matched for age, gender, and ethnicity. All subjects in this study had normal intelligence, while this was not the case for the other studies reporting changes in serotonin transporter binding.

Serotonergic neurotransmission was also studied using tracers for receptor binding. Murphy et al. (2006) measured 5HT2A receptors in eight men with Asperger's Syndrome (mean age 26 years) using the SPECT tracer [^{123}I]5-I-R91150, compared to 10 healthy age-matched men. The group with Asperger's Syndrome has significantly reduced serotonin receptor binding in total, anterior and posterior cingulate cortex, bilaterally in frontal and superior temporal lobes and in the left parietal lobe. Interestingly, there were significant correlations with qualitative abnormalities in social interaction and binding reductions in anterior and posterior cortices, as well as right frontal cortex. More recently, 5-HT2 receptor distribution was measured with the PET tracer [^{18}F] setoperone in six high functioning autistic adults compared

to ten matched control subjects (Beversdorf et al. 2012). In this study, reduced serotonin receptor binding was found in thalamus, and there was a negative relationship between thalamic binding and history of language impairment. Goldberg et al. (2009) compared the parents of children with autism (19 parents from 11 families, 8 females, 11 males) compared to adults who do not have children with autism (9 females, 8 males). The cortical 5HT2 binding potential, using [^{18}F]setoperone, to measure cortical serotonin type-2 receptor (5-HT2) using PET, was significantly lower in the autism parent group compared to the control group. Furthermore, the 5HT-2 binding potential was inversely correlated with platelet serotonin levels in the parent group. These results are interesting in light of family members having what has been described as the broader phenotype of autism. Finally, Girgis et al. (2011) reported no difference in 5HT2A receptor binding in a PET study using the tracer [^{11}C]MDL 100907 in a group of 17 adults with Asperger's Disorder (mean age 34.3) compared to 17 healthy matched adults.

In summary, molecular imaging studies provide convincing evidence of altered serotonergic neurotransmission in both children and adults with autism, as well as in parents of autistic individuals. Decreased serotonin transporter and 5HT2A binding measured in vivo using molecular imaging techniques are consistent with postmortem binding studies (Oblak et al. 2013). These studies are discordant with postmortem immunocytochemistry studies reporting increased serotonin immunoreactivity (Azmitia et al. 2011a, b). The reason for the discrepancy between the binding and immunocytochemistry studies might offer clues regarding the nature of alterations in serotonergic fibers. For example, these results considered together, might indicate fewer serotonergic fibers with remaining serotonergic fibers showing higher density of transporters.

13.5 Serotonin and Functional Imaging

In addition to molecular imaging studies that use radiolabeled ligands and substrates to measure serotonergic metabolism, transport and receptor systems, functional MRI (fMRI) has been applied in adults with ASD following pharmacological manipulation of the serotonergic system using either tryptophan depletion or treatment with the selective serotonin reuptake inhibitor (SSRI) fluoxetine. Following treatment to decrease blood tryptophan, which has been long demonstrated to decrease brain serotonin (Moja et al. 1988, 1989), Daly et al. (2012) reported altered brain activation as measured by blood oxygenation labeled dependent (BOLD) response measured with fMRI to face emotion in adults with ASD compared to matched control subjects. Furthermore, the differences in brain activation varied by emotion, and brain regions showing altered brain activation in the ASD group include brain regions showing focal abnormalities in serotonin synthesis, and decreased numbers of receptors and transporter in the molecular imaging studies. The same group (Chantiluke et al. 2014) studied the effects of fluoxetine on medial prefrontal activation during a reward reversal learning task in boys with autism compared to a

matched group of typically developing boys. This study showed that while placebo treatment was associated with decreased activation of the medial prefrontal cortex during the performance of the task in the ASD group, fluoxetine treatment lead to normalization of the activation in boys with ASD compared to controls. Despite normalization of brain activation in this study, the fluoxetine treatment did not improve behavioral performance on the task. Altered function related to serotonergic function in medial prefrontal cortex is consistent with decreased serotonin transporter binding in this region as reported by Makkonen et al. (2008).

The pharmacological treatments combined with fMRI measure of brain activation complement the postmortem and molecular imaging studies by assessing the functional significance of altered serotonergic neurotransmission in different brain regions. These results might be due to differences during brain development and ongoing changes in serotonergic mechanisms.

13.6 Genetic Evidence Linking Serotonin to Autism

Because of the long-standing link between serotonin and ASD, the serotonin transporter, its receptors and pathway enzymes have been studied as candidate genes for ASD. The serotonin transporter has 2 isoforms, the short and the long forms, that are common in the general population. The short variant of the 5HTT has 30–40% lower mRNA expression and 50% lower serotonin uptake than the long form (Lesch et al. 1996). There have been conflicting reports regarding the relative roles of the short and long forms in autism (reviewed in Devlin et al. 2005). More recently, the short allele of the 5HTT receptor was associated with higher gray matter volume in young boys with autism (Wassink et al. 2007). Altered serotonin synthesis, turnover and dynamic regulation in multiple brain regions have been reported in mice lacking the serotonin transporter (Kim et al. 2005). Veenstra-VanderWeele et al. (2012) reported that the SERT Ala56 variant was over-transmitted to ASD probands, but that it was also seen in some unaffected individuals, suggesting that associated ASD risk is influenced by the interaction of the SERT variant with other genetic factors. Subsequently, the same group showed that mice expressing the SERT Ala56 variant on a 129S6/S4 genetic background display multiple biochemical, physiological and behavioral changes, including hyperserotonemia, altered 5-HT receptor sensitivity, and abnormal social, communication, and repetitive behavior. The same group (Kerr et al. 2013) examined the effect of this same mutation on different mouse backgrounds. Presence of the SERT Ala56 variant on the B6 background caused a significant increase in mutant pup ultrasonic vocalizations, whereas vocalizations were decreased on the 129 background. Further, hyperserotonemia, 5-HT receptor hypersensivity, and repetitive behavior were not observed on the B6 background. These studies combined, demonstrated how epistatic interactions can modulate the effect of genetic mutations and how genes may modulate the risk of ASD.

Blood serotonin levels have been linked to an interaction of the serotonin transporter gene (SLC6A4) and integrin beta3 (ITGB3) (Weiss et al. 2006a, b). This

finding was recently replicated and additional interactions between these 2 genes were found, including an additive effect of the HTR5A gene, an interaction of TPH1 and SLC6A4, and an interaction between 5HTR1D and SLC6A4 for blood serotonin level (Coutinho et al. 2007). Whyte et al. (2014) examined the effect of Itgb3 on SERT function using a mouse genetic approach. Using isolated synaptoneurosomes and citalopram binding, they reported significant Slc6a4-driven reductions in SERT expression in midbrain synapses, but no significant changes in hippocampal or cortical regions. They also measured 5-HT uptake activity in synaptoneurosomal preparations. Itgb3 heterozygous mice displayed significant reductions in 5HT Vmax, with no changes in Km, in midbrain preparations. These results support an interaction of SLC6A4 and ITGB3 in the regulation of serotonergic systems in midbrain but not cortical and hippocampal serotonin synapses.

Several studies report association of genes that may impact serotonin metabolism in autism. Single nucleotide polymorphisms in introns 1 and 4 of TPH2, the brain specific form of tryptophan hydroxylase, showed an association with autism (Coon et al. 2005). Brain serotonin synthesis in mouse strains differs depending on a single nucleotide polymorphism in the Tph2 gene (Siesser et al. 2010). Based upon this Tph2 polymorphism, the BALB/c strain shows lower brain serotonin synthesis, although serotonin levels were not significantly altered. Behavioral studies of the BALB/c strain have shown low sociability and other phenotypes relevant to ASD (Brodkin 2007). Kane et al. (2012) analyzed mice lacking brain serotonin via a null mutation in the Tph2 gene for behaviors that are relevant to ASD. Mice lacking brain TPH2 showed substantial deficits in validated tests of social interaction and communication, as well as repetitive and compulsive behaviors. Newborn TPH22/2 mutant mice showed diminished preference for maternal scents over the scent of an unrelated female. The authors conclude that lack of serotonin during development could lead to ASD behavioral traits.

There are several studies linking genetic variation in the MAOA gene to ASD. Cortical enlargement in autism was reported to be associated with the “low activity” allele of the MAOA gene (Davis et al. 2008). Jones et al. (2004) reported that maternal genotypes containing specific polymorphisms at the MAOA locus show significant negative effects on the intelligence quotient (IQ) in children with autism. These results are consistent with those of a study which found that a low activity MAOA allele, due to an upstream variable-number tandem repeat region, is associated with both lower IQ and more severe autistic behavior in children, as compared to the high-activity allele (Cohen et al. 2003). Tassone et al. (2011) also reported an association of ASD with repeats in the MAOA promoter. They found that boys carrying 4 tandem repeats in the promoter region of the MAOA gene showed a 2-fold higher risk of autism or other forms of ASD than those carrying allele 3. They also found that mothers homozygous for the 4 tandem repeat allele showed at least a 3-fold higher risk for having a child with ASD than mothers homozygous for allele 3. Tassone et al. concluded that the functional MAOA promoter allelles in male children and mothers play a role in the risk for ASD. Finally, Verma et al. (2013) analyzed 8 MAOA markers in 421 subjects including cases and ethnically-matched controls from West Bengal. MAOA marker, rs6323 and haplotypes formed

between the markers showed a significant association with the ASD. In addition, gender stratification showed significant genetic effect of rs6323 with low activity T allele posing higher risk of ASD in males, but not in females. Taken together, genetic differences in genes for enzymes involved in the synthesis and degradation of serotonin play a role in the risk for ASD and associated phenotypes such as brain enlargement and intellectual disability. In addition, these studies suggest that serotonin alterations in mothers and gender may also play a role in the risk for ASD.

Metabolism of tryptophan by other metabolic pathways may also affect serotonin synthesis, due to the dependence of brain serotonin synthesis on plasma tryptophan levels (Fernstrom and Wurtman 1971). One such study reports the presence of a susceptibility mutation in a promoter variant of the tryptophan 2,3 dioxygenase gene (Nabi et al. 2004). Tryptophan 2,3-dioxygenase is a rate-limiting enzyme in the metabolism of tryptophan by the kynurenine pathway. A mutation that results in decreased activity of this enzyme could decrease the metabolism of tryptophan by this pathway and increase the level of whole body serotonin content.

13.7 Altered Serotonin in Autism Caused by Genes not Related to Serotonin

In addition to genetic variation in genes directly involved with serotonergic function, there is evidence that serotonergic function is altered in humans and in animal models having genetic changes associated with ASD. These genetic changes include mutations involved in causation of Rett syndrome, 15q duplication syndrome and Fragile X syndrome. There are several studies showing alterations in serotonergic neurotransmission in Rett syndrome, as well as in the MECP2-null mouse model of Rett syndrome. Decreased levels of the serotonin metabolite 5HIAA were reported in the CSF from 32 patients with Rett syndrome compared to age matched control subjects (Zoghbi et al. 1989). Serotonin concentrations in whole brains from mecp2-null mice showed no differences at birth, but were significantly lower at 42 days of age compared to wild-type mice (Ide et al. 2005). However, these changes were not specific to serotonin and changes in other transmitters were also reported. Further evidence that the serotonergic system is involved in MeCP2, is the report that the selective serotonin uptake inhibitor fluoxetine induced up regulation of Mecp2_e1 and Mecp2_e2 transcripts in adult rat brain (Cassel et al. 2006). Samaco et al. (2009) confirmed lower serotonin metabolite 5HIAA in the cerebral spinal fluid of 64 individuals with Rett syndrome and decreased serotonin in Mecp2-null male mice. In addition, this study deleted Mecp2 in serotonergic neurons positive for the marker PET-1 and showed decreased levels of serotonin and decreased expression of TPH2. These data suggest that Mecp2 is involved in the regulation of serotonin synthesis by promoting the transcription of TPH2.

The duplication of human chromosome 15q11-13 is a chromosome rearrangement associated with ASD (Cook et al. 1997). Tamada et al. (2010) studied serotonin signaling in a mouse model in which chromosome 7C (orthologous to human

chromosome 15q11-13) was duplicated. In this study, compared to wild type mice, there were decreased serotonin levels measured at 1, 2 and 3 weeks of age in cerebellum, cerebral cortex, hippocampus, hypothalamus, midbrain and pons. In adult animals, serotonin levels remained decreased in midbrain.

Fragile X syndrome is caused by a CGG expansion greater than 200 repeats in the 5' untranslated region in the fragile X mental retardation 1 gene, encoding the fragile X mental retardation 1 protein (FMRP), and is a leading single gene cause of autism (Hagerman et al. 2010). Zhang et al. (2005) used a Drosophila model and a proteomic approach to find targets of dFMRP in the brain. They found two upregulated enzymes, phenylalanine hydroxylase (Henna) and GTP cyclohydrolase (Punch), which mediate the dopamine and serotonin synthetic pathways. They confirmed a nearly 2-fold elevation of Punch activity in dfmr1 null mutants and significantly increased dopamine and serotonin in dfmr1 null mutants.

Thus, serotonin mechanisms may be impacted by disparate genetic risks for ASD. These examples illustrate that in the different disorders serotonin levels can be decreased or increased. Furthermore, changes in serotonergic function may differ depending on the developmental stage. These results have implications for pharmacological treatments aimed at serotonergic function.

13.8 Environmental Exposures and ASD

There is evidence for increased risk of autism with several environmental exposures. Three types of exposure will be discussed here as they relate to serotonin: prenatal antidepressants acting at the serotonin transporter (SSRIs), prenatal sodium valproate and maternal infection/inflammation. A recent epidemiology study reported a modest increased risk for ASD in offspring of mothers taking antidepressants, which inhibit serotonin uptake by the serotonin transporter (Croen et al. 2011). However, Hviid et al. (2013) did not replicate a significant association between maternal use of SSRIs during pregnancy and autism spectrum disorder in the offspring, although their study could not rule out a relative risk of up to 1.61. Animal studies of SSRI treatment during pregnancy have demonstrated changes in brain development and behavior. For example, Smit-Rigter et al. (2012) reported changes in cortical cytoarchitecture and anxiety in mice exposed to the SSRI fluoxetine in utero. Further study is required to determine the risk for the development of ASD with antidepressant treatment in pregnant women.

Epidemiological studies have shown increased risk of developing ASD due to in utero exposure to the anticonvulsant sodium valproate (Christensen et al. 2013; Bromley et al. 2013). Among other changes, the rodent valproate model of ASD shows changes in serotonergic function (Wang et al. 2013). In this model, there was increase tryptophan hydroxylase immunoreactivity in the dorsal and medial raphe nuclei and an increase number of TPH immunoreactive neurons in the medial raphe but not in the dorsal raphe of the valproate-exposed group. Using the SERT ligand [^{123}I]ADAM and small animal SPECT, valproate exposed animals

showed an increase in the uptake of [^{123}I]ADAM in the amygala. Furthermore, long term potentiation (LTP) was shown to be enhanced in the lateral amygdala at thalamic-amygdala synapses (Lin et al. 2013). Treatment of amygdala slices with the 5HT1A agonist 5-OH-DPAT reversed the excitatory/inhibitory imbalance in tissue from VPA-exposed animals, as measured by the miniature excitatory postsynaptic currents and paired pulse facilitation. Furthermore, chronic treatment with 5-OH-DPAT improved social deficits and fear extinction memory in the valproate exposed animals.

Case reports and epidemiological studies have linked autism risk to prenatal viral infections, such as cytomegalovirus, herpes simplex virus, rubella, measles, and mumps (Chess 1971; Deykin and MacMahon 1979; Ghaziuddin et al. 1992; Mason-Brothers et al. 1990; Yamashita et al. 2003). In a more recent epidemiologic study, Zerbo et al. (2013) showed no overall association between diagnoses of any maternal infection during pregnancy and ASD (Zerbo et al. 2013). However, this study did find an increased risk for ASD when maternal infection was diagnosed during a hospital admission. The risk was higher for bacterial infections. Maternal intrauterine inflammation resulting in immune dysfunction during development has been implicated in the development of neurodevelopmental disorders such as autism, schizophrenia, and cerebral palsy (Fatemi et al. 2008). A common link among these disorders appears to be the presence of activated microglia and evidence for immune dysregulation in the developing brain (Patterson 2009). Brain tissues of autistic patients were found to have increased number of activated microglia and astrocytes along with an increase in the levels of proinflammatory cytokines (Vargas et al. 2005). One link between maternal infection and intrauterine inflammation and fetal brain development may be the transport and metabolism of serotonin and its precursor tryptophan in the placenta. As kynurenine pathway enzyme IDO is induced by the cytokine interferon-γ, it is upregulated in the placenta with maternal infection (Mackler et al. 2003). Intrauterine infections in women are associated with upregulation of kynurenine pathway enzyme mRNA expression in the placenta (Manuelpillai et al. 2005). Increased metabolism of tryptophan by the kynurenine pathway at the placenta may result in abnormalities in fetal brain development because of decreased tryptophan available for serotonin synthesis and the neurotoxic effects of kynurenine metabolites (Stone 2001). ASD animal models of maternal inflammation have shown alterations in brain serotonin in the postnatal brains. Viral infections in the prenatal period have been associated with alterations in serotonin in the cerebellum of P14 mice (Winter et al. 2008). Maternal intrauterine endotoxin administration in a rabbit model results in microglial activation in the brain of the newborn rabbit (Kannan et al. 2007) and decreased serotonin synthesis in the cortex of the neonatal rabbit (Kannan et al. 2011). Kannan et al. (2011) demonstrated that maternal intrauterine endotoxin administration decreased multiple serotonergic markers in the offspring. Tryptophan metabolism to serotonin as measured by AMT PET in vivo, and serotonin levels in the frontal and parietal cortices measured in vitro, were significantly decreased following intrauterine endotoxin exposure. In addition to the decreased serotonin content, there was a loss of serotonin-immunoreactive terminals and decreased expression of 5HTT measured in the parietal sensory

cortex of endotoxin-exposed. In contrast, serotonergic raphe nuclei cell bodies and TPH2-positive cortical fibers were intact in the endotoxin-treated kits. The authors concluded that the loss of serotonin-immunoreactive fibers in the cortex was due to loss of thalamic neurons and thalamocortical afferents that transiently express 5HTT to uptake and store serotonin during development. Diminished serotonin signaling during development due to intrauterine inflammatory mechanisms may result in defects in thalamocortical circuit formation. These results are significant because they demonstrate that maternal intrauterine inflammation can disrupt the serotonergic developmental regulation of thalamocortical innervation in somatosensory cortex of newborn rabbit kits.

13.9 Conclusions

Although a role for serotonin in ASD has been known for over 50 years, mechanisms by which serotonin may be altered in ASD are only now being elucidated. This new understanding of the role of serotonin in autism has been aided by technological advances, including the discovery of autism risk genes and the ability to model these genetic variants in animal models. Molecular and functional imaging techniques now offer insight into altered serotonin function in vivo in human brain. Understanding of how prenatal factors such as maternal inflammation, maternal antidepressant use, and maternal genetic factors such as MAOA variants may lead to new strategies for the prevention of ASD. Understanding how different genetic factors affect serotonin may lead to individualized pharmacotherapy targeting different aspects of serotonergic function. Finally, understanding how serotonergic function during development differs in ASD may guide treatments aimed at particular points during development.

References

Anderson GM, Freedman DX, Cohen DJ, Volkmar FR, Hoder EL, McPhedran P, Minderaa RB, Hansen CR, Young JG (1987) Whole blood serotonin in autistic and normal subjects. J Child Psychol Psychiatry 28:885–900

Anderson GM, Hertzig ME, McBride PA (2012) Brief report: platelet-poor plasma serotonin in autism. J Autism Dev Disord 42:1510–1514

Azmitia EC, Singh JS, Whitaker-Azmitia PM (2011a) Increased serotonin axons (immunoreactive to 5-HT transporter) in postmortem brains from young autism donors. Neuropharmacology 60:1347–1354

Azmitia EC, Singh JS, Hou XP, Wegiel J (2011b) Dystrophic serotonin axons in postmortem brains from young autism patients. Anat Rec, 294:1653–1662

Balkovetz DF, Tiruppathi C, Leibach FH, Mahesh VB, Ganapathy V (1989) Evidence for an imipramine-sensitive serotonin transporter in human placental brush-border membranes. J Biol Chem 264:2195–2198

Berger M, Gray JA, Roth BL (2009) The expanded biology of serotonin. Annu Rev Med 60:355–366.
Berman NEJ, Puri V, Chandrala S, Puri S, Macgregor R, Liverman CS, Klein RM (2006) Serotonin in trigeminal ganglia of female rodents: relevance to menstrual migraine. Headache 46:1230–1245
Beversdorf DQ, Nordgren RE, Bonab AA, Fischman AJ, Weise SB, Dougherty DD, Felopulos GJ, Zhou FC, Bauman ML (2012) 5-HT2 receptor distribution shown by [18F]setoperone PET in high-functioning autistic adults. J Neuropsychiatry Clin Neurosci 24:191–197
Bonnin A, Goeden N, Chen K, Wilson ML, King J, Shih JC, Blakely RD, Deneris ES, Levitt P (2011) A transient placental source of serotonin for the fetal forebrain. Nature 472:347–350
Bortolato M, Chen K, Shih JC (2008) Monoamine oxidase inactivation: from pathophysiology to therapeutics. Adv Drug Deliv Rev 60:1527–1533
Brodkin ES (2007) BALB/c mice: low sociability and other phenotypes that may be relevant to autism. Behav Brain Res 176:53–65
Bromley RL, Mawer GE, Briggs M, Cheyne F, Clayton-Smith J, García-Fiñana M, Kneen R, Lucas SB, Shallcross R, Baker GA; Liverpool and Manchester Neurodevelopment Group (2013) The prevalence of neurodevelopmental disorders in children prenatally exposed to antiepileptic drugs. J Neurol Neurosurg Psychiatry 84:637–643
Cassel S, Carouge D, Gensburger C, Anglard P, Burgun C, Dietrichm JB, Aunis D, Zwiller J (2006) Fluoxetine and cocaine induce the epigenetic factors MeCP2 and MBD1 in adult rat brain. Mol Pharmacol 70:487–492
Chandana SR, Behen ME, Juhasz C, Muzik O, Rothermel RD, Mangner TJ, Chakraborty PK, Chugani HT, Chugani DC (2005) Significance of abnormalities in developmental trajectory and asymmetry of cortical serotonin synthesis in autism. Int J Dev Neurosci 23:171–182
Chantiluke K, Barrett N, Giampietro V, Brammer M, Simmons A, Murphy DG, Rubia K (2014) Inverse effect of fluoxetine on medial prefrontal cortex activation during reward reversal in ADHD and autism. Cereb Cortex [Epub ahead of print]
Chess S (1971) Autism in children with congenital rubella. Journal of Autism and Childhood Schizophrenia 1:33–47
Christenson JG, Dairman W, Udenfriend S (1972) On the identity of DOPA decarboxylase and 5-hydroxytryptophan decarboxylase (immunological titration-aromatic L-amino acid decarboxylase-serotonin-dopamine-norepinephrine). Proc Nat Acad Sci U S A 69:343–347
Christensen J, Grønborg TK, Sørensen MJ, Schendel D, Parner ET, Pedersen LH, Vestergaard M (2013) Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. JAMA 309:1696–1703
Chugani DC, Muzik O, Rothermel R, Behen M, Chakraborty P, Mangner T, da Silva EA, Chugani HT (1977) Altered serotonin synthesis in the dentatothalamo-cortical pathway in autistic boys. Ann Neurol 14:666–669
Chugani DC, Muzik O, Behen M, Rothermel R, Janisse JJ, Lee J, Chugani HT (1999) Developmental changes in brain serotonin synthesis capacity in autistic and nonautistic children. Ann Neurol 45:287–295
Cohen IL, Liu X, Schutz C, White BN, Jenkins EC, Brown WT, Holden JJ (2003) Association of autism severity with a monoamine oxidase a functional polymorphism. Clin Genet 64:190–197
Cook EH Jr Charak DA Arida J Spohn JA Roizen NJ Leventhal BL (1994) Depressive and obsessive–compulsive symptoms in hyperserotonemic parents of children with autistic disorder. Psychiatry Res 52:25–33
Cook EH Jr, Lindgren V, Leventhal BL, Courchesne R, Lincoln A, Shulman C, Lord C, Courchesne E (1997) Autism or atypical autism in maternally but not paternally derived proximal 15q duplication. Am J Hum Genet 60:928–934
Cook EH Jr Leventhal BL Heller W Metz J Wainwright M Freedman DX (1990) Autistic children and their first-degree relatives: relationships between serotonin and norepinephrine levels and intelligence. J Neuropsychiatry Clin Neurosci 2:268–274

Coon H, Dunn D, Lainhart J, Miller J, Hamil C, Battaglia A, Tancredi R, Leppert MF, Weiss R, McMahon W (2005) Possible association between autism and variants in the brain-expressed tryptophan hydroxylase gene (TPH2). Am J Med Genet B Neuropsychiatr Genet 135B:42–46

Correa RR, Barrilari SE, Guimaraes CS, Rossi e Silva RC, Olegario JG, Cavellani CL, Oliveira FA, Salge AK, Teixeira VP, Castro EC (2009) Expression of the melatonin receptor and tryptophan hydroxylase in placentas of the fetus with intra-uterine stress. Eur J Obstet Gynecol Reprod Biol 147:234–236

Cote F, Fligny C, Bayard E, Launay JM, Gershon MD, Mallet J, Vodjdani G (2007) Maternal serotonin is crucial for murine embryonic development. Proc Natl Acad Sci U S A 104:329–334

Coutinho AM, Sousa I, Martins M, Correia C, Morgadinho T, Bento C, Marques C, Ataíde A, Miguel TS, Moore, JH, Oliveira G, Vicente AM (2007) Evidence for epistasis between SLC6A4 and ITGB3 in autism etiology and in the determination of platelet serotonin levels. Hum Genet 121:243–256

Croen LA, Grether JK, Yoshida CK, Odouli R, Hendrick V (2011) Antidepressant use during pregnancy and childhood autism spectrum disorders. Arch Gen Psychiatry 68:1104–1112

Daly EM, Deeley Q, Ecker C, Craig M, Hallahan B, Murphy C, Johnston P, Spain D, Gillan N, Brammer M, Giampietro V, Lamar M, Page L, Toal F, Cleare A, Surguladze S, Murphy DG (2012) Serotonin and the neural processing of facial emotions in adults with autism: an fMRI study using acute tryptophan depletion. Arch Gen Psychiatry 69:1003–1013

Davis LK, Hazlett HC, Librant AL, Nopoulos P, Sheffield VC, Piven J, Wassink TH (2008) Cortical enlargement in autism is associated with a functional VNTR in the monoamine oxidase a gene. Am J Med Genet B Neuropsychiatr Genet 147B:1145–1151

Devlin B, Cook EH Jr, Coon H, Dawson G, Grigorenko EL, McMahon W, Minshew N, Pauls D, Smith, M, Spence MA, Rodier PM, Stodgell C, Schellenberg GD (2005) CPEA Genetics Network Autism and the serotonin transporter, the long and short of it. Mol Psychiatry 10:1110–1116

Deykin EY, MacMahon B (1979) Viral exposure and autism. Am J Epidemiol 109:628–638

Diksic M, Nagahiro S, Sourkes TL, Yamamoto YL (1990) A new method to measure brain serotonin synthesis in vivo. I. Theory and basic data for a biological model. J Cereb Blood Flow Metab 9:1–12

Eddahibi S, Guignabert C, Barlier-Mur AM, Dewachter L, Fadel E, Dartevelle P, Humbaert M, Simonneau G, Hanoun N, Saurini F, Hamon M, Adnot S (2006) Cross talk between endothelial and smooth muscle cells in pulmonary hypertension. Circulation 113:1857–1864

Fatemi SH, Reutiman TJ, Folsom TD, Huang H, Oishi K, Mori S, Smee DF, Pearce DA, Winter C, Sohr R, Juckel G (2008) Maternal infection leads to abnormal gene regulation and brain atrophy in mouse offspring: implications for genesis of neurodevelopmental disorders. Schizophr Res 99:56–70

Fernstrom JD, Wurtman RJ (1971) Brain serotonin content: physiological dependence on plasma tryptophan levels. Science 173:149–151

Gershon MD, Tack J (2007) The serotonin signaling system: from basic understanding to drug development for functional GI disorders. Gastroenterology 132:397–414

Ghaziuddin M, Tsai LY, Eilers L, Ghaziuddin N (1992) Brief report: Autism and herpes simplex encephalitis. J Autism Dev Disord 22:107–113

Girgis RR, Slifstein M, Xu X, Frankle WG, Anagnostou E, Wasserman S, Pepa L, Kolevzon A, Abi-Dargham A, Laruelle M, Hollander E (2011) The 5-HT(2A) receptor and serotonin transporter in Asperger's disorder: A PET study with [^{11}C]MDL 100907 and [^{11}C]DASB. Psychiatry Res 194:230–234

Goldberg J, Anderson GM, Zwaigenbaum L, Hall GB, Nahmias C, Thompson A, Szatmari P (2009) Cortical serotonin type-2 receptor density in parents of children with autism spectrum disorders. J Autism Dev Disord 2009:97–104

Hagerman R, Hoem G, Hagerman P (2010) Fragile X and autism: Intertwined at the molecular level leading to targeted treatments. Mol Autism 1:12

Hamon M, Bourgoin S, Artaud FEl, Mestikawy S (1981) The respective roles of tryptophan uptake and tryptophan hydroxylase in the regulation of serotonin synthesis in the central nervous system. J Physiol 77:269–279

Hoshino Y, Yamamoto T, Kaneko M, Tachibana R, Watanabe M, Ono Y, Kumashiro H (1984) Blood serotonin and free tryptophan concentration in autistic children. Neuropsychobiol 11:22–27

Hviid A, Melbye M, Pasternak B (2013) Use of selective serotonin reuptake inhibitors during pregnancy and risk of autism. N Engl J Med 369:2406–2415

Ide S, Itoh M, Goto Y (2005) Defect in normal developmental increase of the brain biogenic amine concentration in the mecp2-null mouse. Neurosci Lett 386:14–17

Ikeda K, Tojo K, Otsubo C, Udagawa T, Kumazawa K, Ishikawa M, Tokudome G, Hosoya T, Tajima N, Claycomb WC, Nakao K, Kawamura M (2005) 5-hydroxytryptamine synthesis in HL-1 cells and neonatal rat cardiocytes. Biochem Biophys Res Commun 328:522–525

Jones MB, Palmour RM, Zwaigenbaum L, Szatmari P (2004) Modifier effects in autism at the MAO-A and DBH loci. Am J Med Genet B Neuropsychiatr Genet 126:58–65

Kane MJ, Angoa-Peréz M, Briggs DI, Sykes CE, Francescutti DM, Rosenberg DR, Kuhn DM (2012) Mice genetically depleted of brain serotonin display social impairments, communication deficits and repetitive behaviors: possible relevance to autism. PLoS One 7:e48975

Kannan S, Saadani-Makki F, Muzik O, Chakraborty P, Mangner TJ, Janisse J, Romero R, Chugani DC (2007) Microglial activation in perinatal rabbit brain induced by intrauterine inflammation: detection with 11C-(R)-PK11195 and small-animal PET. J Nucl Med 48:946–954

Kannan S, Saadani-Makki F, Balakrishnan B, Dai H, Chakraborty PK, Janisse J, Muzik O, Romero R, Chugani DC (2011) Decreased cortical serotonin in neonatal rabbits exposed to endotoxin in utero. J Cereb Blood Flow Metab 31:738–749

Kerr TM, Muller CL, Miah M, Jetter CS, Pfeiffer R, Shah C, Baganz N, Anderson GM, Crawley JN, Sutcliffe JS, Blakely RD, Veenstra-Vanderweele J (2013) Genetic background modulates phenotypes of serotonin transporter Ala56 knock-in mice. Mol Autism 4:35

Kim DK, Tolliver TJ, Huang S J, Martin BJ, Andrews AM, Wichems C, Holmes A, Lesch KP, Murphy DL (2005) Altered serotonin synthesis, turnover and dynamic regulation in multiple brain regions of mice lacking the serotonin transporter. Neuropharmacol 49:798–810

Kudo Y, Boyd CA (2002) Human placental amino acid transporter genes: expression and function. Reproduction 124:593–600

Leboyer M, Philippe A, Bouvard M, Guilloud-Bataille M, Bondoux D, Tabuteau F, Feingold J, Mouren-Simeoni MC, Launay JM (1999) Whole blood serotonin and plasma beta-endorphin in autistic probands and their first-degree relatives. Biol Psychiatry 45:158–163

Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J Müller, CR Hamer DH, Murphy DL (1996) Association of anxiety-related with a polymorphism in the serotonin transporter gene regulatory region. Science 274:1527–1531

Leventhal BL, Cook Jr, EH, Morford M, Ravitz A, Freedman DX (1990) Relationships of whole blood serotonin and plasma norepinephrine within families. J Autism Dev Disord, 20:499–511

Ligam P, Manuelpillai U, Wallace EM, Walker D (2005) Localisation of indoleamine 2,3-dioxygenase and kynurenine hydroxylase in the human placenta and decidua: implications for role of the kynurenine pathway in pregnancy. Placenta 26:498–504

Lin HC, Gean PW, Wang CC, Chan YH, Chen PS (2013) The amygdala excitatory/inhibitory balance in a valproate-induced rat autism model. PLoS One 8:e55248

Mackler AM, Barber EM, Takikawa O, Pollard JW (2003) Indoleamine 2,3-dioxygenase is regulated by IFN-gamma in the mouse placenta during Listeria monocytogenes infection. J Immunol 170:823–830

Makkonen I, Riikonen R, Kokki H, Airaksinen MM, Kuikka JT (2008) Serotonin and dopamine transporter binding in children with autism determined by SPECT. Dev Med Child Neurol 50:593–597

Manuelpillai U, Ligam P, Smythe G, Wallace EM, Hirst J, Walker DW (2005) Identification of kynurenine pathway enzyme mRNAs and metabolites in human placenta: up-regulation by inflammatory stimuli and with clinical infection. Am J Obstet Gynecol 192:280–288

Mason-Brothers A, Ritvo ER, Pingree C, Petersen PB, Jenson WR, McMahon WM, Freeman BJ, Jorde LB, Spencer MJ, Mo A (1990) The UCLA-University of Utah epidemiologic survey of autism: prenatal, perinatal, and postnatal factors. Pediatrics 86:514–519

Matsuda M, Imaoka T, Vomachka AJ, Gudelsky GA, Hou Z, Mistry M, Bailey JP, Nieport KM, Walther DJ, Bader M, & Horseman ND (2004) Serotonin regulates mammary gland development via an autocrine–paracrine loop. Development Cell 6:193–203

Moja EA, Stoff DM, Gessa GL, Castoldi D, Assereto R, Tofanetti O (1988) Decrease in plasma tryptophan after tryptophan-free amino acid mixtures in man. Life Sci 42:1551–1556

Moja EA, Cipolla P, Castoldi D, Tofanetti O (1989) Dose-response decrease in plasma tryptophan and in brain tryptophan and serotonin after tryptophan-free amino acid mixtures in rats. Life Sci 44:971–976

Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, Brown C, Mellor AL (1998) Prevention of allogeneic fetal rejection by tryptophan catabolism. Science 281:1191–1193

Murphy DG, Daly E, Schmitz N, Toal F, Murphy K, Curran S, Erlandsson K, Eersels J, Kerwin R, Ell P, Travis M (2006) Cortical serotonin 5-HT2A receptor binding and social communication in adults with Asperger's syndrome: an in vivo SPECT study. Am J Psychiatry 163:934–936.

Nabi R, Serajee FJ, Chugani DC, Zhong H, Huq AH (2004) Association of tryptophan 2,3 dioxygenase gene polymorphism with autism. Am J Med Genet B Neuropsychiatr Genet 125:63–68

Nakamura K, Sekine Y, Ouchi Y, Tsujii M, Yoshikawa E, Futatsubashi M, Tsuchiya KJ, Sugihara G, Iwata Y, Suzuki K, Matsuzaki H, Suda S, Sugiyama T, Takei N, Mori N (2010) Brain serotonin and dopamine transporter bindings in adults with high-functioning autism. Arch Gen Psychiatry 67:59–68

Ni W, Watts SW (2006) 5-hydroxytryptamine in the cardiovascular systems: focus on the serotonin transporter (SERT). Clin Exp Pharmacol Physiol 33:575–583

Ni W, Geddes TJ, Priestley JR, Szasz T, Kuhn DM, Watts SW (2008) The existence of a local 5-hydroxytryptaminergic system in peripheral arteries. Br J Pharmacol 154:663–674

Nocito A, Dahm F, Jochum W, Jang JH, Georgiev P, Bader M, Renner EL, Clavien PA (2007) Serotonin mediates oxidative stress and mitochondrial toxicity in a murine model of nonalcoholic steatohepatitis. Gastroenterology 133:608–618

Oblak A, Gibbs TT, Blatt GJ (2013) Reduced serotonin receptor subtypes in a limbic and a neocortical region in autism. Autism Res 6:571–583

Oritz-Alvarado R, Guzmán-Quevedo O, Meracado-Camargo R, Haertle T, Vignes C, Bolaños-Jiménez F (2006) Expression of tryptophan hydroxylase in developing mouse taste papillae. FEBS Letters 580:5371–5376

Pardridge, WM (1977) Kinetics of competitive inhibition of neutral amino acid transport across the blood-brain barrier. J Neurochem 28:103–108

Patterson PH (2009) Immune involvement in schizophrenia and autism: etiology, pathology and animal models. Behav Brain Res 204:313–321

Piven J, Tsai GC, Nehme E, Coyle JT, Chase GA, Folstein SE (1991) Platelet serotonin, a possible marker for familial autism. J Autism Dev Disord 21:51–59

Sakowski SA, Geddes TJ, Thomas DM, Levi E, Hatfield JS, Kuhn DM (2006) Differential tissue distribution of tryptophan hydroxylase isoforms 1 and 2 as revealed with monospecific antibodies. Brain Res 1085:11–18

Samaco RC, Mandel-Brehm C, Chao HT, Ward CS, Fyffe-Maricich SL, Ren J, Hyland K, Thaller C, Maricich SM, Humphreys P, Greer JJ, Percy A, Glaze DG, Zoghbi HY, Neul JL (2009) Loss of MeCP2 in aminergic neurons causes cell-autonomous defects in neurotransmitter synthesis and specific behavioral abnormalities. Proc Natl Acad Sci U S A 106:21966–21971

Schain RJ, Freedman DX (1961) Studies on 5-hydroxyindole metabolism in autism and other mentally retarded children. J Pediatr 59:315–320

Siesser WB, Zhang X, Jacobsen JP, Sotnikova TD, Gainetdinov RR, Caron MG (2010) Tryptophan hydroxylase 2 genotype determines brain serotonin synthesis but not tissue content in C57Bl/6 and BALB/c congenic mice. Neurosci Lett 481:6–11

Slominski A, Pisarchik A, Semak I, Sweatman T, Worstman J, Szczesniewski A, Slugocki G, McNulty J, Kauser S, Tobin DJ (2002) Serotoninergic and melatoninergic systems are fully expressed in human skin. FASEB J 16:896–898

Smith QR, Monna S, Aoyagi M, & Rapoport SI (1987) Kinetics of neutral amino acid transport across the blood-brain barrier. J Neurochem 49:1651–1658

Smit-Rigter LA, Noorlander CW, von Oerthel L, Chameau P, Smidt MP, van Hooft JA (2012) Prenatal fluoxetine exposure induces life-long serotonin 5-HT3 receptor-dependent cortical abnormalities and anxiety-like behaviour. Neuropharmacology 62:865–870

Stone TW (2001) Endogenous neurotoxins from tryptophan. Toxicon 39:61–73

Stull MA, Pai V, Vomachka AJ, Marshall AM, Joacb GA, Horseman ND (2007) Mammary gland homeostasis employs serotonergic regulation of epithelial tight junctions. Proc Natl Acad Sci 104:16708–16713

Tamada K, Tomonaga S, Hatanaka F, Nakai N, Takao K, Miyakawa T, Nakatani J, Takumi T (2010) Decreased exploratory activity in a mouse model of 15q duplication syndrome; implications for disturbance of serotonin signaling. PLoS One 5e:15126

Tassone F, Qi L, Zhang W, Hansen RL, Pessah IN, Hertz-Picciotto I (2011) MAOA, DBH and SLC6A4 variants in CHARGE: A case control study of autism spectrum disorders. Autism Res 4:250–261

Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA (2005) Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann Neurol 57:67–81

Veenstra-VanderWeele J, Muller CL, Iwamoto H, Sauer JE, Owens WA, Shah CR, Cohen J, Mannangatti P, Jessen T, Thompson BJ, Ye R, Kerr TM, Carneiro AM, Crawley JN, Sanders-Bush E, McMahon DG, Ramamoorthy S, Daws LC, Sutcliffe JS, Blakely RD (2012) Autism gene variant causes hyperserotonemia, serotonin receptor hypersensitivity, social impairment and repetitive behavior. Proc Natl Acad Sci U S A 109:5469–5474

Verma D, Chakraborti B, Karmakar A, Bandyopadhyay T, Singh AS, Sinha S, Chatterjee A, Ghosh S, Mohanakumar KP, Mukhopadhyay K, Rajamma U (2013) Sexual dimorphic effect in the genetic association of monoamine oxidase A (MAOA) markers with autism spectrum disorder. Prog Neuropsychopharmacol Biol Psychiatry 50C:11–20

Walther DJ, Peter JU, Winter S, Höltje M, Paulmann N, Grohmann M, Vowinckel J, Alamo-Bethencourt V, Wilhelm CS, Ahnert-Hilger G, Bader M (2003) Serotonylation of small GTPases is a signal transduction pathway that triggers platelet alpha-granule release. Cell 115:851–862

Walther DJ, Stahlberg S, Vowinckel J (2011) Novel roles for biogenic monoamines: from monoamines in transglutaminase-mediated post-translational protein modification to monoaminylation deregulation diseases. FEBS J 278:4740–4755

Wang CC, Lin HC, Chan YH, Gean PW, Yang YK, Chen PS (2013) 5-HT1A-receptor agonist modified amygdala activity and amygdala-associated social behavior in a valproate-induced rat autism model. Int J Neuropsychopharmacol 16:2027–2039

Wassink TH, Hazlett HC, Epping EA, Arndt S, Dager SR, Schellenberg GD, Dawson, G, Piven J (2007) Cerebral cortical gray matter overgrowth and functional variation of the serotonin transporter gene in autism. Arch Gen Psychiatry 64:709–717

Weiss LA, Ober C, Cook EH Jr (2006a) ITGB3 shows genetic and expression interaction with SLC6A4. Hum Genet 120:93–100

Weiss LA, Kosova G, Delahanty RJ, Jiang L, Cook EH, Ober C, Sutcliffe JS (2006b) Variation in ITGB3 is associated with whole-blood serotonin level and autism susceptibility. Eur J Hum Genet 14:923–931

Whyte A, Jessen T, Varney S, Carneiro AM (2014) Serotonin transporter and integrin beta 3 genes interact to modulate serotonin uptake in mouse brain. Neurochem Int 73:122–126

Winter C, Reutiman TJ, Folsom TD, Sohr R, Wolf RJ, Juckel G, Fatemi SH (2008) Dopamine and serotonin levels following prenatal viral infection in mouse—implications for psychiatric disorders such as schizophrenia and autism. Eur Neuropsychopharmacol 18:712–716

Yamashita, Y, Fujimoto, C, Nakajima E, Isagai, T, Matsuishi, T (2003) Possible association between congenital cytomegalovirus infection and autistic disorder. J Autism Dev Disord 33:455–459

Zerbo O, Qian Y, Yoshida C, Grether JK, Van de Water J, Croen LA (2013) Maternal infection during pregnancy and autism spectrum disorders. J Autism Dev Disord [Epub ahead of print]

Zhang X, Beaulieu, JM, Sotnikova TD, Gainetdinov RR, Caron MG (2004) Tryptophan hydroxylase-2 controls brain serotonin synthesis. Science 305:217

Zhang YQ, Friedman DB, Wang Z, Woodruff E 3rd, Pan L, O'Donnell J, Broadie K (2005) Protein expression profiling of the drosophila fragile X mutant brain reveals up-regulation of monoamine synthesis. Mol Cell Proteomics 4:278–290

Zhao X, Leotta A, Kustanovich V, Lajonchere C, Geschwind DH, Law K, Law P, Qiu S, Lord C, Sebat, J, Ye K, Wigler M (2007) A unified genetic theory for sporadic and inherited autism. Proc Nat Acad Sci U S A 104:12831–12836

Zoghbi HY, Milstien S, Butler IJ, Smith EO, Kaufman S, Glaze DG, Percy AK (1989) Cerebrospinal fluid biogenic amines and biopterin in Rett syndrome. Ann Neurol 25:56–60

Chapter 14

Oxytocin and Vasopressin in Autism and Genetic Syndromes

Tal Levin-Decanini, Sunday M. Francis, Angela Sagar, Wensheng Liu, C. Sue Carter and Suma Jacob

Abstract Oxytocin (OT) and arginine vasopressin (AVP) are two small, related neuropeptides found in many mammalian species, including humans. These neuropeptides are associated with a range of social behaviors and their dysregulation has been associated with deficits in social behavior. In particular, the OT neuropeptide system has been investigated in Autism Spectrum Disorder (ASD), as well as in Prader-Willi Syndrome (PWS), Williams Syndrome (WS) and Fragile X Syndrome (FXS), all of which are characterized by marked social deficits. PWS, WS and FXS are caused by identified genetic mutations and provide insight into the developmental influences of the OT system. In particular, FXS is caused by a mutation in a single gene and up to 47 % of patients with FXS are diagnosed with ASD or also have autism related behaviors. Animal models of genetic neurodevelopmental disorders (NDD) are becoming a valuable tool to examine the role and relatedness of OT and AVP in the developing brain. We provide an example of how OT and AVP systems are altered with a mutation in the mouse Fragile X mental retardation 1 (*Fmr1*) gene which leads to FXS-like symptoms in *Fmr1* knockout (KO) mice. By studying the OT and AVP systems in these rare disorders, we may further understand their mechanisms of action in ASD and in typical development. This chapter will summarize the current data pertaining to these NDD and the systems of OT and AVP.

Keywords Oxytocin · Vasopressin · Autism · Prader-Willi · Williams · Fragile X

S. Jacob (✉) · T. Levin-Decanini · S. M. Francis
Department of Psychiatry, University of Minnesota, School of Medicine, Wallin Medical Biosciences Building, 2101 6th Street SE, 55455 Minneapolis, MN, USA
e-mail: sjacob@umn.edu

A. Sagar
Psychiatry, UCLA Health, Los Angeles, CA, USA

W. Liu
Center for Advanced Microscopy, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

C. S. Carter
Department of Psychiatry, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

S. Hossein Fatemi (ed.), *The Molecular Basis of Autism,*
Contemporary Clinical Neuroscience, DOI 10.1007/978-1-4939-2190-4_14

14.1 Introduction to OT & AVP Neuropeptide Hormones

Oxytocin (OT) and arginine vasopressin (AVP) are small mammalian neuropeptides nine amino acids in length, differing from each other by only two amino acids. OT is produced primarily in hypothalamic nuclei, including the supraoptic (SON) and paraventricular nuclei (PVN). AVP is also synthesized in the PVN and SON. However in males, additional brain regions including the amygdala and the bed nucleus of the stria terminalis (BNST) also produce AVP. OT and AVP of hypothalamic origins are transported from the SON and PVN to the mammalian posterior pituitary by neurosecretion where they are released into the blood stream, acting as hormones on target tissues. In addition, both OT and AVP are capable of moving throughout the central nervous system (CNS) via diffusion in the cerebral spinal fluid (CSF; Neumann and Landgraf 2012). The peptide producing oxytocin gene (OXT) is homologous with its evolutionarily related, vasopressin (AVP) gene. The human OXT and AVP genes are linked on chromosome 20p13 and are positioned in opposite transcriptional orientations, while separated by only 12 kb of DNA. Both have specific receptors, but their close evolutionary relationship permits cross-talk and interacting molecular systems. These neuropeptide hormones have receptors in various brain regions and throughout the body, including areas that are important for regulating social behavior and reactivity to stressors.

In both, the human and mouse genomes OT and AVP peptide genes are located adjacently on the same chromosome. Often, the blood levels of both peptides are highly correlated (Dai et al. 2012) suggesting a coordinated release. The receptors for both peptides are localized in specific areas of the nervous system, particularly in the brainstem and areas that play a role in social, adaptive behaviors, or in the regulation of the hypothalamic-pituitary-adrenal axis (HPA) and autonomic nervous system (ANS; Lim et al. 2004). Because OT and AVP are closely related and have the ability to act on the other's receptors, it has been proposed that they evolved to interact and sometimes have opposing physiological effects. For example, both hormones have been shown to affect the control of the autonomic nervous system (ANS), with OT having primarily parasympathetic actions, and AVP serving as both a central and peripheral regulatory component of the sympathetic nervous system and HPA axis (Sawchenko and Swanson 1985; Kenkel et al. 2012). However, high levels of neuropeptides can be partial agonists for their homologous receptors and may result in AVP and OT pathway interactions (Chini et al. 1996).

Of particular importance in neurodevelopmental disorders (NDD) is the fact that OT and AVP can modulate social and repetitive behavior, as well as other manifestations of anxiety and state regulation (Carter 2007). Animal research has generally associated OT release or exposure with positive sociality, reduced anxiety and lower levels of reactivity to stressors (Carter 1998; Neumann and Landgraf 2012). AVP can influence anxiety and the regulation of the HPA axis and stress responses. In general, central AVP is described as anxiogenic (Landgraf and Wigger 2003). However, there also is evidence in rats that the effects of AVP are brain region specific and dose-dependent. For example, AVP may be anxiolytic if given in low doses (Appenrodt et al. 1998).

Mouse knockout (KO) studies of the OT receptor (OXTR) or OT regulators have reported decreased social memory or recognition (Jin et al. 2007; Ferguson et al. 2000; Takayanagi et al. 2005). *Oxtr* KO mice also showed decreased cognitive flexibility and a resistance to change learned pattern of behavior, comparable to restricted/repetitive interests (Sala et al. 2011). Both social deficits and behavioral rigidity were ameliorated by OT administration (Sala et al. 2011). The finding that OT continues to have effects in *Oxtr* KO mice supports the hypothesis that OT can influence behavior through other receptors, particularly the AVP receptors (e.g. AVPR1A and/or AVPR1B). Given the influence of these neuropeptides on brain regions affecting both social and repetitive behaviors, modulation of OT and AVP pathways are being explored as treatment targets for Fragile X Syndrome (FXS) and Autism Spectrum Disorders (ASD).

These studies set the stage for a series of recent studies of the effects of exogenous OT treatments in humans (Ebstein et al. 2012; Macdonald and Feifel 2013). For example, intranasal OT (IN-OT) administration in healthy human males increased prosocial behaviors and trust, especially as measured by computerized economic games (Baumgartner et al. 2008; Kirsch et al. 2005; Kosfeld et al. 2005). IN-OT may also increase gaze toward the eye region of faces (Guastella et al. 2008), and has been associated with improved facial memory (Rimmele et al. 2009), enhanced salience of social cues (Shamay-Tsoory et al. 2009), and improved performance on the reading the mind in the eyes task (RMET; Domes et al. 2007).

As previously reviewed, OT has been found to have anxiolytic effects, improve social interactions, reduce fear, and improve the ability of healthy volunteers to interpret subtle social cues (Macdonald and Macdonald 2010). In addition, OT dysfunction has been associated with neuropsychiatric disorders such as ASD in human studies (Ishak et al. 2011; Domes et al. 2007; Winslow and Insel 2004). By 2010, there were over 20 OT administration studies, which included ASD, schizophrenia, postpartum depression, posttraumatic stress disorder (PTSD), and irritable bowel syndrome (Macdonald and Macdonald 2010). As IN-OT has been associated with alterations in social decision-making, processing of social stimuli, certain social behaviors such as eye contact, and social memory, there has been a growing number of studies investigating its abilities to treat a range of neurobehavioral disorders.

14.2 Autism Spectrum Disorder and OT/AVP

In 1943, Leo Kanner described a male patient as having “stereotyped movements [and] … repetitions carried out in exactly the same way in which they had been performed originally” and also noted his social communication such that “he always seemed to be parroting what he had heard said to him at one time or another ….Words to him had a specifically literal, inflexible meaning. He seemed unable to generalize, to transfer an expression to another similar object or situation” (Kanner 1943). This group of symptoms later extended and described in detail is currently known as ASD. As described in the DSM-5 (American Psychiatric Association

2013), it is characterized by persistent deficits in social communication and social interaction across multiple contexts, and the diagnosis requires the presence of restricted, repetitive patterns of behaviors, interests, or activities. ASD is a heritable (Bailey et al. 1995) and highly heterogeneous disorder, caused by familial genetic risks in addition to possible gene-environment interactions during early development (Chaste and Leboyer 2012). Individuals with ASD may also have comorbid anxiety disorders, irritability and aggression, and come to clinical attention due to difficulties at home and school related to their communication deficits and restricted interests.

It was suggested by a number of researchers, including Waterhouse et al. as early as 1996, that dysfunction in the OT and AVP systems might contribute to the atypical social behaviors in ASD (Waterhouse et al. 1996). One of the first examinations of the association of OT specifically and ASD showed that children with ASD have low levels of plasma OT (Modahl et al. 1998). In particular, a subgroup of this sample identified as aloof using Wing's diagnostic topology had the lowest levels, suggesting that those with the most severe socially aloof symptoms had more OT dysfunction. Building on these results with the same sample, it was also shown that there was an increase in OT-Gly, OT-Gly-Lys, and OT-Gly-Lys-Arg peptides, collectively known as OT-X precursors for OT, as well as an increase in the ratio of OT-X/OT, associated with the reduction in OT seen in the patients with ASD (Green et al. 2001). There was also a positive correlation between OT-X and checklist items associated with ASD, including stereotypies, while OT-X correlated negatively with an item describing atypical comfort-giving within the ASD group. Consequently, changes in OT processing, specifically a failure to completely process the prohormone OT-X, might lead to a deficiency in OT and thus exacerbate some of the symptoms of ASD. To our knowledge this study has not been replicated. However, other studies done in older patients have failed to report an OT deficiency, or have reported higher than expected OT levels in their ASD samples (Jansen et al. 2006; Miller et al. 2013). Future research will need to determine if differences in results of OT levels in individuals with ASD compared to controls reflect differences in the study populations and/or methods for assaying OT. It should be noted that these studies often prepared samples differently with varying plasma processing/extraction methods, and used different assay techniques. Additionally, ASD population differences included varying ages and recent data has suggested that some blood biomarkers like OT may change after puberty (Hammock et al. 2012). In addition, ASD is a very heterogeneous disorder and OT level differences may be specific to clinical and etiological subgroups within the broader ASD population.

14.2.1 Intranasal OT Studies in ASD

Currently medications for ASD concentrate on alleviating certain symptoms. Risperidone and aripiprazole may be used for irritability, whereas guanfacine and clonidine are used off label for aggression, and selective serotonin reuptake inhibitors (SSRI; i.e. escitalopram, fluoxetine, and sertraline) are used to treat anxiety or

depression (Owley et al. 2010; Jaselskis et al. 1992). Recently, OT has been investigated to target the treatment of ASD's core symptoms, social deficits and restrictive and repetitive behaviors (RRBs).

Several studies using intravenous or IN-OT in patients with ASD have been conducted. It has been shown that nonapeptides, like AVP and OT, can be measured in CSF after intranasal administration (Born et al. 2002). Ease of giving intranasal drugs makes it preferred for most ASD studies, although more research needs to be conducted on how IN-OT reaches the brain and influences behavior. In addition to measuring OT responses to single dose challenges in ASD (Andari et al. 2010; Guastella et al. 2010), few studies have examined longer term treatment effects (Anagnostou et al. 2012). In addition to varying administration and duration study protocols, studies have often focused on symptom subdomains or defined social tasks such as RRBs (Hollander et al. 2003), emotion recognition (Guastella et al. 2010; Dadds et al. 2014), affective speech comprehension (Hollander et al. 2007), and facial recognition (Domes et al. 2013).

As mentioned above, single dose studies or challenges have been utilized to study the acute and immediate effects of OT. An initial study in ASD examined the effects of a four-hour continuous dose of intravenous OT (Hollander et al. 2003). After 1 h of infusion there was a decrease in RRB (repeating and touching). This decrease lasted when measured after 4 h as well. More recently, a double-blind, randomized, placebo controlled study of IN-OT in 16 males with ASD (ages 12–19 years old) showed that a single IN-OT dose could improve the ability to recognize emotion, particularly in easy queries (Guastella et al. 2010). Single dose IN-OT studies have also been done to examine the effects of OT on trust behavior and visual scanning of faces (Andari et al. 2010). Individuals with ASD given OT had a significant preference for the "good player" (the computer player who tossed the ball back to you) that was similar to the control subjects also performing the task. This preference was further supported by the patients' reporting of trust, towards the "good player" after OT administration. In the visual scanning task, ASD subjects specifically avoided the area of high expression, the eyes, when given placebo, but significantly increased their gaze fixation towards the eyes with OT.

Recently a multi-dose OT administration study was conducted by Dadds et al. (2014). Individuals with high functioning ASD received 12 or 24 IU (depending on the weight of the patient) IN placebo or OT once daily for 5 days. During these 5 days, the participant and their parents received daily parent-child interaction training and assessments of RRB, emotion recognition, social interaction skills, and general behavioral adjustment. While improvements over time were detected in both OT and placebo, there were no differences observed between the two groups. Several proposed possible explanations for these findings were: (1) emotion recognition was measured pre-post changes following multiple exposures versus while the patient was under the influence; (2) lower-order RRB, such as repeating, ordering and touching, respond to OT (Hollander et al. 2003), while higher-order RRB, such as ritualistic behavior and insistence on sameness, do not (Anagnostou et al. 2012); (3) increased eye gaze frequency is usually measured with artificial or computerized faces, while they utilized "real-life" interactions; (4) the OT receptor system

disruptions in some patients with ASD may respond differently than in other ASD patients; and (5) differences between the studies regarding age and diagnostic characteristics of the sample. Positive results were also observed in a 6-week protocol, which showed that IN-OT is well tolerated when given daily and may improve social cognition and decrease RRB in adults (Anagnostou et al. 2012). It may be important to note that reports of individual differences in the response to IN-OT are increasingly observed, although sample sizes in studies will need to be larger to explore individual response variation. While complex, the results from OT administration studies have provided a basis for understanding the role of OT. They aid in the development of study protocols that are focused on the population that will benefit the most from OT therapy.

14.2.2 OT and AVP Genetic Studies in ASD

Over the past decade, many studies have explored the role of genetics in ASD. It is theorized that the genetic heterogeneity of ASD could account for the complexity of its genetic etiology. When studying OT and AVP system genes, researchers often explore subphenotype scores such as social impairments in ASD. In a recent review, genetic polymorphisms of receptor and pathway regulators such as AVPR1a, OXTR, neurophysin I and II, and CD38 were discussed (Ebstein et al. 2009). Ebstein and colleagues presented preliminary data in their review regarding their findings about CD38, a transmembrane glycoprotein involved in OT secretion (Ebstein et al. 2009). Based on their studies of mice, they hypothesized a role for CD38 in ASD and its role in mediating OT, and in promoting nurturing behavior and social familiarity. (Jin et al. 2007). They genotyped 12 tag single nucleotide polymorphisms (SNPs) across CD38 in 170 ASD trios and assessed IQ and social skills via the Vineland Adaptive Behavior Scales (VABS) in this sample. A significant association between categorical ASD measures (ADI-R; Lord et al. 1994 and ADOS-G; Lord et al. 2000) and CD38 SNPs was found, as well as between social skills in ASD (VABS) and CD38 (rs4634217, rs4516711, rs4508877 and rs3796867), haplotypes and VABS, and CD38 mRNA levels and VABS. Further supporting the role of CD38 in ASD, it has also been noted that there is reduced expression of *CD38* in lymphoblastoid cells of patients with ASD (Lerer et al. 2010), and the rs3796863 CD38 SNP has been associated with high functioning ASD in some populations (Munesue et al. 2010). However, this *CD38* SNP has also been shown to correlate with higher activation of fusiform brain regions in healthy males challenged with OT (Sauer et al. 2012).

Candidate genes for ASD have also been culled from known genetic variants that are more broadly related to affiliative behavior (Yrigollen et al. 2008). In a study of 177 ASD probands from 151 families it was found that different OXTR SNPs were associated with stereotyped behavior, communication skills, an ADI-based diagnosis group and the ASD diagnosis acquired from multiple measurements. Specifically, there was a significant SNP in the OXT/AVP region (rs2740204; $p = 0.016$) associated with stereotyped behaviors (Yrigollen et al. 2008).

In summary, many studies have focused on OXTR as a candidate gene for ASD. It has been shown that multiple SNPs are associated with stereotyped behaviors and communication skills (Yrigollen et al. 2008). Studies specifically examining identified populations such as Chinese Han have shown significant associations between ASD and two of its SNPs, rs2254298 and rs53576 (Wu et al. 2005). Also, in a study utilizing a Caucasian sample the rs2254298 SNP was associated with ASD diagnosis (Jacob et al. 2007). It should be noted that there was overtransmission of the G allele to the autistic Caucasian probands versus overtransmission of the A allele in the Chinese Han sample. More recently, the rs2268493, rs1042778 and rs7632287 SNPs have also been associated with ASD in a sample in which 95 % of individuals self identified as Caucasian (Campbell et al. 2011).

14.2.3 OT Blood Levels in ASD

The limitations to direct access of the brain's oxytocinergic pathways have constrained human research. Therefore, peripheral OT levels have been used as proxies for brain OT levels. A widely used measurement is plasma OT, but urine and salivary OT levels have also been examined. Several researchers have observed associations between peripheral, plasma OT and AVP levels and social stimuli (Kenkel et al. 2012; Schneiderman et al. 2012; Schradin et al. 2013; Seltzer et al. 2010; Wismer Fries et al. 2005). In particular, peripheral OT has been associated with human parental care, both maternal and paternal, such that parents display higher levels of OT than non-parents and low plasma OT levels in parents are associated with less parental touch, whereas higher levels correspond to longer durations of gaze synchrony and reporting of greater parental care during the parent's childhood (Feldman et al. 2012).

Studies of adolescents have also utilized peripheral measures of OT as well as AVP. In a recently published preliminary study of high functioning ASD individuals and typically developing controls, Miller et al. (2013) researched the direct connection between peripheral OT/AVP levels and ASD. Higher levels of OT were observed in all girls and were associated with greater anxiety. Across both sexes, higher OT levels were also associated with better pragmatic language. In addition, all boys had significantly higher levels of AVP. Gender differences were also noted within the ASD sample such that there was a positive association between AVP levels and RRB in ASD girls, but this was negatively (although non-significantly) associated with RRB in boys with ASD (Miller et al. 2013). Overall, these results suggest specific and sexually dimorphic mechanisms for OT and AVP with regard to anxiety and RRB.

Another important study that researched the connection between ASD and OT plasma levels was performed in 1998. Modahl et al. (1998), found plasma OT levels in children with ASD were lower than the control group. They also examined how social behaviors were associated with plasma OT levels. Modahl et al. found that oxytocin was positively associated with age for "normal" children but not for

children with ASD. Additionally, physiological variables such as time of food intake were negatively associated with oxytocin level, and physical exercise and the presence of a respiratory condition were also associated with oxytocin level in the control group. The oxytocin levels of the children with ASD were not related to these physiological variables. Later, a study performed by Jansen et al. (2006) involved adults diagnosed with ASD, who were asked to perform a public speaking task. Interestingly, in this study, subjects showed normal cortisol responses and no change in response to the task in norepinephrine, epinephrine, OT or AVP. However both basal OT levels and heart rate were elevated in the ASD group compared to healthy controls. They hypothesized that the interplay between cortisol and OT may influence the effects of social interactions and support.

Peripheral measurement of serotonin (5-HT) has recently been investigated in conjunction with OT, as many studies have reported hyperserotonemia within a subgroup of individuals with ASD (Leventhal et al. 1990; Schain and Freedman 1961; Leboyer et al. 1999; Kuperman et al. 1985; Abramson et al. 1989; Chugani et al. 1999) and both 5-HT and OT are peripheral biomarkers that correspond to systems that interact in the brain. An analysis of whole blood 5-HT and plasma OT levels in children and adolescents with ASD showed a negative correlation, and this negative correlation was more prominent in younger children (Hammock et al. 2012). These results parallel findings in *Oxtr* KO mice which show that these mice had higher concentrations of whole-blood 5-HT, and the relationship between plasma OT levels and whole blood 5-HT levels are stronger in younger individuals (Hammock et al. 2012).

Over the last few years studies measuring peripheral OT have increased. As discussed in McCullough et al. (2013) and Szeto et al. (2011), the methodologies can lead to vastly different results (increased values, decreased values or values differing by an order of magnitude). For example, Modahl et al. (1998) performed plasma extractions and then radioimmunoassays (RIA) whereas Miller et al. (2013) utilized an enzyme immunoassay (EIA) with different plasma preparation methods. Additionally, there is also specific laboratory generated RIA versus commercial EIA and RIA kits. When manufacturer instructions are followed, values obtained have a similar order of magnitude, but it has been noted that some of these kits may also be detecting closely related metabolites. Note that the studies often prepared samples differently with varying plasma processing/extraction methods, and use of different assay techniques. Future research will need to determine if differences in results about OT levels in ASD reflect differences in the study populations and/or methods for assaying OT. ASD population differences include varying ages and OT levels may change during development, especially after puberty. (Hammock et al. 2012). ASD is a very heterogeneous disorder and OT level differences may be specific to clinical and etiological subgroups within the broader ASD population. In addition, there is variability of OT plasma levels across typical and healthy populations. The inherent U-shaped distribution (Zhong et al. 2012) observed in normative populations may also add to variability in OT measurement in ASD studies.

14.3 Prader-Willi Syndrome and OT

Prader-Willi syndrome (PWS) is a complex disorder with multisystem effects and a distinct behavioral phenotype. It occurs in approximately 1/10,000 to 1/30,000 births, and is initially characterized by severe infantile hypotonia and difficulty feeding, although later in infancy and into adolescence individuals with PWS often eat excessively and develop morbid obesity. Other characteristics of PWS include hypogonadism, short stature, small hands and feet, and strabismus. The cognitive phenotype is marked by delayed motor and language development, and behavioral difficulties including compulsive behavior, stubbornness and temper tantrums (Bittel et al. 2007b; Cassidy et al. 2012). The many behavioral and psychiatric manifestations of PWS are evident in early childhood, and are characterized by hyperactivity, impulsivity, temper tantrums, emotional lability, anxiety and repetitive behavior (Borghgraef et al. 1990; Whitman and Accardo 1987; Gross-Tsur et al. 2001). Often this phenotype is suggestive of ASD as well as attention deficit hyperactivity disorder (ADHD; Cassidy et al. 2012). Face processing is also altered in individuals with PWS, as they have difficulty reading facial expressions (Whittington and Holland 2011).

The cause of PWS is the lack of expression of specifically paternal genes located on chromosome 15q11.2-q13. Many of the genes expressed in this region come from the father, as those from the mother are normally inactivated. Consequently, either a lack of expression or absence of the paternal copy of the genes in this region leads to no expression (Saitoh et al. 1997). This may occur through microdeletions in the paternal chromosome, no copy of the paternal chromosome paired with two copies of the maternal chromosome or uniparental disomy (UPD) or imprinting defects due to epigenetic causes (Cassidy et al. 2012). The genes expressed in this region have been studied at length to develop models of PWS and to delineate their roles in the different aspects of the PWS phenotype. Such studies are complicated by differences in the behavioral phenotype between individuals with deletions and those with UPD, as those with UPD have a less severe phenotype (Bittel et al. 2007a) and higher verbal IQ scores (Dimitropoulos et al. 2000).

While the deletion of no one individual gene has been found to cause PWS, research has shown that the lack of expression of multiple genes may be central to the syndrome's expression. Specifically, five polypeptide coding genes, namely *MKRN3, MAGEL2, MAGED1, NECDIN* and *SNURF-SNRPRN,* have been shown to be centrally involved in PWS. Animal models lacking one of these genes have been developed for *Magel2* (Boccaccio et al. 1999), *Maged1* (Dombret et al. 2012), *Necdin* (Lavi-Itzkovitz et al. 2012; Muscatelli et al. 2000) and *Snurf* (Tsai et al. 1999), although none of these individual gene disruption models completely recapitulates the PWS phenotype.

Another line of approach to elucidate the physiological underpinnings of PWS has been to examine the OT system in individuals with PWS as well as in animal models. There is a deficit of OT producing neurons in the PVN in the brains of persons with PWS (Swaab et al. 1995), as well as lower levels of OT in CSF (Martin

et al. 1998). IN-OT administration increases trust in others and decreases disruptive behavior in individuals with PWS (Tauber et al. 2011). In addition, administration of OT has also been shown to rescue behavior in a *Maged1* deletion model of PWS in which there is a decrease in hypothalamic OT (Dombret et al. 2012). Although rescue was not attempted in the *Necdin* model, this mutant also shows a reduction in OT producing neurons in the hypothalamus (Muscatelli et al. 2000). Consequently, there appears to be disruption of the OT system in individuals with PWS, which is recapitulated in different animal models. However, the exact mechanism of OT dysregulation is unclear.

14.4 Williams Syndrome and OT

Williams syndrome (WS) was first described over 50 years ago (Williams et al. 1961). The first reported cases were focused on infants with hypercalcemia, developmental delays, cardiac malformations and dysmorphic facial features (Morris 1993). However, better characterization of this syndrome has elucidated a distinct behavioral phenotype marked by an increased social drive paired with social fearlessness, poor judgment, difficulty forming peer relationships and high anxiety levels (Jarvinen et al. 2013). The cause of this disorder has been determined to be the deletion of 25–30 genes in the q11.23 region of either maternal or paternal chromosome 7 that span approximately 1.5 megabases (Ewart et al. 1993; Lowery et al. 1995; Korenberg et al. 2000; Schubert 2009). *ELN*, the gene for elastin, was the first deleted gene identified and its absence is indicative of a diagnosis of WS. While *ELN* disruption affects connective tissue, particularly of the aorta (Lowery et al. 1995), other genes such as *LIMK1, CYLN2, GTF2I and GTF2IRD1* are involved in the behavioral phenotype of WS (Jarvinen-Pasley et al. 2008). The deletion of *GTF2I* as well as *GTF2IRD1* has been shown to be involved in the social phenotype specifically (Sakurai et al. 2011; Proulx et al. 2010).

The social phenotype associated with WS is striking due to the hypersociability of the affected individuals, as well as the preference for novel social over non-social stimuli (Jarvinen-Pasley et al. 2008, 2010) and increased eye contact (Mervis et al. 2003). In addition, the speech of individuals with WS is marked by high levels of socially engaging language as compared to controls or individuals with other developmental disorders such as Down Syndrome (Jarvinen-Pasley et al. 2010; Jarvinen et al. 2013). However, this does not translate into the development of social relationships as individuals show difficulty with social adjustment (Gosch and Pankau 1994, 1997) and social judgment (Einfeld et al. 1997; Gosch and Pankau 1997). In addition, affected individuals show deficits in social understanding, as evidenced by difficulty identifying affect (Gagliardi et al. 2003; Plesa-Skwerer et al. 2006) or other's mental states (Jarvinen-Pasley et al. 2008).

The high sociability of individuals with WS positions this syndrome as a good mechanism through which to understand the biological underpinnings of social behavior. Mouse models of WS include GTF2I-deficient mice which display increased social interaction with novel mice and diminished social habituation (Saku-

rai et al. 2011) as well as *Gtf2ird1* deletions, that also show increased sociability (Proulx et al. 2010). Recently, de novo duplications of regions of 7q11.23 have been shown to be associated with ASD, whereas deletions of the same region lead to WS (Sanders et al. 2011). Such opposite effects of gene expression leading to markedly contrasting phenotypes raises the issue of dosage effects, but it should be noted that both ASD and WS phenotypes include abnormal social relationships, although through different mechanisms. Whereas individuals with WS show prolonged face gaze, those with ASD display reduced face gaze (Riby and Hancock 2009). In addition, although children with WS and ASD display high levels of anxiety, individuals with ASD have higher levels of RRB as well as greater rates of social phobia and separation anxiety (Cascio et al. 2012).

Dai et al. (2012) examined the possibility of dysregulation of OT in WS as it relates to the contrasting phenotypes of WS or ASD. This was done by examining deletions or increased expression, respectively, of genes in the region defining WS. They show increased baseline levels of OT in individuals with WS as compared to controls. Additionally, OT levels correlated positively with increased approach to strangers as well as decreased adaptive social behaviors. These results suggest that there may be a dose dependent effect of OT, as high levels may impair adaptive social behavior and may partly underlie the maladaptive social phenotype of WS.

14.5 Fragile X Syndrome

Named for the fragile site observed at Xq27.3, Fragile X Syndrome (FXS) is the most common inherited form of intellectual disability and the most common known single gene mutation associated with ASD (O'Donnell and Warren 2002). Worldwide prevalence estimates range from 1 case in 1000-4000 males and 1 case in 4000-6000 females (Brown 1990; Morton et al. 1997; Turner et al. 1996; Webb 2010). This rare genetic disorder is characterized by specific physical features, and cognitive and behavioral phenotypes (Berry-Kravis et al. 2002, 2011; McLennan et al. 2011). The physical features can include a long narrow face with large protruding ears, connective tissue abnormalities (i.e. hyperextensive joints), macroorchidism, macrocephaly, obesity (especially in young males), loose skin over the hands, a high arched palate, a vertical plantar crease and flat feet (Moy et al. 2009; Schapiro et al. 1995). The behavioral and social characteristics of FXS include hyperactivity, attention difficulties, mood lability, compulsive and perseverative behaviors, aggressive outbursts, learning deficits, developmental delays (including delayed speech development), social shyness, gaze avoidance, sensory hypersensitivity, withdrawal from touch and stereotypic movements and behaviors (i.e. hand flapping and rocking), poor motor coordination and echolalia (Hagerman et al. 2009; Hall 2009; Hall et al. 2009; Moy et al. 2009). Many of these behaviors are linked to the anxiety level of the individual, a meaningful link because physiological studies have noted increased sympathetic and decreased parasympathetic activity and poor coordination between the systems (Hall et al. 2009).

Cognitive tests have indicated a specific pattern of strengths and weaknesses. FXS individuals exhibit deficits in visuo-spatial tasks, quantitative skills, short-term and working memory, expressive language skills, sequential processing and executive function (Hall et al. 2012; Cornish et al. 1999; Kwon et al. 2001; Freund and Reiss 1991; Maes et al. 1994; Berry-Kravis et al. 2002) and relative strengths in receptive language skills, visual memory, acquisition of factual information, imitation skills and gestalt processing (Berry-Kravis et al. 2002). This population also has susceptibility to certain other neuropsychiatric disabilities including ASD, ADHD and anxiety disorders, as well as neurological disorders such as epilepsy (Pretorius et al. 1998; Hessl et al. 2001). While the genetic cause of FXS has been found, the neurological basis of FXS symptoms continues to be unknown. MRI studies have found that individuals with FXS have enlarged lateral ventricles and increased caudate nucleus volumes relative to control subjects (Reiss et al. 1995). Anatomical studies of post-mortem brains have revealed that dendritic spines of neocortical pyramidal neurons of FXS subjects are longer and thinner than those of matched controls (Rudelli et al. 1985; Wisniewski et al. 1991; Irwin et al. 2001; Hinton et al. 1991), indicating that the spines fail to mature normally in FXS patients (Irwin et al. 2001).

The majority of individuals with FXS have social anxiety and almost a third have symptoms that overlap with ASD (Hagerman et al. 2010). Published studies have reported the prevalence rate of FXS and autistic behaviors/ASD diagnosis to range from 25–47%, however sample sizes are often small (Hatton et al. 2006; Morton et al. 1997). Like the disorder itself, autistic symptoms are more common in males than females. Individuals with both FXS and ASD often have poorer developmental outcomes, lower cognitive abilities, lower levels of adaptive behavior and more problem behaviors than FXS individuals with fewer ASD related comorbidities. Of the individuals with FXS and autistic behaviors, 15–40% of males and a few females meet the diagnostic criteria for ASD (Berry-Kravis et al. 2002) and present with more severe communication deficits, stereotyped behaviors, and social anxiety versus social disinterest. In addition, males present with more severe developmental delays. Overlapping behaviors between ASD and FXS, such as eye gaze avoidance (Hall et al. 2009), have led many scientists to study FXS as a way to understand and possibly target treatment for ASD.

14.5.1 Molecular Biology of Fragile X Syndrome

Diagnosis is based on DNA analysis that identifies the number of CGG repeats in the fragile X mental retardation 1 (*FMR1*) gene at the Xq27.3 site (Turner et al. 1996). In most affected individuals, this genetic disorder is caused by a trinucleotide (CGG) repeat expansion in the 5′ untranslated (promoter) region of the *FMR1* gene. *FMR1* encodes the fragile X mental retardation protein (FMRP), a 69 kDa protein found in most adult and fetal tissues, with high concentrations in the brain and testes. The expression of FMRP in neural tissue seems to be experience-dependent. It

is produced in the soma and near the synapse of neurons (Berry-Kravis et al. 2002, 2011), and is essential to the shaping of dendritic spines (Davidovic et al. 2011). The protein and network of mRNA targets and interacting proteins contribute to several forms of synaptic plasticity involving learning and memory processes, notably those induced by activation of type I metabotropic glutamate receptor (mGluR; Davidovic et al. 2011). It is hypothesized that a decrease in *Fmr1* functionally affects the protein interaction network with direct consequences on signaling cascade and cellular metabolism (Davidovic et al. 2011).

There are two different *FMR1* mutations, namely full mutation and premutation (Goodrich-Hunsaker et al. 2011a, b). Premutations make up approximately 10% of male and 2–3% of female ASD cases (Wang et al. 2010). These mutations are associated with Fragile X-associated tremor/ataxia syndrome (FXTAS; Wang et al. 2010), have a repeat length of 50–200 and do not usually cause mental deficits, although shyness and anxiety have been known to occur. Premutations influence translation of *FMR1* mRNA (Feng et al. 1995) such that individuals with permutations produce excess *FMR1* mRNA, yet synthesize lower than normal levels of FMRP (Tassone et al. 2000a, b). Upon female transmission the premutation can become a full mutation. FXS is caused by full mutation which is >200 trinucleotide repeats, and results in hypermethylation of the gene and transcriptional silencing (Tassone et al. 2000a). This creates an FMRP deficiency in the brain which leads to FXS presentation (Tassone et al. 2000a; McLennan et al. 2011).

Very rarely have other mutations in the *FMR1* gene involving deletions (Gedeon et al. 1992; Wohrle et al. 1992) or a point mutation (De Boulle et al. 1993) resulted in symptoms identical or even more severe than typical FXS. FMRP is an RNA-binding protein with three RNA-interacting motifs, namely two KH domains and one RGG box (Ashley et al. 1993; Siomi et al. 1993). Findings that a point mutation in one of the KH domains is sufficient to produce severe FXS (Siomi et al. 1994; De Boulle et al. 1993) points to the conclusion that this aspect of the protein is closely related to the clinical symptoms. While mainly found in the cytoplasm (Verheij et al. 1993; Devys et al. 1993), FMRP appears to be able to shuttle in and out of the nucleus (Feng et al. 1997) possibly as a carrier for specific mRNAs.

In the brain, FMRP appears in the cytoplasm of both the soma and dendrites of neurons (Devys et al. 1993; Feng et al. 1997), forms complexes with other proteins including the fragile X-related proteins 1 and 2 (FXR1P and FXR2P), along with mRNA in mRNA-proteins (mRNPs) in association with ribosomes (Feng et al. 1997; Corbin et al. 1997; Ceman et al. 2000; Eberhart et al. 1996; Khandjian et al. 1996). As mentioned earlier, levels of FMRP have been shown to be regulated by sensory experience (Irwin et al. 2005; Todd and Mack 2000; Todd et al. 2003a, b). Supporting this theory is mounting evidence which implicates FMRP in synaptic plasticity and findings showing that mice lacking FMRP have impaired long-term potentiation in somatosensory cortex (Li et al. 2002), visual cortex (Wilson and Cox 2007), olfactory cortex (Larson et al. 2005), cingulate cortex, and amygdala (Zhao et al. 2005), as well as enhanced long-term depression in hippocampus (Huber et al. 2002). In synaptosomal preparations, stimulation of mGluR results in an FMRP-dependent increase in protein synthesis (Weiler et al. 1997, 2004).

14.5.2 Fragile X Syndrome and OT

There are several reasons why treatment with OT has been investigated in FXS. There is evidence that some of the features of ASD and FXS may be reduced by OT (Bartz and Hollander 2006; Hall et al. 2012; Hollander et al. 2007). As described above, OT released endogenously or given exogenously has been associated with positive social behaviors, as well as reductions in anxiety, obsessiveness and stress reactivity. OT may also serve to counter the defensive behavioral strategies associated with stressful experiences, and the central release of AVP and other peptides, such as corticotropin-releasing factor (Carter 2007). IN-OT in males has increased "trust" in a computer game task (Kosfeld et al. 2005) and may reduce endocrine responses to psychosocial stressors in men and women (Heinrichs and Domes 2008).

Similarly to ASD, available treatments for FXS focus on managing symptoms. Stimulants are prescribed for ADHD symptoms, whereas SSRIs and antipsychotics treat aggression associated with anxiety and carbamazepine is used for treatment of seizures (Hampson et al. 2011). Currently, there are no treatments on the market targeting the molecular abnormalities of FXS (Gurkan and Hagerman 2012). However, recent studies have begun to investigate IN-OT due to the autistic-like behaviors observed in FXS and the social and anxiolytic effects of OT.

A 2012 study conducted by Hall and colleagues researched the effects of IN-OT on males with FXS. They hypothesized that the prosocial and anxiolytic effects of OT would reduce, if not alleviate, socially inappropriate behaviors and social anxiety in males with FXS. Of the ten low functioning males between the ages of 13 and 28 years (mean age = 21.3 years) recruited, eight subjects completed the study. All subjects were confirmed by standard Southern blot analysis to have a fully methylated full mutation. The study was set-up as a randomized double-blind placebo-controlled single-dose trial performed with intranasal administration of placebo, 24 IU OT and 48 IU OT.

As previously mentioned, extreme eye gaze avoidance and hyperarousal are exhibited by FXS individuals when experiencing stressful social situations. Hall and colleagues collected eye gaze frequency, heart rate (HR), respiratory sinus arrhythmia (RSA), heart rate variability (HRV) and salivary cortisol during the social challenge (10 min in length), which was conducted 50 min after OT administration. The first 5 min was a social proximity task, where the subjects sat quietly while a female researcher sat opposite with knees almost touching and read a magazine or book. The second five minute section was a social interaction task, here the experimenter while seated had a conversation with the subject, asking questions such as "tell me what movies you like to watch". Before the conversation started, the subjects were instructed to look the experimenter in the eyes as much as possible while talking, they were additionally prompted by the researcher during the conversation when needed, similar to previous studies (Hall et al. 2012).

Confirmation of the hypothesis that OT would have beneficial consequences in FXS would be observed in increased eye gaze frequency, a reduction in physiological arousal, and a decrease in salivary cortisol. The researchers observed a

significant main effect with OT. As compared to a placebo, 24 IU OT led to an increase in eye gaze frequency ($p=0.042$). No differences were noticed between the two tasks of the social challenge. There was also a significant main effect of OT observed in the salivary cortisol levels. Salivary cortisol levels were looked at pre and post challenge, and showed a significant decrease for the 48 IU dose as compared to placebo ($p=0.05$). However, no effects were observed in the physiological measurements (HR, HRV, and RSA). Hall and colleagues hypothesized that the administration of OT may dampen amygdala reactivity towards social stimuli that causes anxiety (Kirsch et al. 2005; Petrovic et al. 2008), decrease HPA axis activation, and increase social motivation (Witt et al. 1992; Witt and Insel 1992). At present, neither OT nor AVP are known to be targets of FMRP. Two points of future investigation that could reveal the interplay between OT and FMRP as proposed by Hall et al. (2012) were: (1) an experiment to determine the localization of mRNAs encoding FMRP and OT precursor in dendritic domains (Smith 2004), and (2) research into the non-coding BC1, a neuron-specific RNA polymerase III transcript and OT in hypothalamo-neurohypophyseal neurons (Tiedge et al. 1993) should be performed.

14.5.3 Animal Models of Fragile X Syndrome Provide Insight on Moderators of Neurodevelopmental Pathways

Animal models have proven indispensible in the understanding of diseases and disorders, and in the development of pharmaceuticals used to treat them. The quality of an animal model is ascertained based on how well it can meet certain criteria of validity, namely construct, face and predictive validity (Bernardet and Crusio 2006). How well the model's behavioral traits resemble the core traits of the disorder is face validity. Predictive validity is established when a drug reduces or improves symptoms in both the model and human. Construct validity is the "quality" of the model, its ability to accurately measure or represent what it claims to be measuring (Cronbach and Meehl 1955). There currently are several FXS animal models, three mice and one drosophila model that meet multiple criteria.

Two homologous genes to *Fmr1* in vertebrates are *Fxr1* (fragile X related gene) and *Fxr2*. Their proteins, FXR1P and FXR2P are both expressed in the neural tissue, specifically in cell bodies, but they are also found in the dendrites near the synapse. Both *Fxr1* and *Fxr2* KO mice have been produced. FXR1P homozygous mice die within 24 hours of birth, while heterozygous mice exhibit abnormal limb musculature. FXR2P deficient mice have a normal lifespan and have learning deficits similar to *Fmr1* KO with some differences and circadian rhythm deficits (Berry-Kravis et al. 2011). The fruit fly model, a mutant lacking dFmr1 (also known as dFxr) protein, exhibits overextension of neurites during development of mushroom bodies (brain region linked with memory) and have a behavioral phenotype that includes circadian rhythm abnormalities and altered courtship behavior (Berry-Kravis et al. 2011; Gatto and Broadie 2009).

One of the most well characterized animal models of FXS was developed in 1994 by the Dutch-Belgian Fragile X Consortium (1994), and is a mouse model

with an identical molecular endpoint of the experimental model and the human disease (i.e., lack of FMRP throughout the lifespan). This mouse was created by inserting a neomycin cassette into exon 5 of the murine *Fmr1* gene. The insert disrupts the transcription of *Fmr1* mRNA causing an absence of FMRP. Even though the cause may not be identical, this mouse model exhibits behavioral similarities to FXS. *Fmr1* KO mice are described as having lower than normal levels of initial social interaction (Mineur et al. 2006), lacking preference for social novelty, and displaying inappropriate social responses (Pietropaolo et al. 2011) versus wild-type (WT). In contrast, OTKO or AVPKO mice display high levels of social contact that does not diminish over time, thus failing to show familiarity (Crawley et al. 2007).

While macroorchidism is observed in the *Fmr1* KO animals (Bakker et al. 1994) as well as in FXS patients, the behavioral patterns differ between the patients and the KO. By most accounts *Fmr1* KO mice appear to have relatively normal behavior, but research has shown that the behavioral and cognitive deficits of the KO are actually quite subtle and parallel FXS patients (Paradee et al. 1999; D'Hooge et al. 1997; Peier et al. 2000; Berry-Kravis et al. 2002). Some behavioral phenotypes displayed in *Fmr1* KO are deficits in object recognition memory (including a failure to habituate to objects), and impairment of spatial memory (Mineur et al. 2002).

Several studies indicate that *Fmr1* KO mice are hyperactive and show indications of increased anxiety (Mineur et al. 2002; Spencer et al. 2005; Bakker et al. 1994), and overreact to sensory stimuli (Chen and Toth 2001; Frankland et al. 2004; Nielsen et al. 2002). *Fmr1* KO mice also exhibit abnormal social interactions, including a general reduction in social contact and a failure to show social recognition (Bernardet and Crusio 2006; Mineur et al. 2006; Spencer et al. 2005; Yan et al. 2004). Also, similar to FXS patients, these mice exhibit sensory hyperresponsiveness, especially to auditory stimuli. Loud tones may induce audiogenic seizures. In some tasks there is variability in the results (i.e. complex visual and auditory discriminant tasks and activity level in an open field). This variability may come from different mouse strains reflecting the effect of different genetic backgrounds on the the expression of the symptoms. This is also hypothesized to be the basis for the variability observed in the symptoms of FXS patients. In work by Pietropaolo et al. (2011), the validity of the *Fmr1* KO mouse on the C57BL/6 (B6) background was tested against WT and *Fmr1* KO on the FVB background. They found the *Fmr1* KO on the B6 background to be a good model for FXS and a suitable model for ASD (Pietropaolo et al. 2011; Yan et al. 2004).

These mice also have neuropathologic phenotypes that are similar to those of FXS patients, including density of dendritic spines of pyramidal neurons in the visual and somatosensory cortices that are greater in adult *Fmr1* KO than WT. In some brain areas, in both mice *Fmr1* KO and FXS patients, the appearance of the spines are more similar to developing versus mature spines (Berry-Kravis et al. 2002). Absence of FMRP in both humans and mice results in improper development of dendritic spines on cortical pyramidal neurons (Comery et al. 1997; Irwin et al. 2000, 2001, 2002). The use of the *Fmr1* KO mouse has also provided some insight into the normal cellular function of FMRP. The subtle cognitive deficits of *Fmr1* KO mice present difficulties for preclinical testing of potential treatments, and high-

light how complex the relationship between the mouse and human phenotypes are. One possibility is that cognitive processes in which FMRP plays a vital role in humans are poorly developed in mice; thus mice lacking FMRP are not particularly disabled, at least compared to severely-affected patients. A second possibility is that other proteins can compensate for FMRP in mice but not in humans. Third, the behavioral paradigms thus far applied to the mouse model may not efficiently assay cognitive domains most affected in FXS.

14.5.4 Examining OT in Neurodevelopmental Animal Models: A Way to Examine Early Effects?

Below we present preliminary data measuring the OT and AVP systems in a mouse model in order to learn more about FXS pathway interactions during development. The animal model was generated with WT and *Fmr1* KO mice from a colony founded with stock obtained from the Jackson Laboratory (Bar Harbor, ME, USA) that was backcrossed onto a B6 background > 10 generations. Mice were genotyped using primers described previously (The Dutch-Belgian Fragile-X Consortium 1994). Cells were stained using the immunocytochemical (ICC) staining procedures, following protocols described in early work on OT and AVP in voles (Yamamoto et al. 2004). All sections were double-stained for NeuN (a marker that stains cell nuclei only in neurons), which allowed precise localization of cytoarchitechtonic boundaries. Stained sections were mounted on subbed slides and examined with OT and AVP antibodies (OT antibodies were generously provided by M. Morris and AVP antibodies were obtained from MP Biomedical #647171, formerly ICN; Solon, OH, USA). Slices of tissue for each animal were categorized as described in Paxinos and Franklin (2004) and carefully matched across subjects to allow comparable sections. The imaged slides captured at 10X, were coded and scored by an experimentally blind scorer using Image J (NIH, Bethesda, MD) software. Cells in the PVN of the hypothalamus regions were stained separately for OT and AVP ($N = 6–7$ mice per group). Boxed sampling areas were: 125×125 μm^2 (PVN total staining density), 250×375 μm^2 (PVN fibers), 93.75×93.75 μm^2 for cell counts bilaterally in both the PVN and SON.

Preliminary results suggest a significant reduction in both OT-positive (Fig. 14.1) and AVP-positive (Fig. 14.2) cells in the PVN of *Fmr1* KO as compared to WT (Table 14.1). While not significant, there is a trend for fewer OT-positive cells in the SON (Table 14.2). We also measured, by cell count, the abundance of OXTR positive cells in the hippocampus, and retrosplenial granular and piriform cortices. None of these areas showed a significant change in cell density as compared to WT mice ($p > 0.05$). Earlier work in voles has suggested that both OT and AVP may support a general tendency toward social contact (Cho et al. 1999). The absence of either OT or AVP in the presence of the other neuropeptide did not produce an "asocial" animal. However, selective social preferences, such as those necessary for pair bond formation, appear to require stimulation of both OT and AVP receptors.

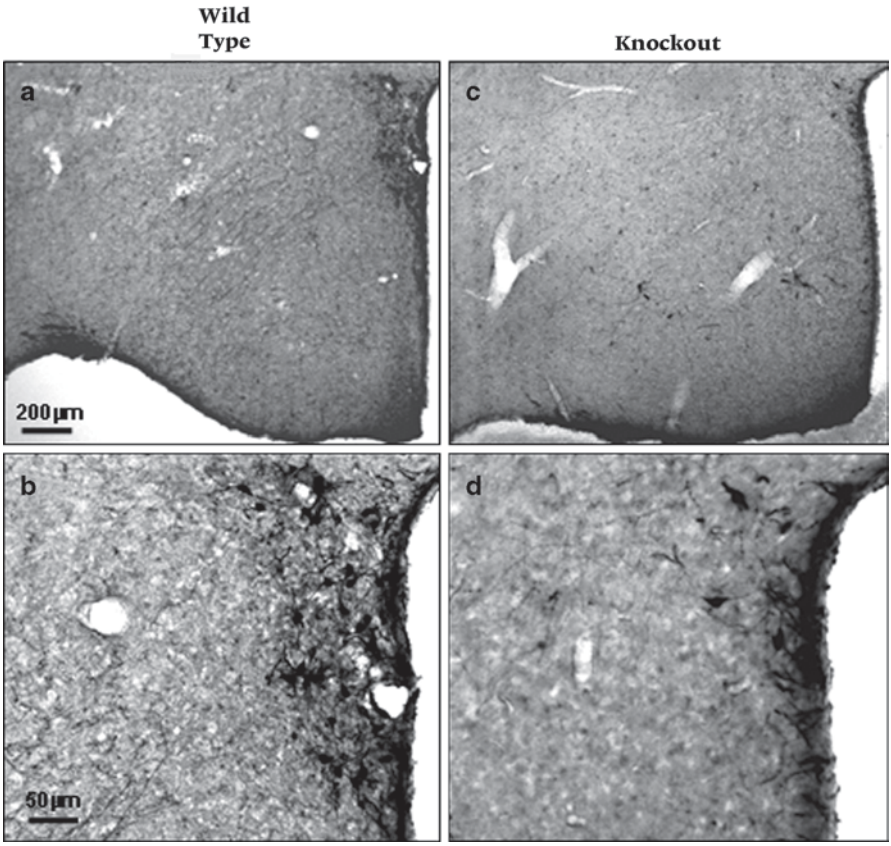


Fig. 14.1 Expression in the paraventricular nucleus (*PVN*) of OT, as measured by ICC, is reduced in *Fmr1* KO mice, compared to the wild-type (*WT*). (Reprinted from Brain Research, Francis et al. 2014, Copyright (2014) with permission from Elsevier)

Although the preliminary data shown here for *Fmr1* KO mice needs to be replicated in a larger sample size and in other animal models, we include these findings as an example of possible approaches to examining the role of peptides, including OT and AVP, in molecularly characterized genetic syndromes. Work across these models also could provide additional insight regarding the role of OT and AVP in early development, especially in syndromes in which atypical trajectories occur.

14.6 Conclusion and Next Steps

While each of the disorders described here (ASD, PWS, WS and FXS) is unique, each one is characterized by atypical social behaviors and in many cases a tendency toward high levels of anxiety. Given the importance of OT and AVP to mamma-

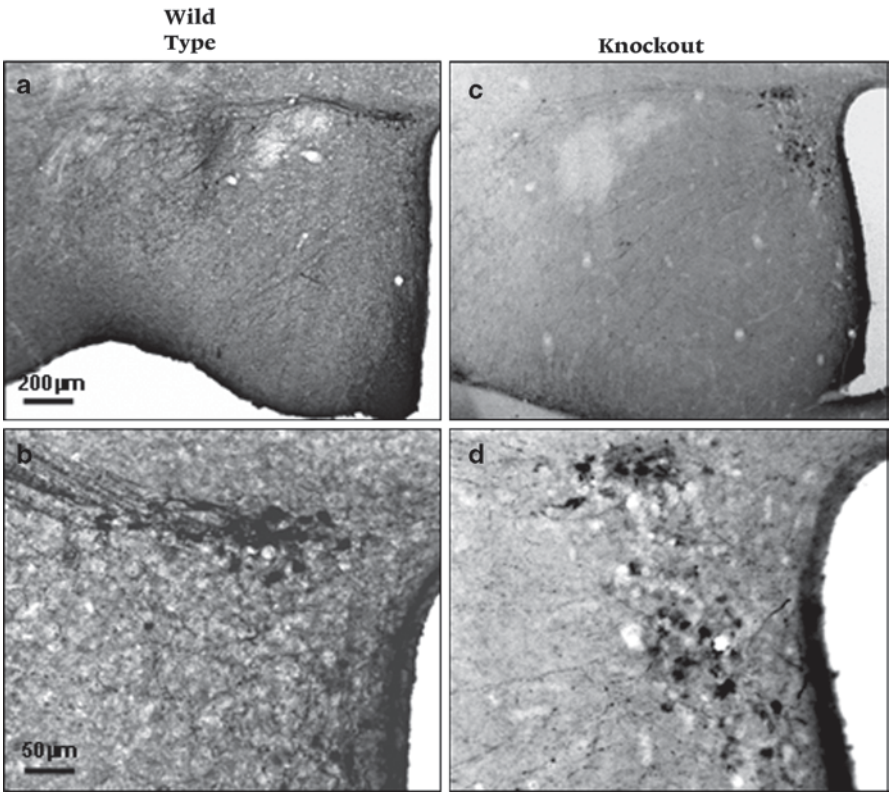


Fig. 14.2 Expression in the paraventricular nucleus (*PVN*) of AVP. In *Fmr1* KO mice, as compared to the wild-type (*WT*) AVP expression is reduced as measured by ICC. (Reprinted from Brain Research, Francis et al. 2014, Copyright (2014) with permission from Elsevier)

lian social behaviors and anxiety, the neuropeptides' investigative value in these syndromes is not unexpected. This review summarized the possible role of OT in these NDD through experiments conducted by others and ourselves (Table 14.3). Each of these early developmental disorders displayed alterations in the OT system and may represent many molecular pathways that lead to a commonly disrupted neuropeptide hormone system. Our preliminary data suggests decreased numbers of OT-positive and AVP-positive cells in the PVN of *Fmr1* KO mice, a mouse model for FXS. Individuals with PWS have shown lower levels of OT in CSF and fewer OT producing cells in the PVN. Lower plasma OT levels have also been detected in some children or a subgroup of ASD affected children. In contrast with WS, which is characterized by hypersociability, a positive correlation was found between OT levels and increased stranger approach and decreased adaptive social behavior. Knowledge of the functionality of the OXTR in WS remains to be studied. Given the rarity of these disorders and the complex animal models needed to research these disorders, many of these studies have small sample sizes. These significant studies, however, can motivate future research on these disorders and other NDD, especially those disorders with dysfunctional social behaviors as a symptom.

Table 14.1 Number of OT and AVP-positive cells in PVN of *Fmr1* KO versus WT mice. (Reprinted from Brain Research, Francis et al. 2014, Copyright (2014) with permission from Elsevier)

	OT			AVP		
	WT	Knockout		WT	Knockout	
PVN	17[a] ± 2[b]	9 ± 3	$p = 0.047$	10 ± 2	4 ± 2	$p = 0.05$

[a] Mean number of positive cell/0.2 mm^2
[b] Mean ± SE (N = 6–7/group)

Table 14.2 Number of OT and AVP-positive cells in SON of *Fmr1* KO versus WT mice. (Reprinted from Brain Research, Francis et al. 2014, Copyright (2014) with permission from Elsevier)

	OT			AVP		
	WT	Knockout		WT	Knockout	
SON	13[a] ± 2[b]	8 ± 3	$p = 0.254$	11 ± 2	6 ± 2	$p = 0.107$

[a] Mean number of positive cell/0.08 mm^2
[b] Mean ± SE (N = 6–7/group)

Table 14.3 A Summary of OT Affects on NDD (Modified from Brain Research, Francis et al., 2014, Copyright (2014) with permission from Elsevier)

Disorder	Neuropeptide system affected
Autism spectrum disorders	↓↑ or atypical levels of OT in blood (human)
	IN-OT ↑ social task performance and ↓ repetitive behaviors (human)
	To be studied:human neuropathology and animal models
Prader-willi syndrome	↓ OT producing cells in the PVN (human)
	↓ level of OT in CSF (human)
	IN-OT ↑ trust and ↓ disruptive behaviors (human)
Williams syndrome	↑ OT levels (human)
	To be studied:human neuropathology and animal models
Fragile X Syndrome	↓ OT+and AVP+cells in the PVN (*Fmr1* KO mice)
	↓ OXTR+cells in several areas of the brain related to learning, memory and emotion (*Fmr1* KO mice)
	IN-OT ↑ eye gaze frequency (human)
	IN-OT ↓ salivary cortisol (human)

ASD, as described above, is the primary NDD presumed to be associated with dysregulation of the OT system. It is striking, however, that other disorders with phenotypes marked by abnormal social behavior as well as anxiety (manifested in RRB) have abnormalities in OT production as measured in blood and CSF (Table 14.3). For example, individuals with PWS, like those in ASD, have difficulty with social competence (Dimitropoulos et al. 2013), are aloof and avoid eye contact (Dimitropoulos et al. 2009). Furthermore, RRB is also evidenced in PWS (Greaves et al. 2006), although to a lesser degree than in ASD as measured using the Repetitive Behavior Scale-Revised (RBS-R; Flores et al. 2011). A subset of the chromosomal region associated with PWS is also associated with an increased risk for ASD, as maternally inherited duplications of the 15q11-13 region are associated with 1–3 % of ASD cases (Bolton et al. 2001; Cook et al. 1997; Vorstman et al. 2006).

Williams syndrome and ASD also share commonalities, namely abnormal social phenotypes and anxiety. Individuals with WS, unlike those with PWS, show a phenotype that is markedly different from ASD. Although both groups are at risk for anxiety, individuals with ASD show higher levels of social phobia and separation anxiety, as well as higher rates of RRB. However, children with WS have higher scores on measures of generalized anxiety (Rodgers et al. 2012). WS is characterized by an increase in OT levels (Dai et al., 2012), as well as a deletion of the 7q11.23 region as opposed to a de novo duplication which leads to ASD (Sanders et al., 2011). Thus, it is likely that some ASD and WS symptoms are related to genetic dosage effects. Studies of FX and ASD mechanism may also inform each other, as mutations in mGluR5 can contribute to the diagnosis of FXS or ASD, and mGluR5 antagonists have shown promise in alleviating ASD symptoms in mouse models (Silverman et al. 2012) as well as Fragile X pathology.

As summarized in this review, animal and human research to date has shown that dysregulation of the OT system is associated with marked deficits in social behavior as well as anxiety. This commonality across multiple NDD may indicate a shared OT pathway that is affected during development. The use of animal models, particularly those developed for FXS, WS and PWS, will provide insight into such a pathway, as these disorders have well characterized genetics, whereas there are over 103 disease genes and 44 genomic loci reported to be involved in ASD (Betancur 2011). However, unlike in ASD, there is a lack of human data on the pathophysiology of FXS, WS and PWS, as well as pharmacological interventions. Ideally, scientists want to identify specific molecular pathways to target distinct syndromes and disorders for treatment. However, many effective treatments modulate common neurochemical or hormone pathways that are downstream from etiologically contributing factors (e.g. drugs for hypertension). Combining the strengths of human and animal model studies across these NDD may provide important clues into the role of OT in development, in addition to elucidating the complex neurophysiology and treatment targets for FXS, PWS, WS and ASD.

Acknowledgements This work was supported in part by NIH K23MH082121 (SJ). The authors would also like to thank Jeanine Leary and Jennifer Speak for their assistance in preparing the text.

References

Abramson RK, Wright HH, Carpenter R, Brennan W, Lumpuy O, Cole E, Young SR (1989) Elevated blood serotonin in autistic probands and their first-degree relatives. J Autism Dev Disord 19:397–407

American Psychiatric Association (2013) Diagnostic and statistical manual of mental disorders, 5th edn. American Psychiatric Publishing, Arlington

Anagnostou E, Soorya L, Chaplin W, Bartz J, Halpern D, Wasserman S, Wang AT, Pepa L, Tanel N, Kushki A, Hollander E (2012) Intranasal oxytocin versus placebo in the treatment of adults with autism spectrum disorders: a randomized controlled trial. Mol Autism 3:16

Andari E, Duhamel JR, Zalla T, Herbrecht E, Leboyer M, Sirigu A (2010) Promoting social behavior with oxytocin in high-functioning autism spectrum disorders. Proc Natl Acad Sci U S A 107:4389–4394

Appenrodt E, Schnabel R, Schwarzberg H (1998) Vasopressin administration modulates anxiety-related behavior in rats. Physiol Behav 64:543–547

Ashley CT Jr, Wilkinson KD, Reines D, Warren ST (1993) FMR1 protein: conserved RNP family domains and selective RNA binding. Science 262:563–566

Bailey A, Le Couteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E, Rutter M (1995) Autism as a strongly genetic disorder: evidence from a British twin study. Psychol Med 25:63–77

Bakker CE, Verheij C, Willemsen R, Vanderhelm R, Oerlemans F, Vermey M, Bygrave A, Hoogeveen AT, Oostra BA, Reyniers E, Deboulle K, Dhooge R, Cras P, Vanvelzen D, Nagels G, Martin JJ, Dedeyn PP, Darby JK, Willems PJ (1994) Fmr1 knockout mice—a model to study fragile-X mental-retardation. Cell 78:23–33

Bartz JA, Hollander E (2006) The neuroscience of affiliation: forging links between basic and clinical research on neuropeptides and social behavior. Horm Behav 50:518–528

Baumgartner T, Heinrichs M, Vonlanthen A, Fischbacher U, Fehr E (2008) Oxytocin shapes the neural circuitry of trust and trust adaptation in humans. Neuron 58:639–650

Bernardet M, Crusio WE (2006) Fmr1 KO mice as a possible model of autistic features. Scientific World Journal 6:1164–1176

Berry-Kravis E, Grossman AW, Crnicz LS, Greenough WT (2002) Understanding fragile X syndrome. Curr Paediatr 12:316–324

Berry-Kravis E, Knox A, Hervey C (2011) Targeted treatments for fragile X syndrome. J Neurodev Disord 3:193–210

Betancur C (2011) Etiological heterogeneity in autism spectrum disorders: more than 100 genetic and genomic disorders and still counting. Brain Res 1380:42–77

Bittel DC, Kibiryeva N, McNulty SG, Driscoll DJ, Butler MG, White RA (2007a) Whole genome microarray analysis of gene expression in an imprinting center deletion mouse model of Prader-Willi syndrome. Am J Med Genet A 143:422–429

Bittel DC, Kibiryeva N, Sell SM, Strong TV, Butler MG (2007b) Whole genome microarray analysis of gene expression in Prader-Willi syndrome. Am J Med Genet A 143:430–442

Boccaccio I, Glatt-Deeley H, Watrin F, Roeckel N, Lalande M, Muscatelli F (1999) The human MAGEL2 gene and its mouse homologue are paternally expressed and mapped to the Prader-Willi region. Hum Mol Genet 8:2497–2505

Bolton PF, Dennis NR, Browne CE, Thomas NS, Veltman MW, Thompson RJ, Jacobs P (2001) The phenotypic manifestations of interstitial duplications of proximal 15q with special reference to the autistic spectrum disorders. Am J Med Genet 105:675–685

Borghgraef M, Fryns JP, Van Den Berghe H (1990) Psychological profile and behavioural characteristics in 12 patients with Prader-Willi syndrome. Genet Counsel 1:141–150

Born J, Lange T, Kern W, McGregor GP, Bickel U, Fehm HL (2002) Sniffing neuropeptides: a transnasal approach to the human brain. Nat Neurosci 5:514–516

Brown WT (1990) The fragile X: progress toward solving the puzzle. Am J Hum Genet 47:175–180

Campbell DB, Datta D, Jones ST, Batey Lee E, Sutcliffe JS, Hammock EA, Levitt P (2011) Association of oxytocin receptor (OXTR) gene variants with multiple phenotype domains of autism spectrum disorder. J Neurodev Disord 3:101–112

Carter CS (1998) Neuroendocrine perspectives on social attachment and love. Psychoneuroendocrinology 23:779–818

Carter CS (2007) Sex differences in oxytocin and vasopressin: implications for autism spectrum disorders? Behav Brain Res 176:170–186

Cascio CJ, Foss-Feig JH, Heacock JL, Newsom CR, Cowan RL, Benningfield MM, Rogers BP, Cao A (2012) Response of neural reward regions to food cues in autism spectrum disorders. J Neurodev Disord 4:9

Cassidy SB, Schwartz S, Miller JL, Driscoll DJ (2012) Prader-Willi syndrome. Genet Med 14:10–26

Ceman S, Nelson R, Warren ST (2000) Identification of mouse YB1/p50 as a component of the FMRP-associated mRNP particle. Biochem Biophys Res Comm 279:904–908
Chaste P, Leboyer M (2012) Autism risk factors: genes, environment, and gene-environment interactions. Dialogue Clin Neurosci 14:281–292
Chen L, Toth M (2001) Fragile X mice develop sensory hyperreactivity to auditory stimuli. Neurosci 103:1043–1050
Chini B, Mouillac B, Balestre MN, Trumpp-Kallmeyer S, Hoflack J, Hibert M, Andriolo M, Pupier S, Jard S, Barberis C (1996) Two aromatic residues regulate the response of the human oxytocin receptor to the partial agonist arginine vasopressin. FEBS Lett 397:201–206
Cho MM, DeVries AC, Williams JR, Carter CS (1999) The effects of oxytocin and vasopressin on partner preferences in male and female prairie voles (Microtus ochrogaster). Behav Neurosci 113:1071–1079
Chugani DC, Muzik O, Behen M, Rothermel R, Janisse JJ, Lee J, Chugani HT (1999) Developmental changes in brain serotonin synthesis capacity in autistic and nonautistic children. Ann Neurol 45:287–295
Comery TA, Harris JB, Willems PJ, Oostra BA, Irwin SA, Weiler IJ, Greenough WT (1997) Abnormal dendritic spines in fragile X knockout mice: maturation and pruning deficits. Proc Natl Acad Sci U S A 94:5401–5404
Cook EH Jr, Lindgren V, Leventhal BL, Courchesne R, Lincoln A, Shulman C, Lord C, Courchesne E (1997) Autism or atypical autism in maternally but not paternally derived proximal 15q duplication. Am J Hum Genet 60:928–934
Corbin F, Bouillon M, Fortin A, Morin S, Rousseau F, Khandjian EW (1997) The fragile X mental retardation protein is associated with poly(A)+mRNA in actively translating polyribosomes. Hum Mol Genet 6:1465–1472
Cornish KM, Munir F, Cross G (1999) Spatial cognition in males with Fragile-X syndrome: evidence for a neuropsychological phenotype. Cortex 35:263–271
Crawley JN, Chen T, Puri A, Washburn R, Sullivan TL, Hill JM, Young NB, Nadler JJ, Moy SS, Young LJ, Caldwell HK, Young WS (2007) Social approach behaviors in oxytocin knockout mice: comparison of two independent lines tested in different laboratory environments. Neuropeptides 41:145–163
Cronbach LJ, Meehl PE (1955) Construct validity in psychological tests. Psychol Bull 52:281–302
D'Hooge R, Nagels G, Franck F, Bakker CE, Reyniers E, Storm K, Kooy RF, Oostra BA, Willems PJ, De Deyn PP (1997) Mildly impaired water maze performance in male Fmr1 knockout mice. Neuroscience 76:367–376
Dadds MR, Macdonald E, Cauchi A, Williams K, Levy F, Brennan J (2014) Nasal oxytocin for social deficits in childhood autism: a randomized controlled trial. J Autism Dev Disord 44:521–531
Dai L, Carter CS, Ying J, Bellugi U, Pournajafi-Nazarloo H, Korenberg JR (2012) Oxytocin and vasopressin are dysregulated in Williams Syndrome, a genetic disorder affecting social behavior. PloS One 7:e38513
Davidovic L, Navratil V, Bonaccorso CM, Catania MV, Bardoni B, Dumas ME (2011) A metabolomic and systems biology perspective on the brain of the fragile X syndrome mouse model. Genome Res 21:2190–2202
De Boulle K, Verkerk AJ, Reyniers E, Vits L, Hendrickx J, Van Roy B, Van den Bos F, de Graaff E, Oostra BA, Willems PJ (1993) A point mutation in the FMR-1 gene associated with fragile X mental retardation. Nat Genet 3:31–35
Devys D, Lutz Y, Rouyer N, Bellocq JP, Mandel JL (1993) The FMR-1 protein is cytoplasmic, most abundant in neurons and appears normal in carriers of a fragile X premutation. Nat Genet 4:335–340
Dimitropoulos A, Feurer ID, Roof E, Stone W, Butler MG, Sutcliffe J, Thompson T (2000) Appetitive behavior, compulsivity, and neurochemistry in Prader-Willi syndrome. Ment Retard Dev Disabil Res Rev 6:125–130

Dimitropoulos A, Ho AY, Klaiman C, Koenig K, Schultz RT (2009) A comparison of behavioral and emotional characteristics in children with autism, Prader-Willi syndrome, and Williams syndrome. J Ment Health Res Intellect Disabil 2:220–243

Dimitropoulos A, Ho A, Feldman B (2013) Social responsiveness and competence in Prader-Willi syndrome: direct comparison to autism spectrum disorder. J Autism Dev Disord 43:103–113

Dombret C, Nguyen T, Schakman O, Michaud JL, Hardin-Pouzet H, Bertrand MJ, De Backer O (2012) Loss of Maged1 results in obesity, deficits of social interactions, impaired sexual behavior and severe alteration of mature oxytocin production in the hypothalamus. Hum Mol Genet 21:4703–4717

Domes G, Heinrichs M, Michel A, Berger C, Herpertz S (2007) Oxytocin improves "mind-reading" in humans. Biol Psychiatry 61:731–733

Domes G, Sibold M, Schulze L, Lischke A, Herpertz SC, Heinrichs M (2013) Intranasal oxytocin increases covert attention to positive social cues. Psychol Med 43:1747–1753

Eberhart DE, Malter HE, Feng Y, Warren ST (1996) The fragile X mental retardation protein is a ribonucleoprotein containing both nuclear localization and nuclear export signals. Hum Mol Genet 5:1083–1091

Ebstein RP, Israel S, Lerer E, Uzefovsky F, Shalev I, Gritsenko I, Riebold M, Salomon S, Yirmiya N (2009) Arginine vasopressin and oxytocin modulate human social behavior. Ann N Y Acad Sci 1167:87–102

Ebstein RP, Knafo A, Mankuta D, Chew SH, Lai PS (2012) The contributions of oxytocin and vasopressin pathway genes to human behavior. Horm Behav 61:359–379

Einfeld SL, Tonge BJ, Florio T (1997) Behavioral and emotional disturbance in individuals with Williams syndrome. Am J Ment Retard 102:45–53

Ewart AK, Morris CA, Atkinson D, Jin W, Sternes K, Spallone P, Stock AD, Leppert M, Keating MT (1993) Hemizygosity at the elastin locus in a developmental disorder, Williams syndrome. Nat Genet 5:11–16

Feldman R, Zagoory-Sharon O, Weisman O, Schneiderman I, Gordon I, Maoz R, Shalev I, Ebstein RP (2012) Sensitive parenting is associated with plasma oxytocin and polymorphisms in the OXTR and CD38 genes. Biol Psychiatry 72:175–181

Feng Y, Zhang F, Lokey LK, Chastain JL, Lakkis L, Eberhart D, Warren ST (1995) Translational suppression by trinucleotide repeat expansion at FMR1. Science 268:731–734

Feng Y, Absher D, Eberhart DE, Brown V, Malter HE, Warren ST (1997) FMRP associates with polyribosomes as an mRNP, and the I304N mutation of severe fragile X syndrome abolishes this association. Mol Cell 1:109–118

Ferguson JN, Young LJ, Hearn EF, Matzuk MM, Insel TR, Winslow JT (2000) Social amnesia in mice lacking the oxytocin gene. Nat Genet 25:284–288

Flores CG, Valcante G, Guter S, Zaytoun A, Wray E, Bell L, Jacob S, Lewis MH, Driscoll DJ, Cook EH Jr, Kim SJ (2011) Repetitive behavior profiles: consistency across autism spectrum disorder cohorts and divergence from Prader-Willi syndrome. J Neurodev Disord 3:316–324

Francis SM, Sagar A, Levin-Decanini T, Liu W, Carter CS, Jacob S. (2014) Brain Res. 11;1580:199–218

Frankland PW, Wang Y, Rosner B, Shimizu T, Balleine BW, Dykens EM, Ornitz EM, Silva AJ (2004) Sensorimotor gating abnormalities in young males with fragile X syndrome and Fmr1-knockout mice. Mol Psychiatry 9:417–425

Freund LS, Reiss AL (1991) Cognitive profiles associated with the fra(X) syndrome in males and females. Am J Med Genet 38:542–547

Gagliardi C, Frigerio E, Burt DM, Cazzaniga I, Perrett DI, Borgatti R (2003) Facial expression recognition in Williams syndrome. Neuropsychologia 41:733–738

Gatto CL, Broadie K (2009) Temporal requirements of the fragile X mental retardation protein in modulating circadian clock circuit synaptic architecture. Front Neural Circuits 3:8

Gedeon AK, Baker E, Robinson H, Partington MW, Gross B, Manca A, Korn B, Poustka A, Yu S, Sutherland GR, Mulley JC (1992) Fragile X syndrome without CCG amplification has an FMR1 deletion. Nat Genet 1:341–344

Goodrich-Hunsaker NJ, Wong LM, McLennan Y, Srivastava S, Tassone F, Harvey D, Rivera SM, Simon TJ (2011a) Young adult female fragile X premutation carriers show age- and genetically-modulated cognitive impairments. Brain Cogn 75:255–260

Goodrich-Hunsaker NJ, Wong LM, McLennan Y, Tassone F, Harvey D, Rivera SM, Simon TJ (2011b) Adult female fragile X premutation carriers exhibit age- and CGG repeat length-related impairments on an attentionally based enumeration task. Front Hum Neurosci 5:63

Gosch A, Pankau R (1994) Social-emotional and behavioral adjustment in children with Williams-Beuren syndrome. Am J Med Genet 53:335–339

Gosch A, Pankau R (1997) Personality characteristics and behaviour problems in individuals of different ages with Williams syndrome. Dev Med Child Neurol 39:527–533

Greaves N, Prince E, Evans DW, Charman T (2006) Repetitive and ritualistic behaviour in children with Prader-Willi syndrome and children with autism. J Intellect Disabil Res 50:92–100

Green L, Fein D, Modahl C, Feinstein C, Waterhouse L, Morris M (2001) Oxytocin and autistic disorder: alterations in peptide forms. Biol Psychiatry 50:609–613

Gross-Tsur V, Landau YE, Benarroch F, Wertman-Elad R, Shalev RS (2001) Cognition, attention, and behavior in Prader-Willi syndrome. J Child Neurol 16:288–290

Guastella AJ, Mitchell PB, Dadds MR (2008) Oxytocin increases gaze to the eye region of human faces. Biol Psychiatry 63:3–5

Guastella AJ, Einfeld SL, Gray KM, Rinehart NJ, Tonge BJ, Lambert TJ, Hickie IB (2010) Intranasal oxytocin improves emotion recognition for youth with autism spectrum disorders. Biol Psychiatry 67:692–694

Gurkan CK, Hagerman RJ (2012) Targeted treatments in autism and fragile X syndrome. Res Autism Spectr Disord 6:1311–1320

Hagerman RJ, Berry-Kravis E, Kaufmann WE, Ono MY, Tartaglia N, Lachiewicz A, Kronk R, Delahunty C, Hessl D, Visootsak J, Picker J, Gane L, Tranfaglia M (2009) Advances in the treatment of fragile X syndrome. Pediatrics 123:378–390

Hagerman R, Hoem G, Hagerman P (2010) Fragile X and autism: intertwined at the molecular level leading to targeted treatments. Mol Autism 1:12

Hall SS (2009) Treatments for fragile X syndrome: a closer look at the data. Dev Disabil Res Rev 15:353–360

Hall SS, Lightbody AA, Huffman LC, Lazzeroni LC, Reiss AL (2009) Physiological correlates of social avoidance behavior in children and adolescents with fragile x syndrome. J Am Acad Child Adolesc Psychiatry 48:320–329

Hall SS, Lightbody AA, McCarthy BE, Parker KJ, Reiss AL (2012) Effects of intranasal oxytocin on social anxiety in males with fragile X syndrome. Psychoneuroendocrinology 37:509–518

Hammock E, Veenstra-VanderWeele J, Yan Z, Kerr TM, Morris M, Anderson GM, Carter CS, Cook EH, Jacob S (2012) Examining autism spectrum disorders by biomarkers: example from the oxytocin and serotonin systems. J Am Acad Child Adolesc Psychiatry 51:712–721

Hampson DR, Adusei DC, Pacey LK (2011) The neurochemical basis for the treatment of autism spectrum disorders and Fragile X Syndrome. Biochem Pharmacol 81:1078–1086

Hatton DD, Sideris J, Skinner M, Mankowski J, Bailey DB Jr, Roberts J, Mirrett P (2006) Autistic behavior in children with fragile X syndrome: prevalence, stability, and the impact of FMRP. Am J Med Genet A 140A:1804–1813

Heinrichs M, Domes G (2008) Neuropeptides and social behaviour: effects of oxytocin and vasopressin in humans. Prog Brain Res 170:337–350

Hessl D, Dyer-Friedman J, Glaser B, Wisbeck J, Barajas RG, Taylor A, Reiss AL (2001) The influence of environmental and genetic factors on behavior problems and autistic symptoms in boys and girls with fragile X syndrome. Pediatrics 108:E88

Hinton VJ, Brown WT, Wisniewski K, Rudelli RD (1991) Analysis of neocortex in three males with the fragile X syndrome. Am J Med Genet 41:289–294

Hollander E, Novotny S, Hanratty M, Yaffe R, DeCaria CM, Aronowitz BR, Mosovich S (2003) Oxytocin infusion reduces repetitive behaviors in adults with autistic and Asperger's disorders. Neuropsychopharmacology 28:193–198

Hollander E, Bartz J, Chaplin W, Phillips A, Sumner J, Soorya L, Anagnostou E, Wasserman S (2007) Oxytocin increases retention of social cognition in autism. Biol Psychiatry 61:498–503
Huber KM, Gallagher SM, Warren ST, Bear MF (2002) Altered synaptic plasticity in a mouse model of fragile X mental retardation. Proc Natl Acad Sci U S A 99:7746–7750
Irwin SA, Galvez R, Greenough WT (2000) Dendritic spine structural anomalies in fragile-X mental retardation syndrome. Cereb Cortex 10:1038–1044
Irwin SA, Patel B, Idupulapati M, Harris JB, Crisostomo RA, Larsen BP, Kooy F, Willems PJ, Cras P, Kozlowski PB, Swain RA, Weiler IJ, Greenough WT (2001) Abnormal dendritic spine characteristics in the temporal and visual cortices of patients with fragile-X syndrome: a quantitative examination. Am J Med Genet 98:161–167
Irwin SA, Idupulapati M, Gilbert ME, Harris JB, Chakravarti AB, Rogers EJ, Crisostomo RA, Larsen BP, Mehta A, Alcantara CJ, Patel B, Swain RA, Weiler IJ, Oostra BA, Greenough WT (2002) Dendritic spine and dendritic field characteristics of layer V pyramidal neurons in the visual cortex of fragile-X knockout mice. Am J Med Genet 111:140–146
Irwin SA, Christmon CA, Grossman AW, Galvez R, Kim SH, DeGrush BJ, Weiler IJ, Greenough WT (2005) Fragile X mental retardation protein levels increase following complex environment exposure in rat brain regions undergoing active synaptogenesis. Neurobiol Learn Mem 83:180–187
Ishak WW, Kahloon M, Fakhry H (2011) Oxytocin role in enhancing well-being: a literature review. J Affect Disord 130:1–9
Jacob S, Brune CW, Carter CS, Leventhal BL, Lord C, Cook EH Jr (2007) Association of the oxytocin receptor gene (OXTR) in Caucasian children and adolescents with autism. Neurosci Lett 417:6–9
Jansen LM, Gispen-de Wied CC, Wiegant VM, Westenberg HG, Lahuis BE, van Engeland H (2006) Autonomic and neuroendocrine responses to a psychosocial stressor in adults with autistic spectrum disorder. J Autism Dev Disord 36:891–899
Jarvinen-Pasley A, Bellugi U, Reilly J, Mills DL, Galaburda A, Reiss AL, Korenberg JR (2008) Defining the social phenotype in Williams syndrome: a model for linking gene, the brain, and behavior. Dev Psychopathol 20:1–35
Jarvinen-Pasley A, Adolphs R, Yam A, Hill KJ, Grichanik M, Reilly J, Mills D, Reiss AL, Korenberg JR, Bellugi U (2010) Affiliative behavior in Williams syndrome: social perception and real-life social behavior. Neuropsychologia 48:2110–2119
Jarvinen A, Korenberg JR, Bellugi U (2013) The social phenotype of Williams syndrome. Curr Opin Neurobiol 23:414–422
Jaselskis CA, Cook EH Jr, Fletcher KE, Leventhal BL (1992) Clonidine treatment of hyperactive and impulsive children with autistic disorder. J Clin Psychopharmacol 12:322–327
Jin D, Liu HX, Hirai H, Torashima T, Nagai T, Lopatina O, Shnayder NA, Yamada K, Noda M, Seike T, Fujita K, Takasawa S, Yokoyama S, Koizumi K, Shiraishi Y, Tanaka S, Hashii M, Yoshihara T, Higashida K, Islam MS, Yamada N, Hayashi K, Noguchi N, Kato I, Okamoto H, Matsushima A, Salmina A, Munesue T, Shimizu N, Mochida S, Asano M, Higashida H (2007) CD38 is critical for social behaviour by regulating oxytocin secretion. Nature 446:41–45
Kanner L (1943) Autistic disturbances of affective contact. Nerv Child 2:217–250
Kenkel WM, Paredes J, Yee JR, Pournajafi-Nazarloo H, Bales KL, Carter CS (2012) Neuroendocrine and behavioural responses to exposure to an infant in male prairie voles. J Neuroendocrinol 24:874–886
Khandjian EW, Corbin F, Woerly S, Rousseau F (1996) The fragile X mental retardation protein is associated with ribosomes. Nat Genet 12:91–93
Kirsch P, Esslinger C, Chen Q, Mier D, Lis S, Siddhanti S, Gruppe H, Mattay VS, Gallhofer B, Meyer-Lindenberg A (2005) Oxytocin modulates neural circuitry for social cognition and fear in humans. J Neurosci 25:11489–11493
Korenberg JR, Chen XN, Hirota H, Lai Z, Bellugi U, Burian D, Roe B, Matsuoka R (2000) VI. Genome structure and cognitive map of Williams syndrome. J Cogn Neurosci 12(Suppl 1):89–107
Kosfeld M, Heinrichs M, Zak PJ, Fischbacher U, Fehr E (2005) Oxytocin increases trust in humans. Nature 435:673–676

Kuperman S, Beeghly JH, Burns TL, Tsai LY (1985) Serotonin relationships of autistic probands and their first-degree relatives. J Am Acad Child Psychiatry 24:186–190

Kwon H, Menon V, Eliez S, Warsofsky IS, White CD, Dyer-Friedman J, Taylor AK, Glover GH, Reiss AL (2001) Functional neuroanatomy of visuospatial working memory in fragile X syndrome: relation to behavioral and molecular measures. Am J Psychiatry 158:1040–1051

Landgraf R, Wigger A (2003) Born to be anxious: neuroendocrine and genetic correlates of trait anxiety in HAB rats. Stress 6:111–119

Larson J, Jessen RE, Kim D, Fine AK, du Hoffmann J (2005) Age-dependent and selective impairment of long-term potentiation in the anterior piriform cortex of mice lacking the fragile X mental retardation protein. J Neurosci 25:9460–9469

Lavi-Itzkovitz A, Tcherpakov M, Levy Z, Itzkovitz S, Muscatelli F, Fainzilber M (2012) Functional consequences of necdin nucleocytoplasmic localization. PLoS One 7:e33786

Leboyer M, Philippe A, Bouvard M, Guilloud-Bataille M, Bondoux D, Tabuteau F, Feingold J, Mouren-Simeoni MC, Launay JM (1999) Whole blood serotonin and plasma beta-endorphin in autistic probands and their first-degree relatives. Biol Psychiatry 45:158–163

Lerer E, Levi S, Israel S, Yaari M, Nemanov L, Mankuta D, Nurit Y, Ebstein RP (2010) Low CD38 expression in lymphoblastoid cells and haplotypes are both associated with autism in a family-based study. Autism Res 3:293–302

Leventhal BL, Cook EH Jr, Morford M, Ravitz A, Freedman DX (1990) Relationships of whole blood serotonin and plasma norepinephrine within families. J Autism Dev Disord 20:499–511

Li J, Pelletier MR, Perez Velazquez JL, Carlen PL (2002) Reduced cortical synaptic plasticity and GluR1 expression associated with fragile X mental retardation protein deficiency. Mol Cell Neurosci 19:138–151

Lim MM, Wang Z, Olazabal DE, Ren X, Terwilliger EF, Young LJ (2004) Enhanced partner preference in a promiscuous species by manipulating the expression of a single gene. Nature 429:754–757

Lord C, Rutter M, Le Couteur A (1994) Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. J Autism Dev Disord 24:659–685

Lord C, Risi S, Lambrecht L, Cook EH Jr, Leventhal BL, DiLavore PC, Pickles A, Rutter M (2000) The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. J Autism Dev Disord 30:205–223

Lowery MC, Morris CA, Ewart A, Brothman LJ, Zhu XL, Leonard CO, Carey JC, Keating M, Brothman AR (1995) Strong correlation of elastin deletions, detected by FISH, with Williams syndrome: evaluation of 235 patients. Am J Hum Genet 57:49–53

Macdonald K, Feifel D (2013) Helping oxytocin deliver: considerations in the development of oxytocin-based therapeutics for brain disorders. Front Neurosci 7:35

Macdonald K, Macdonald TM (2010) The peptide that binds: a systematic review of oxytocin and its prosocial effects in humans. Harv Rev Psychiatry 18:1–21

Maes B, Fryns JP, Van Walleghem M, Van den Berghe H (1994) Cognitive functioning and information processing of adult mentally retarded men with fragile-X syndrome. Am J Med Genet 50:190–200

Martin A, State M, Anderson GM, Kaye WM, Hanchett JM, McConaha CW, North WG, Leckman JF (1998) Cerebrospinal fluid levels of oxytocin in Prader-Willi syndrome: a preliminary report. Biol Psychiatry 44:1349–1352

McCullough, ME, Churchland, PS, Mendez, AJ (2013). Problems with measuring peripheral oxytocin: can the data on oxytocin and human behavior be trusted? Neurosci Biobehav Rev 37:1485–1492

McLennan Y, Polussa J, Tassone F, Hagerman R (2011) Fragile X syndrome. Curr Genomics 12:216–224

Mervis CB, Morris CA, Klein-Tasman BP, Bertrand J, Kwitny S, Appelbaum LG, Rice CE (2003) Attentional characteristics of infants and toddlers with Williams syndrome during triadic interactions. Dev Neuropsychol 23:243–268

Miller M, Bales KL, Taylor SL, Yoon J, Hostetler CM, Carter CS, Solomon M (2013) Oxytocin and vasopressin in children and adolescents with autism spectrum disorders: sex differences and associations with symptoms. Autism Res 6:91–102

Mineur YS, Sluyter F, de Wit S, Oostra BA, Crusio WE (2002) Behavioral and neuroanatomical characterization of the Fmr1 knockout mouse. Hippocampus 12:39–46

Mineur YS, Huynh LX, Crusio WE (2006) Social behavior deficits in the Fmr1 mutant mouse. Behavioural Brain Research 168:172–175

Modahl C, Green L, Fein D, Morris M, Waterhouse L, Feinstein C, Levin H (1998) Plasma oxytocin levels in autistic children. Biol Psychiatry 43:270–277

Morris CA (1993) Williams syndrome. In: Pagon RA, Adam MP, Bird TD, Dolan CR, Fong CT, Stephens K (eds) Gene reviews. University of Washington-Seattle, Seattle

Morton JE, Bundey S, Webb TP, MacDonald F, Rindl PM, Bullock S (1997) Fragile X syndrome is less common than previously estimated. J Med Genet 34:1–5

Moy SS, Nadler JJ, Young NB, Nonneman RJ, Grossman AW, Murphy DL, D'Ercole AJ, Crawley JN, Magnuson TR, Lauder JM (2009) Social approach in genetically engineered mouse lines relevant to autism. Genes Brain Behav 8:129–142

Munesue T, Yokoyama S, Nakamura K, Anitha A, Yamada K, Hayashi K, Asaka T, Liu HX, Jin D, Koizumi K, Islam MS, Huang JJ, Ma WJ, Kim UH, Kim SJ, Park K, Kim D, Kikuchi M, Ono Y, Nakatani H, Suda S, Miyachi T, Hirai H, Salmina A, Pichugina YA, Soumarokov AA, Takei N, Mori N, Tsujii M, Sugiyama T, Yagi K, Yamagishi M, Sasaki T, Yamasue H, Kato N, Hashimoto R, Taniike M, Hayashi Y, Hamada J, Suzuki S, Ooi A, Noda M, Kamiyama Y, Kido MA, Lopatina O, Hashii M, Amina S, Malavasi F, Huang EJ, Zhang J, Shimizu N, Yoshikawa T, Matsushima A, Minabe Y, Higashida H (2010) Two genetic variants of CD38 in subjects with autism spectrum disorder and controls. Neurosci Res 67:181–191

Muscatelli F, Abrous DN, Massacrier A, Boccaccio I, Le Moal M, Cau P, Cremer H (2000) Disruption of the mouse Necdin gene results in hypothalamic and behavioral alterations reminiscent of the human Prader-Willi syndrome. Hum Mol Genet 9:3101–3110

Neumann ID, Landgraf R (2012) Balance of brain oxytocin and vasopressin: implications for anxiety, depression, and social behaviors. Trends Neurosci 35:649–659

Nielsen DM, Derber WJ, McClellan DA, Crnic LS (2002) Alterations in the auditory startle response in Fmr1 targeted mutant mouse models of fragile X syndrome. Brain Res 927:8–17

O'Donnell WT, Warren ST (2002) A decade of molecular studies of fragile X syndrome. Annu Rev Neurosci 25:315–338

Owley T, Brune CW, Salt J, Walton L, Guter S, Ayuyao N, Gibbons RD, Leventhal BL, Cook EH (2010) A pharmacogenetic study of escitalopram in autism spectrum disorders. Autism Res 3:1–7

Paradee W, Melikian HE, Rasmussen DL, Kenneson A, Conn PJ, Warren ST (1999) Fragile X mouse: strain effects of knockout phenotype and evidence suggesting deficient amygdala function. Neuroscience 94:185–192

Paxinos G, Franklin KBJ (2004) The mouse brain in stereotaxic coordinates, compact 2nd edn. Elsevier Academic Press, Amsterdam

Peier AM, McIlwain KL, Kenneson A, Warren ST, Paylor R, Nelson DL (2000) (Over)correction of FMR1 deficiency with YAC transgenics: behavioral and physical features. Hum Mol Genet 9:1145–1159

Petrovic P, Kalisch R, Singer T, Dolan RJ (2008) Oxytocin attenuates affective evaluations of conditioned faces and amygdala activity. J Neurosci 28:6607–6615

Pietropaolo S, Guilleminot A, Martin B, D'Amato FR, Crusio WE (2011) Genetic-Background Modulation of Core and Variable Autistic-Like Symptoms in Fmr1 Knock-Out Mice. PloS One 6:e17073

Plesa-Skwerer D, Faja S, Schofield C, Verbalis A, Tager-Flusberg H (2006) Perceiving facial and vocal expressions of emotion in individuals with Williams syndrome. Am J Ment Retard 111:15–26

Pretorius PH, King MA, Pan TS, de Vries DJ, Glick SJ, Byrne CL (1998) Reducing the influence of the partial volume effect on SPECT activity quantitation with 3D modelling of spatial resolution in iterative reconstruction. Phys Med Biol 43:407–420

Proulx E, Young EJ, Osborne LR, Lambe EK (2010) Enhanced prefrontal serotonin 5-HT(1A) currents in a mouse model of Williams-Beuren syndrome with low innate anxiety. J Neurodev Disord 2:99–108

Reiss AL, Abrams MT, Greenlaw R, Freund L, Denckla MB (1995) Neurodevelopmental effects of the FMR-1 full mutation in humans. Nat Med 1:159–167

Riby D, Hancock PJ (2009) Looking at movies and cartoons: eye-tracking evidence from Williams syndrome and autism. J Intellect Disabil Res 53:169–181

Rimmele U, Hediger K, Heinrichs M, Klaver P (2009) Oxytocin makes a face in memory familiar. J Neurosci 29:38–42

Rodgers J, Riby DM, Janes E, Connolly B, McConachie H (2012) Anxiety and repetitive behaviours in autism spectrum disorders and Williams syndrome: a cross-syndrome comparison. J Autism Dev Disord 42:175–180

Rudelli RD, Brown WT, Wisniewski K, Jenkins EC, Laure-Kamionowska M, Connell F, Wisniewski HM (1985) Adult fragile X syndrome. Clinico-neuropathologic findings. Acta neuropathologica 67:289–295

Saitoh S, Buiting K, Cassidy SB, Conroy JM, Driscoll DJ, Gabriel JM, Gillessen-Kaesbach G, Glenn CC, Greenswag LR, Horsthemke B, Kondo I, Kuwajima K, Niikawa N, Rogan PK, Schwartz S, Seip J, Williams CA, Nicholls RD (1997) Clinical spectrum and molecular diagnosis of Angelman and Prader-Willi syndrome patients with an imprinting mutation. Am J Med Genet 68:195–206

Sakurai T, Dorr NP, Takahashi N, McInnes LA, Elder GA, Buxbaum JD (2011) Haploinsufficiency of Gtf2i, a gene deleted in Williams Syndrome, leads to increases in social interactions. Autism Res 4:28–39

Sala M, Braida D, Lentini D, Busnelli M, Bulgheroni E, Capurro V, Finardi A, Donzelli A, Pattini L, Rubino T, Parolaro D, Nishimori K, Parenti M, Chini B (2011) Pharmacologic rescue of impaired cognitive flexibility, social deficits, increased aggression, and seizure susceptibility in oxytocin receptor null mice: a neurobehavioral model of autism. Biol Psychiatry 69:875–882

Sanders SJ, Ercan-Sencicek AG, Hus V, Luo R, Murtha MT, Moreno-De-Luca D, Chu SH, Moreau MP, Gupta AR, Thomson SA, Mason CE, Bilguvar K, Celestino-Soper PB, Choi M, Crawford EL, Davis L, Wright NR, Dhodapkar RM, DiCola M, DiLullo NM, Fernandez TV, Fielding-Singh V, Fishman DO, Frahm S, Garagaloyan R, Goh GS, Kammela S, Klei L, Lowe JK, Lund SC, McGrew AD, Meyer KA, Moffat WJ, Murdoch JD, O'Roak BJ, Ober GT, Pottenger RS, Raubeson MJ, Song Y, Wang Q, Yaspan BL, Yu TW, Yurkiewicz IR, Beaudet AL, Cantor RM, Curland M, Grice DE, Gunel M, Lifton RP, Mane SM, Martin DM, Shaw CA, Sheldon M, Tischfield JA, Walsh CA, Morrow EM, Ledbetter DH, Fombonne E, Lord C, Martin CL, Brooks AI, Sutcliffe JS, Cook EH Jr, Geschwind D, Roeder K, Devlin B, State MW (2011) Multiple recurrent de novo CNVs, including duplications of the 7q11.23 Williams syndrome region, are strongly associated with autism. Neuron 70:863–885

Sauer C, Montag C, Worner C, Kirsch P, Reuter M (2012) Effects of a common variant in the CD38 gene on social processing in an oxytocin challenge study: possible links to autism. Neuropsychopharmacology 37:1474–1482

Sawchenko PE, Swanson LW (1985) Localization, colocalization, and plasticity of corticotropin-releasing factor immunoreactivity in rat brain. Fed Proc 44:221–227

Schain RJ, Freedman DX (1961) Studies on 5-hydroxyindole metabolism in autistic and other mentally retarded children. J Pediatr 58:315–320

Schapiro MB, Murphy DG, Hagerman RJ, Azari NP, Alexander GE, Miezejeski CM, Hinton VJ, Horwitz B, Haxby JV, Kumar A, White B, Grady CL (1995) Adult fragile X syndrome: neuropsychology, brain anatomy, and metabolism. Am J Med Genet 60:480–493

Schneiderman I, Zagoory-Sharon O, Leckman JF, Feldman R (2012) Oxytocin during the initial stages of romantic attachment: relations to couples' interactive reciprocity. Psychoneuroendocrinology 37:1277–1285

Schradin C, Kenkel W, Krackow S, Carter CS (2013) Staying put or leaving home: endocrine, neuroendocrine and behavioral consequences in male African striped mice. Horm Behav 63:136–143

Schubert C (2009) The genomic basis of the Williams-Beuren syndrome. Cell Mol Life Sci 66:1178–1197

Seltzer LJ, Ziegler TE, Pollak SD (2010) Social vocalizations can release oxytocin in humans. Proc Biol Sci 277:2661–2666

Shamay-Tsoory SG, Fischer M, Dvash J, Harari H, Perach-Bloom N, Levkovitz Y (2009) Intranasal administration of oxytocin increases envy and schadenfreude (gloating). Biol Psychiatry 66:864–870

Silverman JL, Smith DG, Rizzo SJ, Karras MN, Turner SM, Tolu SS, Bryce DK, Smith DL, Fonseca K, Ring RH, Crawley JN (2012) Negative allosteric modulation of the mGluR5 receptor reduces repetitive behaviors and rescues social deficits in mouse models of autism. Sci Trans Med 4:131ra151

Siomi H, Siomi MC, Nussbaum RL, Dreyfuss G (1993) The protein product of the fragile X gene, FMR1, has characteristics of an RNA-binding protein. Cell 74:291–298

Siomi H, Choi M, Siomi MC, Nussbaum RL, Dreyfuss G (1994) Essential role for KH domains in RNA binding: impaired RNA binding by a mutation in the KH domain of FMR1 that causes fragile X syndrome. Cell 77:33–39

Smith R (2004) Moving molecules: mRNA trafficking in Mammalian oligodendrocytes and neurons. Neuroscientist 10:495–500

Spencer CM, Alekseyenko O, Serysheva E, Yuva-Paylor LA, Paylor R (2005) Altered anxiety-related and social behaviors in the Fmr1 knockout mouse model of fragile X syndrome. Genes Brain Behav 4:420–430

Swaab DF, Purba JS, Hofman MA (1995) Alterations in the hypothalamic paraventricular nucleus and its oxytocin neurons (putative satiety cells) in Prader-Willi syndrome: a study of five cases. J Clin Endocrinol Metab 80:573–579

Szeto, A, McCabe, PM, Nationmm DA, Tabak, BA, Rossetti, MA, McCullough, ME, Schneiderman, N, Mendez, AJ. (2011) Evaluation of enzyme immunoassay and radioimmunoassay methods for the measurement of plasma oxytocin. Psychosom Med 73:393–400

Takayanagi Y, Yoshida M, Bielsky IF, Ross HE, Kawamata M, Onaka T, Yanagisawa T, Kimura T, Matzuk MM, Young LJ, Nishimori K (2005) Pervasive social deficits, but normal parturition, in oxytocin receptor-deficient mice. Proc Natl Acad Sci U S A 102:16096–16101

Tassone F, Hagerman RJ, Chamberlain WD, Hagerman PJ (2000a) Transcription of the FMR1 gene in individuals with fragile X syndrome. Am J Med Genet 97:195–203

Tassone F, Hagerman RJ, Taylor AK, Gane LW, Godfrey TE, Hagerman PJ (2000b) Elevated levels of FMR1 mRNA in carrier males: a new mechanism of involvement in the fragile-X syndrome. Am J Hum Genet 66:6–15

Tauber M, Mantoulan C, Copet P, Jauregui J, Demeer G, Diene G, Roge B, Laurier V, Ehlinger V, Arnaud C, Molinas C, Thuilleaux D (2011) Oxytocin may be useful to increase trust in others and decrease disruptive behaviours in patients with Prader-Willi syndrome: a randomised placebo-controlled trial in 24 patients. Orphanet J Rare Dis 6:47

The Dutch-Belgian Fragile-X Consortium (1994) Fmr1 knockout mice: a model to study fragile X mental retardation. The Dutch-Belgian Fragile X Consortium. Cell 78:23–33

Tiedge H, Zhou A, Thorn NA, Brosius J (1993) Transport of BC1 RNA in hypothalamo-neurohypophyseal axons. J Neurosci 13:4214–4219

Todd PK, Mack KJ (2000) Sensory stimulation increases cortical expression of the fragile X mental retardation protein in vivo. Brain Res Mol Brain Res 80:17–25

Todd PK, Mack KJ, Malter JS (2003a) The fragile X mental retardation protein is required for type-I metabotropic glutamate receptor-dependent translation of PSD-95. Proc Natl Acad Sci U S A 100:14374–14378

Todd PK, Malter JS, Mack KJ (2003b) Whisker stimulation-dependent translation of FMRP in the barrel cortex requires activation of type I metabotropic glutamate receptors. Brain Res Mol Brain Res 110:267–278

Tsai TF, Jiang YH, Bressler J, Armstrong D, Beaudet AL (1999) Paternal deletion from Snrpn to Ube3a in the mouse causes hypotonia, growth retardation and partial lethality and provides evidence for a gene contributing to Prader-Willi syndrome. Hum Mol Genet 8:1357–1364

Turner G, Webb T, Wake S, Robinson H (1996) Prevalence of fragile X syndrome. Am J Med Genet 64:196–197

Verheij C, Bakker CE, de Graaff E, Keulemans J, Willemsen R, Verkerk AJ, Galjaard H, Reuser AJ, Hoogeveen AT, Oostra BA (1993) Characterization and localization of the FMR-1 gene product associated with fragile X syndrome. Nature 363:722–724

Vorstman JA, Staal WG, van Daalen E, van Engeland H, Hochstenbach PF, Franke L (2006) Identification of novel autism candidate regions through analysis of reported cytogenetic abnormalities associated with autism. Mol Psychiatry 11:1, 18–28

Wang LW, Berry-Kravis E, Hagerman RJ (2010) Fragile X: leading the way for targeted treatments in autism. Neurotherapeutics 7:264–274

Waterhouse L, Fein D, Modahl C (1996) Neurofunctional mechanisms in autism. Psychol Rev 103:457–489

Webb S (2010) Drugmakers dance with autism. Nat Biotechnol 28:772–774

Weiler IJ, Irwin SA, Klintsova AY, Spencer CM, Brazelton AD, Miyashiro K, Comery TA, Patel B, Eberwine J, Greenough WT (1997) Fragile X mental retardation protein is translated near synapses in response to neurotransmitter activation. Proc Natl Acad Sci U S A 94:5395–5400

Weiler IJ, Spangler CC, Klintsova AY, Grossman AW, Kim SH, Bertaina-Anglade V, Khaliq H, de Vries FE, Lambers FA, Hatia F, Base CK, Greenough WT (2004) Fragile X mental retardation protein is necessary for neurotransmitter-activated protein translation at synapses. Proc Natl Acad Sci U S A 101:17504–17509

Whitman BY, Accardo P (1987) Emotional symptoms in Prader-Willi syndrome adolescents. Am J Med Genet 28:897–905

Whittington J, Holland T (2011) Recognition of emotion in facial expression by people with Prader-Willi syndrome. J Intellect Disabil Res 55:75–84

Williams JC, Barratt-Boyes BG, Lowe JB (1961) Supravalvular aortic stenosis. Circulation 24:1311–1318

Wilson BM, Cox CL (2007) Absence of metabotropic glutamate receptor-mediated plasticity in the neocortex of fragile X mice. Proc Natl Acad Sci U S A 104:2454–2459

Winslow JT, Insel TR (2004) Neuroendocrine basis of social recognition. Curr Opin Neurobiol 14:248–253

Wismer Fries AB, Ziegler TE, Kurian JR, Jacoris S, Pollak SD (2005) Early experience in humans is associated with changes in neuropeptides critical for regulating social behavior. Proc Natl Acad Sci U S A 102:17237–17240

Wisniewski KE, Segan SM, Miezejeski CM, Sersen EA, Rudelli RD (1991) The Fra(X) syndrome: neurological, electrophysiological, and neuropathological abnormalities. Am J Med Genet 38:476–480

Witt DM, Insel TR (1992) Central oxytocin antagonism decreases female reproductive behavior. Ann N Y Acad Sci 652:445–447

Witt DM, Winslow JT, Insel TR (1992) Enhanced social interactions in rats following chronic, centrally infused oxytocin. Pharmacol Biochem Behav 43:855–861

Wohrle D, Kotzot D, Hirst MC, Manca A, Korn B, Schmidt A, Barbi G, Rott HD, Poustka A, Davies KE et al (1992) A microdeletion of less than 250 kb, including the proximal part of the FMR-I gene and the fragile-X site, in a male with the clinical phenotype of fragile-X syndrome. Am J Hum Genet 51:299–306

Wu S, Jia M, Ruan Y, Liu J, Guo Y, Shuang M, Gong X, Zhang Y, Yang X, Zhang D (2005) Positive association of the oxytocin receptor gene (OXTR) with autism in the Chinese Han population. Biol Psychiatry 58:74–77

Yamamoto Y, Cushing BS, Kramer KM, Epperson PD, Hoffman GE, Carter CS (2004) Neonatal manipulations of oxytocin alter expression of oxytocin and vasopressin immunoreactive cells in the paraventricular nucleus of the hypothalamus in a gender-specific manner. Neuroscience 125:947–955

Yan QJ, Asafo-Adjei PK, Arnold HM, Brown RE, Bauchwitz RP (2004) A phenotypic and molecular characterization of the fmr1-tm1Cgr fragile X mouse. Genes Brain Behav 3:337–359

Yrigollen CM, Han SS, Kochetkova A, Babitz T, Chang JT, Volkmar FR, Leckman JF, Grigorenko EL (2008) Genes controlling affiliative behavior as candidate genes for autism. Biol Psychiatry 63:911–916

Zhao MG, Toyoda H, Ko SW, Ding HK, Wu LJ, Zhuo M (2005) Deficits in trace fear memory and long-term potentiation in a mouse model for fragile X syndrome. J Neurosci 25:7385–7392

Zhong, S, Monakhov, M, Mok, HP, Tong, T, Lai, PS, Chew, SH, Ebstein RP (2012) U-shaped relation between plasma oxytocin levels and behavior in the trust game. PLoS One 7:e51095

Chapter 15

Molecular Basis of Cholinergic Changes in Autism Spectrum Disorders and Relevance for Treatment Interventions

Elizabeta B. Mukaetova-Ladinska and Elaine K. Perry

Abstract The causes of autism are heterogeneous and still largely unknown. Cholinergic abnormalities are reported in molecular pathological studies conducted on brain tissues from adults with autism and may explain the numerous cognitive and behavioural changes seen in the autism spectrum disorders (ASD), including impairment in various cognitive domains, memory and attention. Currently available treatments for the behavioural problems frequently reported in children and adults with ASD are largely for symptomatic relief of irritability, hyperactivity and repetitive stereotyped behaviour.

In this review we address current knowledge about the cholinergic changes in ASD and how these are relevant in clinical setting. In particular, we review the prospect of the use of cholinesterase inhibitors and other cholinomimetics (chemicals that can act by either directly stimulating the nicotinic or muscarinic receptors, or promote acetylcholine release) in ASD for treatment of both cognitive and behavioural changes, based on their benefits in neurodegenerative (e.g. Alzheimer's disease and Lewy Body Spectrum Diseases) and neurodevelopmental disorders (e.g. schizophrenia and Down syndrome). As a result, we provide an overview of the current use of cholinesterase inhibitors (donepezil, galantamine and rivastigmine) and cholinomimetics (e.g. nicotine) in the treatment of cognitive and behavioral symptoms in ASD, and discuss developments of novel cholinergic drug interventions that can safely target core disease mechanisms as early as possible.

Keywords Autistic spectrum disorder · Treatment · Acetylcholine · Cholinergic changes · Cholinesterase inhibitors · Cholinomimetics

Autism is a pervasive neurodevelopmental disorder characterized by atypical development in socialization, communication, and behaviour (American Psychiatric Association (APA) 2000). It represents a clinically heterogeneous group of disorders, commonly referred to as "autism spectrum disorders" (ASD). ASD prevalence rates are currently predicting that 1 in 88 children will be affected with this disorder

E. B. Mukaetova-Ladinska (✉) · E. K. Perry
Institute of Neuroscience, Campus for Ageing and Vitality, Newcastle University, Westgate Rd, Newcastle upon Tyne NE4 5PL, UK
e-mail: Elizabeta.Mukaetova-Ladinska@ncl.ac.uk

S. Hossein Fatemi (ed.), *The Molecular Basis of Autism,*
Contemporary Clinical Neuroscience, DOI 10.1007/978-1-4939-2190-4_15

(based on 8-year olds: 1:54 boys and 1:252 girls; CDCP 2012), with the latest revised estimate (based on the number of diagnosed ASD people between ages 6 and 17 in 2011 and 2012) being 1:50 (Blumberg et al. 2013), placing the prevalence rate of ASD currently to 2%. The latest research indicates that the ASD prevalence rate may not change overtly with age, with the prevalence rates even for older ASD people remaining at 1%. (Brugha et al. 2011).

The characteristic clinical phenotype of ASD is associated with developmental delays in multiple areas of cognitive and behavioural functioning. These include abnormalities in social interactions, sensorimotor and perceptual performance impairment in attentional processes and motivation, aberrant communication skills, and restricted and repetitive stereotyped behaviours. The aggregation of autistic syndromes, symptoms, or traits varies in the ASD population. This ranges from a full diagnosis of autistic disorder to milder autistic syndromes (e.g. Asperger Syndrome and Pervasive Developmental Disorder Not Otherwise Specified) and sub clinical behavioral features of the autistic syndrome (e.g. social and communication deficits and stereotyped behaviours), to the 'broader autism phenotype'. The latter includes language impairments in childhood and social deficits persisting into adulthood (Le Couteur et al. 1996), personality traits (including increased expression of anxiety, impulsiveness, shyness, over-sensitivity, irritability and eccentricity traits; Murphy et al. 2000), that are akin to autistic symptoms, to developmental impairments or delays involving more specifically the language command, e.g. communication-related forms of expression (Pickles et al. 2000, also reviewed in Constantino 2011).

In addition to the abundance of characteristic behavioural and cognitive changes, various medical and psychiatric comorbidities are frequently reported in autism. These include movement abnormalities, a number of gastrointestinal problems (e.g. abdominal problems, constipation or diarrhea), headaches and migraines, ear, nose and throat problems, increased incidence in allergies and food intolerance, obsessive compulsive disorder, epilepsy, Tourette syndrome and attention deficit hyperactivity disorder (Gillberg and Billstedt 2000). Anxiety and depression, similarly, have been extensively documented (White et al. 2009), and they have even higher prevalence rates in adults with ASD (Stuart-Hamilton et al. 2009).

15.1 Abnormal Brain Development in Autism

The heterogeneity of the clinical phenotypic presentations in ASD suggests involvement of numerous brain structures, both at macro- and microscopic levels. Indeed, a variety of changes in brain volume and functions have been demonstrated by functional Magnetic Resonance Imaging (fMRI) and Positron Emission Tomography (PET) in ASD subjects. These changes appear to be age-dependent, and are more pronounced in younger (2–12.5 years) autistic children (Courchesne et al. 2001; Mills Schumann et al. 2004; Hazlett et al. 2012), whereas older ASD children and adolescents (above the age of 12 years), appear to have normal brain volumes,

similar to their control counterparts (Aylward et al. 2002). One explanation for this is the well documented cerebral overgrowth of either the white (Herbert et al. 2004) and/or grey matter (Palmen et al. 2004) in younger ASD children, which is not the case for the ASD adolescents (Aylward et al. 2002).

The mechanisms behind the brain overgrowth in autism are unknown, and may be related to alterations in neuroregulatory proteins, neurotransmitters, as well as inflammation. Thus, maternal inflammation may play a role in the autistic child brain overgrowth. This, in turn, may influence activation of foetal microglia that will result in changes to, for example, cholinergic neurons in the basal forebrain projecting to medial temporal lobe and various cortical areas, and leading to excessive numbers of cholinergic neurons and 6–8 fold higher levels of choline acetyltransferase (Acevido et al. 2007). Furthermore, the levels of various growth-related hormones, involved in stimulating acetylcholine release, e.g. insulin-like growth factors (IGF-1 and IGF-2), insulin-like growth binding protein IGFB-3 and growth hormone binding protein (GHBP) are significantly higher in autistic children than in age-matched control subjects (Mills et al. 2007).

15.1.1 Excitatory-Inhibitory Circuit Plasticity in ASD

Neurophysiological studies have confirmed that children with autism have 'noisy and unstable cortical networks' (Lewine et al. 1999; Wheless et al. 2002), suggesting involvement of excitatory–inhibitory circuit-plasticity mechanisms underlying the ASD clinical phenotype. The enlarged brain in autism, containing excess neurons, will result in an excess of excitatory neurons causing an imbalance to the excitatory/inhibitory impulse ratio in various neural circuits. The hyperexcitability of neural circuits with increased excitatory impulses will prevent correct functional differentiation of neurons or pruning (Rubenstein and Merzenich 2003) and will result in unstable and 'noisy' circuitry. This hypothesis has been recently confirmed in the mutant mouse model of autism which contains excess climbing fibres due to ineffective pruning in early development leading to Purkinje cell loss (Mariani 1982). Since neocortical areas in autism are also affected by overgrowth, the neural circuits responsible for social interactions and memory will also be affected in this way, resulting in the characteristic clinical phenotype of autism.

15.1.2 Excitatory-Inhibitory Circuit Plasticity and Neurotransmission

Acetylcholine influences attention, short-term memory, sleep–waking cycle through modulatory influence on cortical neurons (reviewed in Pepeu and Giovannini 2004). Furthermore, acetylcholine can be either an excitatory or inhibitory neuromodulator for neurons with postsynaptic G-protein linked muscarinic acetylcholine receptors.

If the postsynaptic cell has nicotinic acetylcholine receptors, acetylcholine can act as a potent rapid excitatory neurotransmitter. It has been proposed that the behavioural state changes, mediated by the acetylcholine, result from its selective effects on the intrinsic membrane properties of cortical inhibitory interneurons (Levy et al. 2008). However, our current knowledge is insufficient to predict whether acetylcholine activity will result in changes in neuronal excitation or inhibition.

Acetylcholine has been shown to reduce the strength of excitatory (glutamatergic) synapses. An increase in excitatory activity could be caused by genetic defects in the glutamate signalling pathway. The association between polymorphisms in the glutamate 6 receptor gene (chromosomal location 6q21) and autism has been documented in several studies, including a family-based association study (Jamain et al. 2002; Shuang et al. 2004). A recent report demonstrated the effect of acetylcholine on local cortical neurons necessary for shaping sensory processing, via reduction of local excitatory input to inhibitory neurons by acetylcholine (Levy et al. 2008).

15.2 Cholinergic System

Acetylcholine released from growing axons, regulates growth, differentiation, and plasticity of the developing central nervous system (Lauder and Schambra 1999) and modulates neurite outgrowth in developing neurons (Tata et al. 2003). Brain derived neutrophic factor (BDNF) encourages the growth of developing neurons and maintains mature neurons. The cholinergic basal forebrain projections to cerebral cortex and hippocampus are associated with maintenance of cognitive abilities, including memory and learning, whereas the brainstem nuclear projections to the thalamus and cerebellum are involved in arousal, including changes in the sleep/wake cycle.

Acetylcholine changes are reported in various animal models of neurodevelopmental disorders, including autism. In particular, rodents with neonatal basal forebrain cholinergic lesions have impaired cognitive function (Hohmann and Berger-Sweeney 1998), and are used as animal models for autism (Walker et al. 2007). This connection is further strengthened by recent genetic studies linking several neuropsychiatric disorders, including schizophrenia (Levinson et al. 2012), attention deficit hyperactivity disorder (Williams et al. 2012), epilepsy (reviewed in Mulley and Mefford 2011) and autism (Yasui et al. 2011; Chilian et al. 2013) to abnormalities in the alpha7-nicotinic acetylcholine receptor subunit gene (CHRNA7) (Riley et al. 2002).

15.2.1 Brain Cholinergic Receptors

There are two classes of cholinergic receptors in the brain: muscarinic and nicotinic, each including a range of subtypes. Both classes appear to co-exist within various

neocortical and subcortical areas, whereas few subcortical areas contain only one acetylcholine receptor class.

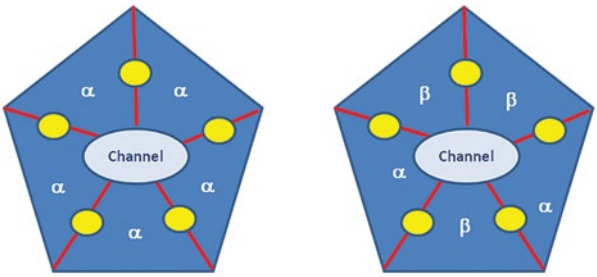
15.2.1.1 Nicotinic Receptors

The nicotinic receptors are involved in regulating neuronal development, including neurogenesis, migration, differentiation and synaptogenesis. One of the earliest neuroregulatory systems to occur in the developing brain (as early as the first half of the first trimester) is the nicotinic acetylcholine receptors (nAchRs). This is followed by development of dopamine and norepinephrine neurons in the following 7 weeks, with the cholinergic fibres entering the brain cortex from 28 to 40 weeks of gestation (reviewed in Dwyer et al. 2008). nAChRs may mediate neuronal pruning and regulate neural pattern formation by decreasing overproduction of cells and outgrowth and guiding remaining neurons to their targets.

The neuronal nicotinic receptors are pentamers consisting of α- and β-subunits (α2–α10 and β2–β4; reviewed in Metherte 2004; Gotti et al. 2006; Dwyer et al. 2008). They can be either heteromeric receptors (consisting of a combination of α- and β-subunits) or homomeric nicotinic receptors (consisting of all five α-subunits (Fig. 15.1). Out of different combinations of α- and β-subunits, eleven can be found in the central nervous system, predominantly consisting of α4β2 heteromeric receptors and α7 homomeric receptors.

The composition of the nicotinic receptor is important, since distinct subunits not only play a role in different functional conformation characteristics of the receptor, but also regulate cholinergic neurotransmission in distinct brain areas. Thus, α7 containing receptors are associated with low affinity for nicotine and a rapid desensitization in presence of agonists, whereas α4β2 receptors have higher affinity for nicotine and slower rates of desensitization (reviewed in Metherte 2004). Incorporation of the alpha5 nicotinic acetylcholine receptor (nAChR) subunit into the α4β2 nAChRs increases the excitatory response and influences the maturation of layer VI prefrontal cortex neurons (Bailey et al. 2012) and is required for normal attentional behaviours in adult mice (Bailey et al. 2010). The α3β2 subunits are expressed in the visual pathway with α4α6β2β3 receptor subtypes being present in visual and mesostriatal pathways, and most likely having specific roles in the brain function (reviewed in Steinlein and Bertrand 2008), whereas α4β3 heteromeric receptors play a role in controlling glutamate release (Alkondon and Albuquerque 2004).

The α7 nicotinic receptors are highly enriched in hippocampal neurons, co-localizing with GABA-ergic neurons (Kawai et al. 2002) and dendrites (Sylvester Vizi et al, 2004) and mediate GABA release, whereas nicotinic agonists against the α7 nicotinic receptors modulate dendritic growth (Torrao et al. 2003). Thus, the documented association of microdeletion of chromosome 15q13.3 that encompasses several genes, including the gene for α7 nicotinic acetylcholine receptor (CHRNA7) results in decrease in hippocampal α7 receptor density, abnormal hippocampal auditory sensory processing and increased hippocampal CA3 pyramidal neuronal activity in animal studies (Adams et al. 2012), further providing support that reduced



	Homomeric receptors		Heteromeric receptors	
Location	α9	Cochlea, olfactory bulb	α2	Interpeduncular cortex
	α8	Found only in avian species	α3β4	PNS, habenulo-interpeduncular system
	α7	Cortex, limbic system	α4β2	Cortex, thalamus
	α5	Drosophila only	α5	Cortex, monoamine containing nuclei
			α6β3	Monoamine containing nuclei

Fig. 15.1 Nicotinic receptors. Graphic presentation of homomeric and heteromeric receptors and their location. Most functional nAChRs contain more than one subunit, and are therefore heteromeric. The different subunit combinations determine the receptor properties in terms of affinities for different ligands electrophysiological properties, Ca2+ permeability and ability to be 'unregulated' by nicotine. *Yellow circles* refere to acetylcholine binding sites. (Reprinted in a modified format from Taly et al. (2009, pp. 733–750). Copyright (2009) with permission from Nature Publishing Group; and from Gotti et al. (2006). Copyright with permission from Elsevier

CHRNA7 expression may contribute to the hippocampal abnormalities of inhibitory circuits, as observed in ASD. Interestingly, the allosteric potentiation of α7 nAChR may mediate the antipsychotic-like effect of the cholinesterase inhibitor galantamine as an adjunctive treatment (Wiker et al. 2008), providing further support for development of novel α7nAChR selective antipsychotic treatment that could be used in schizophrenia and other related psychiatric disorders, including ASD.

15.2.1.2 Muscarinic Acetylcholine Receptors

The muscarinic acetylcholine receptors (mAChR) mediate most of the action of acetylcholine not only in the central and peripheral nervous system, but also in the end organs of the parasympathetic nerves (e.g. cardiac and smooth muscles and secretory glands).

mAChRs are implicated in learning and memory. Out of the five mAChR (M1-M5) identified in mammals, M1 receptors are important for cognitive processes, including working memory (Felder et al. 2000; Auld et al. 2002) and activation of M1 receptors has cognition-enhancing effects, including restoring impaired learning (Shirey et al. 2009). In contrast, neurodegenerative processes, e.g. amyloid

deposits, can decrease the neurotransmission signals of these receptors, resulting in decreased cholinergic activity (Auld et al. 2002).

M2 mAChR is located presynaptically, and similar to M1 mChAR, is predominantly found in the cerebral cortex and hippocampus, but in contrast to M1, is present both on cholinergic and noncholinergic terminals (reviewed in Nathanson 2008). In contrast to the M1 and M2 mAChRs that are found within the brain tissue, M3 receptors are localised to the peripheral nervous system, and contribute to the cholinergic stimulation of gastrointestinal motility (Matsui et al. 2000), mediate cholinergic-induced dilatation of coronary arteries (Lamping et al. 2004), and detrusor bladder contractions (reviewed in Abrams et al. 2006), stimulate parathyroid gland secretion (Culp et al. 1996) and mediate salivation (Matsui et al. 2000). Interestingly, a recent case report (Petersen et al. 2013) identified an individual with autism who had a deletion in 1q43 comprising only one coding gene, CHRM3, for mAChR M3. This finding expands upon previous animal knock-out M3 receptor studies, on the link between the M3 muscarinic receptor pathophysiology and the neurocognitive phenotype of ASD.

The M4 mAChRs in contrast are inhibitory autoreceptors for acetylcholine. Their activation inhibits acetylcholine release in the striatum. The depression in M4 activity (as demonstrated in M4 knock-out mice) results in abnormal social behaviour, including locomotor hyperactivity, reduction in social contacts, sensory motor gating deficits (Koshimizu et al. 2012), similar to those found in ASD subjects and schizophrenia. The M5 mAChR receptors are also found in the hippocampus and various subcortical areas, including substantia nigra, ventromedial hypothalamic nucleus, mammillary bodies, and ventral tegmental area (Vilaró et al. 1990) and may play a role in the regulation of the dopaminergic nigrostriatal pathway, and also up-regulate the expression of immunoglobulin G and proinflammatory cytokines (Kawashima et al. 2012).

15.3 Post-Mortem Studies

15.3.1 Muscarinic Receptors in Autism

Muscrininic receptor changes have not been extensively investigated in autism. Our own post-mortem study has found 30% decrease in M1 receptor binding in the cortical regions of autistic subjects (Perry et al. 2001; Table 15.1). This could be attributed to epilepsy, as 40% of autistic children are estimated to suffer from epilepsy (Minshew et al. 1997). Furthermore, low numbers of M1 receptors have been reported in hippocampal sclerosis, linked with temporal lobe epilepsy (Pennell et al. 1999) and in the hippocampal CA1 region in aged epileptic animals (Cavarsan et al. 2011). However, the findings of Perry et al. (2001) were not due to presence of epilepsy within the series of autism individuals examined. The findings from the latter study are similar to those reported for schizophrenia, where one of the more consistent neurotransmitter abnormalities is loss of the various muscarinic receptors

Table 15.1 Summary of post-mortem studies focusing on the cholinergic system in adults with ASD

Study	Subjects	Main findings in adult subjects with autism compared to control
Perry et al. 2001	7 autistic adults (diagnosed by DSM-IV criteria), 6 MR adults, 3 adults with Down's syndrome, 6 controls	Lower cortical M1 receptor binding (up to 30 %) and decrease in nicotinic receptors by 65–73 % present in the parietal and frontal cortices
		Lower levels of α4 and β2 nicotinic receptor subunits were found in the parietal cortex
		BDNF expression was three-fold higher in the basal forebrain
Lee et al. 2002	8 autistic MR adults (diagnosed by DSM-IV criteria), 11 non-autistic MR adults, 10 controls	A reduction of 40–50 % nicotinic receptor binding to the agonist epibatidine (subunits α3, α4, β2) in the granular, Purkinje and molecular layers
		A three-fold increase in the nicotinic receptor binding of α-bungarotoxin (α7 subunit) in the granule cell layer
		A decrease in α4 receptor subunits in Purkinje and other cell layers
		A non significant increase in α7 subunit in the granule cell layer
Martin-Ruiz et al. 2004	6 autistic adults (diagnosed with DSM-IV criteria), 8 controls	Lower expression of α4 and β2 subunit mRNA levels, protein expression and receptor binding density in the parietal cortex
		α4 subunit mRNA levels were higher and the protein expression and receptor density decreased in the cerebellum
		Non significant increases in α7 subunit mRNA and protein expression levels and significant increases in receptor binding density in the cerebellum
Ray et al. 2005	3 autistic adults (diagnosed by DSM-IV criteria), 3 controls	Decrease in α7 and β2 immunoreactive neurons in the paraventricular nucleus (PV) and nucleus reuniens
		No difference between autistic and control adults in co-expression of α7 and glutamic acid decarboxylase in the PV
		The above suggests that the loss of α7 subunits in autism is not due to a loss of GABA-ergic neurons

DSM-IV diagnostic and statistical manual of mental disorders, *MR* mentally retarded, *M1*, muscarinic acetylcholine receptor M_1, *BDNF* brain derived neutrophic factor, *PV* paraventricular nucleus

(e.g. M1 and M4) in different brain areas, including the superior frontal and temporal gyri and hippocampus (Mancama et al. 2003; Deng and Huang 2005; Scarr et al. 2007; Kang et al. 2009).

15.3.2 Nicotinic Receptor Changes in Autism

We have demonstrated significant loss of nicotinic receptors (40–50 and 65–73 % for the cerebellar cortex, and the parietal and frontal cortices, respectively) in autistic adult subjects compared to control counterparts (Perry et al. 2001; Table 15.1). In

the parietal cortex specifically, there were 30 % lower levels of α4 and β2 nicotinic receptor subunits ($p < 0.05$). Similar changes were also reported for the thalamus, with a significant decrease in numbers of α7 and β2 immunoreactive neurons in the paraventricular nucleus and nucleus reuniens in autistic subjects (Ray et al. 2005).

The profound involvement of the nicotinic cholinergic system in autism was further supported by a study in the cerebellar cortex, that reported lower nicotinic receptor binding for the agonist epibatidine (α3, α4, β2) restricted to the granule cell, Purkinje and molecular layers in the analysed autistic subjects (Fig. 15.2). In contrast, in the granule cell layer, there was a three-fold increase in the nicotinic receptor binding for the agonist α-bungarotoxin (α7 subunit), despite choline acetyltransferase (ChAT) levels being similar in autistic and control subjects (Lee et al. 2002). This suggests that the presynaptic cholinergic system is not altered in autistic subjects.

Our other studies concentrated on investigating the levels of mRNA, protein expression and receptor binding densities of the various nicotinic receptor subunits in both autistic and control subjects (Figs. 15.2 and 15.3). The α4 mRNA levels, protein expression and receptor binding densities were all lower in the parietal cortices of autistic subjects than in controls ($p < 0.05$; Martin-Ruiz et al. 2004; Table 15.1). β2 protein expression was similarly found to be 5-fold lower in this brain area in the autistic subjects, but did not reach statistical significance. Although the cerebellar α4 mRNA levels increased, the protein expression and receptor binding densities

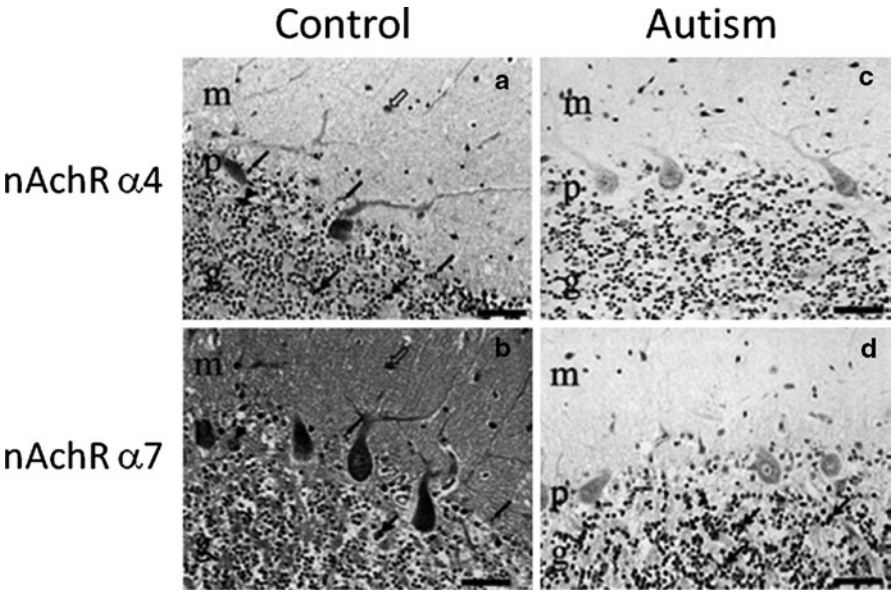


Fig. 15.2 nAChR subunit immunochemistry in the cerebellar cortex. nAChR α4 (**a** and **c**) and α7 (**b** and **d**) subunits in autistic (**c** and **d**) compared to control (**a** and **b**). *M* molecular layer, *p* Purkinje cell layer, *g* granule cell layer. (Reprinted from Mukaetova-Ladinska et al. (2010, pp. 129–161). Copyright (2010) with permission of Springer Science + Business Media

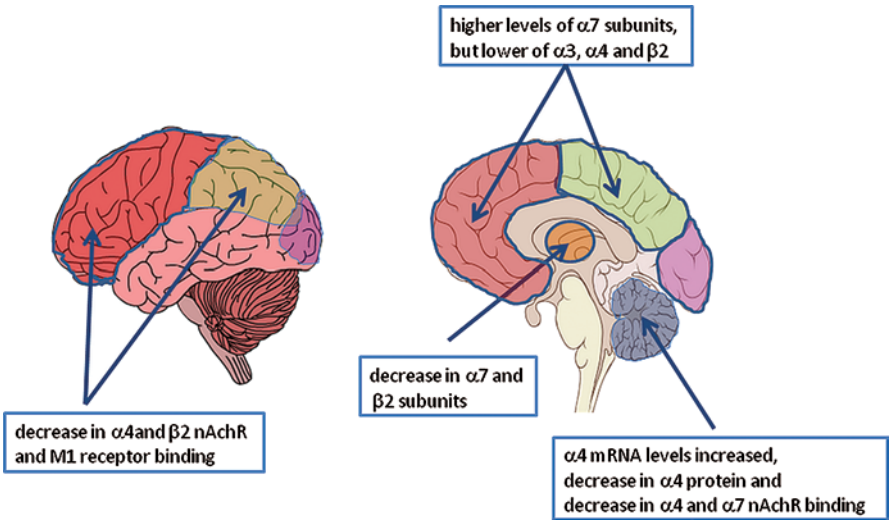


Fig. 15.3 Location of cholinergic deficits in the autistic brain. Summary of findings listed in the text. (Reprinted from Mukaetova-Ladinska et al. (2010, pp. 129–161). Copyright (2010) with permission of Springer Science + Business Media

decreased in the autistic children ($p=0.02$). Levels of α7 receptor subunit binding density were also increased in the cerebellum ($p<0.05$), as well as non-significant increases in mRNA levels and protein expression (Martin-Ruiz et al. 2004).

The significance of loss of nicotinic receptors in neocortical, cerebellar, thalamic and striatal regions in adult autistic individuals (Perry et al. 2001; Lee et al. 2002; Martin-Ruiz et al. 2004; Figs. 15.2 and 15.3) is unknown. Since these receptors are widely localised pre- and post-synaptically, reduced expression of nAChR (especially α4β2 nAChR, Martin-Ruiz et al. 2004) indicate widespread nicotinic receptor dysfunction and disconnectivity. These cholinergic system abnormalities could be related to cortical cell loss or dysfunction, including changes caused by abnormal cortical neuronal morphology (for example synaptic and dendritic abnormalities, Mukaetova-Ladinska et al. 2004), similar to other developmental disorders (such as schizophrenia in which nicotinic receptors have also been implicated).

Neurotrophins mediate acetylcholine release from cholinergic neurons (Huh et al. 2008). In particular, the brain-derived nerve growth factor (BDNF) contributes to both pre- and postnatal brain development, and influences nAChR activity (Fernandes et al. 2008). This strongly argues for its association with a number of diseases associated with cognitive changes, including autism. A recent report studying autistic babies found 62 out of 64 to have increased blood levels of BDNF (Nelson et al. 2000). Furthermore, animal studies have confirmed that impairment in BDNF release at axonal level results in 'autism-like' behavior, with profound deficits in social and anxiety-related behaviors (Sadakata et al. 2012). However, the BDNF measurements in the human autistic brain tissue are not conclusive. Thus, both increase in BDNF-immunoreactivity in the fusiform gyrus (Garcia et al. 2012), and

decrease in BDNF cortical concentration (Sheikh et al. 2010) in autistic children have been reported. These neurobiochemical measures closely follow the inconclusive pattern of peripheral blood BDNF measures in ASD subjects (Hashimoto et al. 2006; Nelson et al. 2006; Katoh-Semba et al. 2007; Croen et al. 2008; Abdallah et al. 2012). Interestingly, no changes in the nerve growth factor (NGF) were noted in the postmortem study (Perry et al. 2001). Since neurotrophic factors regulate the expression of nicotinic receptors (e.g α7 nAChR; Massey et al. 2006), more detailed studies are warranted to address the brain and the peripheral BDNF content in ASD.

A recent study also implicated the neurexin-1β dysfunction in relation to the α4β2 synaptic targeting to the presynaptic neuronal terminals (Cheng et al. 2009), thus further providing support for the role of altered synaptic changes in ASD. Interestingly, the β2-containing nAChRs have been shown to regulate executive and social behaviours (as demonstrated in a β2 nAChR subunit knock-out mice; Granon et al. 2003), which is very similar to the significant reduction of the α4β2 nAChR expression that we have described in the parietal and the cerebellar cortex.

Since nAChRs are known to modulate the release of other neurotransmitters, e.g. GABA and glutamate (Lavine et al. 1997; Baulac et al. 2001), the deficit of nicotinic receptors may denote the early imbalance between the excitatory (glutamatergic) and inhibitory (GABAergic) interneurons in the autistic brain, and thus would increase the excitatory/inhibitory impulse ratio further, and consequently result in worsening of the clinical presentation of autism. To explore these possibilities immunohistochemical double labelling studies using antibodies to specific nicotinic receptor subunits together with GABA or glutamate markers are needed.

15.4 Cholinergic Therapies for Autism

15.4.1 Acetylcholinesterase Inhibitors (ChEI)

These cholinergic abnormalities in the adult autistic brain tissue may be relevant to the clinical phenotype of ASD, especially cognitive deficits. Augmentation of acetylcholine, therefore, may help regulate some of these deficits; acetylcholinesterase inhibitors (ChEIs) decrease the rate at which acetylcholine is broken down. Donepezil, galantamine and rivastigmine are all ChEIs, licensed for the treatment of Alzheimer's and Parkinson's disease dementia, and also relevant to treatment of other neuropsychiatric disorders such as Lewy Body Dementia, Down syndrome, delirium, schizophrenia, depression, mania, traumatic brain injury, attention deficit hyperactivity disorder etc. (reviewed in Yoo et al. 2007). In the case of dementia, ChEIs have been shown to improve both cognitive and behavioural changes, global function and activities of daily living (reviewed in Farlow et al. 2008).

ChEIs have been predominantly used in adults and older people. Their safety for younger people has been recently demonstrated in children with Down syndrome (DS). Heller et al. (2004) reported significant improvement of expressive

and receptive language performance in 7 DS children (ages 8–13 years) in a 16 week open-label trial (Donepezil, 2.5–5 mg/day, followed by 6 weeks of washout period). These findings were confirmed when the study was extended to a 22 week open-label trial (Donepezil 2.5 mg/day and 5 mg/day; Spiridigliozzi et al. 2007). Overall, Donepezil was well tolerated; subjects had language improvement, though some of the participants exhibited increased irritability and/or assertiveness. However, a later study by Kishnani et al. (2010), also conducted on young DS subjects (10–17 years of age, with mild to moderate degree of intellectual disability) failed to replicate these findings.

Similar results of improvement of adaptive function, attention, memory and language have been reported for another ChEI, rivastigmine, in 11 DS children and adolescents (ages 10–17 years; Heller et al. 2006). However, in the follow-up study, the long-term use of rivastigmine (38 months) in the same group of DS subjects did not result in significant improvement of cognitive and language performance (Heller et al. 2010). These results, coming from a small number of participants in these trials, argue that the initial benefits of the ChEIs seen in the DS children may level off with the ageing process. However, larger controlled studies that will employ tests that will cover the performance across multiple psychological domains, including functional ability of the DS subjects are required.

Studies in young DS children treated with ChEIs did not highlight overt side effects, and were consistent with the adverse events noted for ChEIs in older adults. Thus, mild, transient, worsening of gastrointestinal functions, e.g. diarrhea, nausea and vomiting, decreased appetite and moodiness appeared with the initiation of the treatment, whereas problems with sleep, fatigue and emotional lability (e.g. rivastigmine 4.5 mg daily; Heller et al. 2006), cramps and hypotension (donepezil 10 mg daily; Heller et al. 2004) were transient late adverse effects, associated with increasing ChEI dose and resolved within few days.

15.4.2 ChEIs Treatment in Autism

The studies of the use of ChEIs in DS confirmed the good tolerability of these drugs in children, and their potential in regulating some of the similar behavioral and cognitive symptoms in DS and ASD. Over the last decades, all 3 ChEIs have been used in behavioral and cognitive studies in both animal models of autism, as well as children and adolescents with a clinical diagnosis of ASD (Table 15.2).

15.4.2.1 Donepezil

Donepezil inhibits acetylcholinesterase activity and improves memory and spatial learning. In a recent animal study, an intraperitonal injection of donepezil in a mouse model of autism (BTBR mice) resulted in significant improvement of autism-relevant phenotypes, including decreasing cognitive rigidity, improving

Table 15.2 Review of studies using cholinesterase inhibitors for treatment of ASD subjects. All currently published studies on use of ChEIs (donepezil, rivastigmine and galantamine) in ASD reviewed.

ChEI	Study	Trial/Subjects	Criteria for Diagnosis	Main Findings
Donepezil	Hardan and Handen, 2002	8 autistic children and adolescents (7-19 years) Open trial	DSM-IV	50% of subjects had improvement on ABC and Clinical Global impression scale.
	Chez et al, 2003	43 children with autism or pervasive developmental disorder. Double blind study over first 6 weeks, followed by open label 6 week study	DSM-IV	Improvements in receptive and expressive language scores, using CARS
	Hertzman, 2003	1 adult treated with 5 mg/day donepezil	DSM-IV	Verbal and behavioural regression after 1 month of treatment.
	Handen et al, 2011	34 children (8-17 years) with autism (IQ>75), treated with 5-10mg donepezil. Double-blind, placebo-controlled trial over 10 weeks, followed by a 10-week open label trial for placebo non-responders.	DSM-IV	Improvement in a number of executive function measures, but no statistically significant between-group differences were found
	Srivastava et al, 2011	A 5 years old boy (IQ90) treated with donepezil 5mg as an add-on therapy to risperidone 0.5mg Open trial	ICD-10	CARS score improved from 50 to 38 within 6 weeks
	Buckley et al, 2011	5 boys (2.5-6.9yeras) Open label trial, 1.25mg donepezil titrated to 5mg	DSM-IV	Significant decrease in REM latency and increase in REM sleep) (p=0.02)

Table 15.2 (continued)

ChEI	Study	Trial/Subjects	Criteria for Diagnosis	Main Findings
Rivastigmine	Chez et al, 2004	Open labelled study. 32 children (unspecified age)	DSM-IV	Improvements in autistic behaviour, particularly verbalization. Assessments done using Childhood Autistic Rating Scale, Gardner's Expressive and Receptive One-Word Picture Vocabulary tests, and the Conners' Parent Rating Scale.
Galantamine	Niederhofer et al, 2002	20 boys (mean age 7.4 years). Dose and duration of treatment not specified.	ICD-10	Improvements in hyperactivity, inadequate eye contact and inappropriate speech. ABC used.
	Hertzman 2003	3 autistic adults treated with 4mg/day of galantamine, the dose increased to 12mg/day after 2 months.	DSM IV	Improvement in expressive language and communication
	Nicolson et al, 2006	13 children (mean age 8.8 years). 12 weeks open-label trial	DSM-IV	61.5% children responded. Improvements in irritability, social withdrawal, emotional lability, inattention and aggression. ABC, Conner's Parent Rating Scale-Revised, Children's Psychiatric Rating Scale and Clinical Global Impressions Scale used.
	Cubells et al, 2011	39 year male with 15q13.3 deletion syndrome (IQ=55). Galantamine initiated 4mg daily, and dose titrated over 2 weeks to 12mg bid, and followed over 10 months period.	DSM-IV	Dramatic decline in frequency and intensity of rage outbursts

social preference, and enhancing social interaction, whereas when injected in the dorsomedial striatum, donepezil improved cognitive-rigidity and social-deficiency phenotypes (Karvat and Kimchi 2013). These findings provide further evidence for the key role of the cholinergic system in ASD, and suggest that increased cognitive flexibility, as a result of acetylcholine augmentation, may result in enhanced social attention.

There are few studies assessing the value of treating autistic subjects with donepezil (Table 15.2). Hardan and Handen (2002) studied the effects of donepezil on eight autistic children and adolescents (7–19 years age). All of the patients were diagnosed using DSM-IV criteria and underwent an open-label trial with donepezil, to assess benefits on their behaviour. Four of them showed improvements in their irritability and hyperactivity; however, assessments of their cognitive functioning and memory were not conducted. Only two side effects were reported: one patient had gastrointestinal problems and another one a mild increase in irritability. Chez et al. (2003) conducted a 6 week double blind study with donepezil, followed by a 6 week open trial with donepezil on a group of 43 children with autism (2–10 years of age) or pervasive developmental disorder. After 6 weeks, the donepezil treated group showed, improvements in overall autistic behaviour, and receptive and expressive language compared to the placebo group.

Although these two studies suggest that donepezil may be effective in treating children and adolescents with autism, the results are not conclusive and more double blind placebo studies need to be carried out. A recent study also dealt with use of donepezil in regulation of Rapid Eye Movement (REM) sleep disorder in young children with autism (Buckley et al. 2011). Since REM sleep duration is greatest in the developing brain and represents the privileged time for neuroplasticity, REM sleep augmentation in children with autism may also have an impact on their cognition and behaviour. This warrants further studies.

15.4.2.2 Galantamine

Besides being a ChEI, galantamine has also allosteric nicotinic receptor modulatory effect which could be particularly relevant in autism in view of nicotinic receptor pathology. As with donepezil, there are few studies of galantamine and autistic patients (Table 15.2). A placebo controlled, double blind crossover, randomized trial with galantamine was conducted by Niederhofer et al. in 2002. Twenty boys (mean age = 7.4 years) with autistic spectrum disorder, diagnosed by ICD-10 criteria, were treated with placebo or galantamine. On average, the subjects receiving the placebo scored slightly higher than the subjects receiving galantamine. Improvements were seen in hyperactivity, inadequate eye contact and inappropriate speech. Clinicians' scores were not significantly different between the placebo and galantamine groups. No side effects were reported but most subjects had very limited language capacities. The study concluded that galantamine may be moderately effective in the short term treatment of irritability in autism.

In another study, galantamine improved verbal skills in 3 autistic adults (Hertzman 2003). Each subject exhibited an improvement in verbalization, and in some, social behavior improved. Only one person experienced side effects. An open label trial by Nicolson et al. (2006), evaluated the use of galantamine with thirteen children (mean age = 8.8 years) with autism. Eight of the thirteen children (61.5 %) benefited from treatment with improvements in irritability, social withdrawal, emotional liability, inattention and aggression. There were no side effects reported except headaches in one patient.

15.4.2.3 Rivastigmine

Only one open label study on use of rivastigmine in autism has been published (Chez et al. 2004; Table 15.2). 32 autistic children (unspecified ages) were recruited to take part in a 12 week open label trial, and repeatedly tested at baseline, 6 and 12 weeks. Improvements were seen with regards to autistic behaviour, particularly in verbalization.

15.4.2.4 Combined Use of ChEI with Neuroleptic and Antidepressant Medications

Although the successes of most pharmacological treatments for autistic adults are limited, combining medications may prove to be more effective. Studies on the joint use of ChEIs and neuroleptic medications in adults with schizophrenia are encouraging. Thus adjunctive treatment with donepezil improves cognition in patients with schizophrenia who are stabilized on atypical antipsychotics (Chung et al. 2009). The most recent meta-analysis, based on six open-label and 24 double-blind randomized controlled studies reported that donepezil, rivastigmine and galantamine adjunctive therapy improve specific cognitive deficits, such as memory, motor speed and attentional aspect of executive function in patients with schizophrenia and schizoaffective disorder (Ribeiz et al. 2010). Similar adjunctive treatment approach was reported recently by Srivastava et al. (2011) in a child with autism. In their case report, they discuss the management of behavioral problems (e.g. hyperactivity) in a 5 years old boy with autism, who was initially treated with Risperidone 0.5 mg for 8 weeks with no improvement. The add-on treatment with donepezil (5 mg daily) resulted in significant improvement of his behavior within the next 6 weeks that was characterized as better verbal communication, eye contact and emotional response, as well as decreased hyperactivity. Similar results were recently confirmed in a randomized control study on 40 children with autism (age 4–12 years) in which the add-on therapy of galantamine to risperidone improved irritability, lethargy and social withdrawl (Ghaleiha et al. 2013). This suggests that the adjunctive treatment with ChEIs may find a place in the treatment of various behavioral and cognitive changes even in young children. However these findings need to be replicated in larger studies. Furthermore, this approach can potentially be

extended to concomitant use of ChEIs and antidepressant medications, e.g. SSRIs, similar to that used in elderly with Alzheimer's disease, which had improvements in both cognitive functioning and functions of daily living (Mowla et al. 2007).

15.4.3 Treatment of Autism with Nicotinic Agonists or Antagonists

15.4.3.1 Nicotinic Agonists

In clinical studies, nicotine has been documented to ameliorate some of autistic spectrum symptomatology. Nicotine-agonists (Gaynor and Handley 2001) and nicotine patches improved attention and reduced complex tics in autistic subjects with Tourette's syndrome (Howson et al. 2004). Most recently we also reported on the benefits of cholinomimetic therapy (e.g. nicotine patch) on a vast range of behavioral problems (e.g. hyperactivity, behavioral dysfunction, impulsivity, restlessness and aggression, and a pronounced sleep disorder) in a 22-year old adult with autism who had profound mental disability (Mukaetova-Ladinska et al. 2012). Interestingly, even the use of a single nicotine patch produced a significant reduction of the symptomatology with an average duration of 1–2 weeks post-application (Shytle et al. 1998), whereas prolonged use of the patch also contributes to discontinuation of adjunctive medication, including neuroleptics and antidepressants (Mukaetova-Ladinska et al. 2012). This effect is not mediated via the tryptophan metabolism and/or its catabolites (Gaynor and Handley 2001).

15.4.3.2 Nicotinic Cholinergic Antagonists

Treatment with nicotinic cholinergic antagonists may also alleviate autistic symptoms (Lippiello 2005). The loss of nicotinic receptors from the frontal, cerebellar and thalamic cortices (summarized in Table 15.1) in autistic adults may be a compensatory measure (or a result of negative feedback), for an excess of cholinergic neurons found in the enlarged young autistics' basal forebrain (Baumann and Kemper 1994). Treatment with a nicotinic antagonist could potentially reduce the effects of the excess cholinergic neurons, preventing the reduction of nicotinic receptors in other parts of the brain.

As a nicotinic AChR agonist, nicotine stimulates dopamine release in the brain (Wonnacott et al. 2005). Dopamine agonists (reviewed in Möller et al. 2008) appear to exacerbate autistic symptoms, so a nicotinic antagonist would theoretically have therapeutic benefits. Mecamylamine is a nonselective and noncompetitive antagonist of nicotinic acetylcholine receptors, with similar effects like nicotine. As such, it has a potential to be used in treatment of the clinical phenotype of autism. The evidence from a rodent study provides support for this. Thus, low doses of mecamylamine (0.125 mg/kg) administered to rats significantly improved cognitive

function compared to saline injected controls ($p<0.05$; Levin and Caldwell 2006). Recently, it has also been used safely in a clinical trial in children with ASD. In their placebo controlled pilot trial on 18 children with autism, Arnold et al. (2012) reported modest to moderate improvement in the 10 mecamylamine-treated children, with 4 children having marked reduction in hyperactivity and irritability, as well as better verbalization, although the overall differences between the treated and control groups were negligible.

Similarly, the availability of allosteric modulators of nicotine acetylcholine receptors and selective/partial agonists for the α7 nAChR, such as choline derived from dietary administration of cytidine 5′diphosphocholine and anabasine derivatives (Deutsch et al. 2011) may provide further avenues for clinical trials that would explore the effects of α7 nAChR agonist strategies in improving the behavioural and cognitive changes in ASD subjects. A recent animal study also found that the α7 nAChR partial agonists have a better efficacy in improving memory than the α4β2 nAChR agonists (Kroker et al. 2011) as a result of the additional involvement of the glutamatergic system leading to improved cognition. Varenicline, a α4β2 nAChR agonist, may also find a place in the ASD treatment, but its safety has not yet been determined in children, and the latest review of literature highlighted significant increase in adverse neuropsychiatric syndromes, including suicide in treated adults (Ahmed et al. 2013). However, other published evidence provides lack of serious neuropsychiatric adverse events in subjects with various psychiatric disorders who are administered varenicline (Anthenelli et al. 2013; Fatemi et al. 2013; Gibbons and Mann 2013).

Having said this, Arnold et al. (2013) reported on a single case of a 9 years old boy with autism treated with varenicline, who showed significant improvement in conversational ability, social interactions and reasoning within 1 month of starting the treatment with ongoing atomoxetine co-administration. Within a week of stopping the varenicline (due to overt depressive symptomatology), he regressed to his psychological status prior to initiation of the treatment, which was stabilized again when varenicline was resumed. Furthermore, stoping the atomoxetine helped regulate the affective side effects. No adverse effects, e.g. gastrointestinal side effects and sleep disturbances were noted in this child. The authors also highlighted the sensitivity to lower drug doses in ASD, suggesting a dose-response study, initially in adults and adolescents, as an initial step in exploring the efficacy of varenicline in ASD.

15.5 Conclusions

While it is accepted that the causes of autism are heterogeneous, we are still far from understanding the specific etiologies for this condition and its appropriate treatments. In autism, neuronal migrational arrest and changes in synaptic and dendritic arborisation (Mukaetova-Ladinska et al. 2004) are accompanied by complex relationships between various interactive neurotransmitter systems, of which the cholinergic neurotransmission appears to be substantially affected.

The limited number of cholinergic brain pathology studies in autism have all been conducted on relatively small numbers of adult individuals, in restricted brain areas, and not always related to core or other clinical symptoms. A key question is the extent to which these receptor changes are central to the disorder, and whether they emerge at an early or later stage.

Nevertheless, our work on cholinergic changes in the brain tissue in people with autism provides a research framework for further development of novel diagnostic tools and therapeutic advantages in this area. The documented changes in nicotinic receptors, in particular α7 nAChAR, provide a promising venue for development of novel diagnostic tools, based on PET and receptor microtransplantation (Palma et al. 2012). Such neuroimaging studies in autism are feasible since there are now reliable markers for muscarinic and nicotinic PET or Single Photon Emission Computed Tomography (SPECT) receptor imaging. There are recent reports of increased choline levels in autism (Hardan et al. 2008; Gabis et al. 2008; Vasconcelos et al. 2008) using MRI proton spectroscopy imaging (MR SPECT), that may be relevant to cholinergic dysfunction. However, these findings are not conclusive, and similar acetylcholine levels have been described in both autistic boys aged 6–17 years (De Vitto et al. 2007), adults with autism (Bernardi et al. 2011) and control subjects using similar methodology.

The latest developments in imaging of cholinergic receptors provide further in-depth research tools for use in clinical studies of autism. Thus, the novel radiotracers for *in vivo* imaging of muscarinic M1 agonists (Buiter et al. 2013), molecular markers of α7 nicotinic acetylcholine receptors (Rötering et al. 2013), as well as α4β2 receptors (Pandey et al. 2012; Hillmer et al. 2012, 2013) have a potential to be used in autism not only to address the molecular changes of the cholinergic neurotransmitter system (in particular, the complex interactions of cognitive and behavioural ASD clinical symptoms with cholinergic neuronal networks), but also for diagnosis and development of novel pharmacological treatments for ASD.

Since the etiological causes of ASD remain unknown, the current treatments of the disturbing behavioral profile are largely symptomatic. The cholinergic deficit in autism and the promising results of cholinergic drug trials so far indicate that further testing of ChEIs to regulate the cognitive and associated behavioral changes in autistic subjects is needed. The use of ChEIs (donepezil, galantamine and rivastigmine) in open label trials is reported to improve core symptoms, but double-blind placebo trials are needed to provide more accurate information. A recent animal study using a pharmacologically-induced deficit in prepulse inhibition, suggested that cholinesterase inhibitors may have distinct profiles in regulating auditory sensory gating (Hohnadel et al. 2007), warranting further studies on similar deficits in the autistic spectrum disorder.

The findings of loss of muscarinic receptors in the brain tissue in autism may also provide a further venue for development of novel treatments. These developments should concentrate on the M1 and M2 receptors, both of which are involved in regulating memory and behavioral flexibility. The initial reports of the use of the selective M1 muscarinic agonist CDD-0102A in rats are favourable

in respect to augmenting memory and cognitive deficit (Ragozzino et al. 2012), and thus may find a potential place in the treatment of a variety of neurological and psychiatric disorders, including autism. Although there are several muscarinic agonists already developed, none of them has been approved for pharmacological use in humans, largely as a result of serious adverse effects. This latest muscarinic agonist has the advantage of lack of profound adverse effects, including hypersalivation, and appears more promising as a pharmacological agent to selectively augment the M1 muscarinic receptor in Alzheimer's disease, schizophrenia and autism.

Another therapeutic approach that can be implemented very early on is modifying the neurotransmission via a change in diet. Thus, regulating the intake of tryptophan, tyrosine and choline can be useful in regulating sleep and mood (Zeisel 1986). Similarly, use of cholinomimetics may find a place in the treatment of some of the symptoms of the autistic spectrum disorders. Thus, a Russian study (Krasnoperova et al. 2004) explored the use of choline alfoscerate (CA; 400 mg/day) in 20 children (aged 3–8 years) with mild to moderate autism. The treatment lasted 8 weeks, alongside with maintenance therapy with neuroleptics. Positive therapeutic effect was observed in 89% of treated autistic subjects: 61% had significant improvement, whereas minimal efficacy was observed in 28%. The clinical benefits included improvement of behavior, development of social and communicative skills, reduction of marked speech disturbances, enhancement of learning activity and productivity. The findings of this study suggest that CA may be an effective and safe medicine for treatment of cognitive and behavioral disturbances in autism, and could be used safely in combination with additional neuroleptic therapy. This is of importance, since children with autism have low dietary intake of choline (James et al. 2011).

A parallel approach to cholinergic therapies involves use of cholinergic precursor loading strategy, involving choline and lecithin (Amenta and Tayebati 2008). There is one open label trial of choline in children with autism with positive outcome although how this can be reconciled with the imaging data on elevated choline (above) is unclear. What we now need is controlled trials of diet in the autistic spectrum disorders fully supported with biochemistry to establish the role of change in diet on behavioral and neurotransmitter changes in autism.

In addition to possible dietary modulation of the cholinergic system there are also herbal agents with relevant bioactivities. For example numerous plants have cholinesterase inhibitory activity (Houghton et al. 2006). Some of these such as sage (*Salvia officinalis*), lemon balm (*Melissa officinalis*) and kampo herbal remedies (e.g. Yokukansan, which is composed of seven herbs) improve cognition, sleep and mood in healthy adults (Kennedy et al. 2003; Tildesley et al. 2003, 2005; Scholey et al. 2008; de Caires and Steenkamp 2010). As these herbs are without adverse side effects at standard doses, they may be worthy of consideration in treatment of autism spectrum disorders.

Although the laboratory-based work on the cholinergic deficit in ASD largely derives from animal studies, the limited number of neuropathological studies

conducted on human postmortem brain tissue of autistic adults has broadened our understanding of the clinical relevance of both muscarinic and nicotinic receptor abnormalities in autism. Furthermore, these studies have also resulted in further application of the pharmacological potential, of cholinesterase inhibitors, and further development of similar novel pharmacological agents that can correct cognitive and behavioral abnormalities in ASD.

References

Abdallah MW, Mortensen EL, Greaves-Lord K, Larsen N, Bonefeld-Jørgensen EC, Nørgaard-Pedersen B, Hougaard DM, Grove J (2012) Neonatal levels of neurotrophic factors and risk of autism spectrum disorders. Acta Psychiatr Scand 128:61–69

Abrams P, Andersson KE, Buccafusco JJ, Chapple C, de Groat WC, Fryer AD, Kay G, Laties A, Nathanson NM, Pasricha PJ, Wein AJ (2006) Muscarinic receptors: their distribution and function in body systems, and the implications for treating overactive bladder. Br J Pharmacol 148:565–578

Acevido NL, Muralidharan B, Paadala N, To J, Jonakait GM (2007) Toll-like receptor ligands and CD154 stimulate microglia to produce a factor(s) that promote excess cholinergic differentiation in the developing rat basal forebrain: implications for neurodevelopmental disorders. Pediatr Res 61:15–20

Adams CE, Yonchek JC, Schulz KM, Graw SL, Stitzel J, Teschke PU, Stevens KE (2012) Reduced Chrna7 expression in mice is associated with decreases in hippocampal markers of inhibitory function: implications for neuropsychiatric diseases. Neuroscience 207:274–282

Ahmed AI, Ali AN, Kramers C, Härmark LV, Burger DM, Verhoeven WM (2013) Neuropsychiatric adverse events of varenicline: a systematic review of published reports. J Clin Psychopharmacol 33:55–62

Alkondon M, Albuquerque EX (2004) The nicotinic acetylcholine receptor subtypes and their function in the hippocampus and cerebral cortex. Prog Brain Res 145:109–120

Amenta F, Tayebati SK (2008) Pathways of acetylcholine synthesis, transport and release as targets for treatment of adult-onset cognitive dysfunction. Curr Med Chem 15:488–498

American Psychiatric Association (APA) (2000) Diagnostic and statistical manual of mental disorders, (4th ed., text revision). Washington, DC: American Psychiatric Association.

Anthenelli RM, Morris C, Ramey TS, Dubrava SJ, Tsilkos K, Russ C, Yunis C (2013) Effects of varenicline on smoking cessation in aduts with stably treated current or past major depression: a randomized control trial. Ann Int Med 159: 390–400

Arnold LE, Aman MG, Hollway J, Hurt E, Bates B, Li X, Farmer C, Anand R, Thompson S, Ramadan Y, Williams C (2012) Placebo-controlled pilot trial of mecamylamine for treatment of autism spectrum disorders. J Child Adolesc Psychopharmacol 22:198–205

Arnold LE, Anand R, Aman M (2013) Varenicline in autistic disorder: hypothesis and case report of single-patient crossover. J Child Adolesc Psychopharmacol 23:61–64

Auld DS, Kornecook TJ, Bastianetto S, Quirion R (2002) Alzheimer's disease and the basal forebrain cholinergic system: Relations to beta-amyloid peptides, cognition, and treatment strategies. Prog Neurobiol 68:209–245

Aylward EH, Minshew NJ, Field K, Sparks BF, Singh N (2002) Effects of age on brain volume and head circumference in autism. Neurology 59:175–183

Bailey CD, De Biasi M, Fletcher PJ, Lambe EK (2010) The nicotinic acetylcholine receptor alpha5 subunit plays a key role in attention circuitry and accuracy. J Neurosci 30:9241–9252

Bailey CD, Alves NC, Nashmi R, De Biasi M, Lambe EK (2012) Nicotinic α5 subunits drive developmental changes in the activation and morphology of prefrontal cortex layer VI neurons. Biol Psychiatry 71:120–128

Baulac S, Huberfeld G, Gourfinkel-An I, Mitropoulou G, Beranger A, Prud'homme JF, Baulac M, Brice A, Bruzzone R, LeGuerm E (2001) First genetic evidence of GABA9A receptor dysfunction in epilepsy: a mutation in the gamma-2-subunit gene. Nat Genet 28:46–48

Bauman ML, Kemper TL (1994) Neuroanatomical observations of the brain in autism. In: Bauman ML, Kemper TL (eds) The Neurobiology of Autism . The Johns Hopkins University Press, Baltimore pp 119–145.

Bernardi S, Anagnostou E, Shen J, Kolevzon A, Buxbaum JD, Hollander E, Hof PR, Fan J (2011) In vivo 1H-magnetic resonance spectroscopy study of the attentional networks in autism. Brain Res 1380:198–205

Blumberg SJ, Bramlett MD, Kogan MD, Schive LA, Jones JR, Lu MC (2013) Changes in prevalence of parent-reported autism spectrum disorder in school-aged US children: 2007 to 2011–2012. Nat Heath Stat Reports 65:1–12

Brugha TS, McManus S, Bankart J, Scott F, Purdon S, Smith J, Bebbington P, Jenkins R, Meltzer H (2011) Epidemiology of autism spectrum disorders in adults in the community in England. Arch Gen Psychiatry 68:459–465

Buckley AW, Sassower K, Rodriguez AJ, Jennison K, Wingert K, Buckley J, Thurm A, Sato S, Swedo S (2011) An open label trial of donepezil for enhancement of rapid eye movement sleep in young children with autism spectrum disorders. J Child Adolesc Psychopharmacol 21:353–357

Buiter HJ, Windhorst AD, Huisman MC, Yaqub M, Knol DL, Fisher A, Lammertsma AA, Leysen JE (2013) [11C]AF150(S), an agonist PET ligand for M1 muscarinic acetylcholine receptors. EJNMMI Res 23:19. doi:10.1186/2191–219X-3-19

Cavarsan CF, Avanzi RD, Queiroz CM, Xavier GF, Mello LE, Covolan L (2011) M1 acetylcholine receptor expression is decreased in hippocampal CA1 region of aged epileptic animals. Aging Dis 2:301–307

Centers for Disease Control and Prevention (CDCP) (2012) Prevalence of autism spectrum disorders—autism and developmental disabilities monitoring network, 14 sites. United States, 2008. MMWR 61:1–19

Cheng SB, Amici SA, Ren XQ, McKay SB, Treuil MW, Lindstrom JM, Rao J, Anand R (2009). Presynaptic targeting of alpha4beta 2 nicotinic acetylcholine receptors is regulated by neurexin-1beta. J Biol Chem 284:23251–23259

Chez MG, Buchanan TM, Becker M, Kessler J, Aimonovitch MC, Mrazek SR (2003) Donepezil hydrochloride: a double-blind study in autistic children. J Pediatric Neurol 1:83–88

Chez MG, Aimonovitch M, Buchanan T, Meazek S, Tremb RJ (2004) Treating autistic spectrum disorders in children: utility of the cholinesterase inhibitor rivastigmine tartrate. J Child Neurol 19:165–169

Chilian B, Abdollahpour H, Bierhals T, Haltrich I, Fekete G, Nagel I, Rosenberger G, Kutsche K (2013) Dysfunction of SHANK2 and CHRNA7 in a patient with intellectual disability and language impairment supports genetic epistasis of the two loci. Clin Genet 84:560–565

Chung YC, Lee CR, Park TW, Yang KH, Kim KW (2009) Effect of donepezil added to atypical antipsychotics on cognition in patients with schizophrenia: an open-label trial. World J Biol Psychiatry 10:156–162

Constantino JN (2011) The quantitative nature of autistic social impairment. Pediatric Res 69:55–62

Courchesne E, Karus CM, Davis HR, Ziccardi R, Carper RA, Tigue ZD, Chisum HJ, Moses P, Pirce K, Lord C, Lincoln AJ, Pizzo S, Schreibman L, Haas RH, Akshoomoff NA, Courchesne RY (2001) Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. Neurology 57:245–253

Croen LA, Goines P, Braunschweig D, Yolken R, Yoshida CK, Grether JK, Fireman B, Kharrazi M, Hansen RL, Van de Water J (2008) Brain-derived neurotrophic factor and autism: maternal and infant peripheral blood levels in the Early Markers for Autism (EMA) Study. Autism Res 1:130–137

Cubells JF, Deoreo EH, Harvey PD, Garlow SJ, Garber K, Adam MP, Martin CL (2011) Pharmacogenetically guided treatment of recurrent rage outbursts in an adult male with 15q13.3 deletion syndrome. Am J Med Genet A 155:805–810

Culp DJ, Luo W, Richardson LA, Watson GE, Latchney LR (1996) Both M1 and M3 receptors regulate exocrine secretion by mucous acini. Am J Physiol Cell Physiol 271:C1963–C1972

de Caires S, Steenkamp V (2010) Use of Yokukansan (TJ-54) in the treatment of neurological disorders: a review. Phytother Res 24:1265–1270

De Vitto TJ Drost DJ Neufeld RWJ Rajakumar N Pavlosky W Williamson P Nicolson R (2007) Evidence for cortical dysfunction in autism: a proton magnetic resonance spectroscopic imaging study. Biol Psychiatry 61:465–473

Deng C, Huang XF (2005) Decreased density of muscarinic receptors in the superior temporal gyrus in schizophrenia. J Neurosci Res 81:883–890

Deutsch SI, Urbano MR, Burket JA, Herndon AL, Winebarger EE (2011) Pharmacotherapeutic implications of the association between genomic instability at chromosome 15q13.3 and autism spectrum disorders. Clin Neuropharmacol 34:203–205

Dwyer JB, Broide RS, Leslie FM (2008) Nicotine and brain development. Brain Defects Res (PC) 84:30–44

Farlow M, Miller ML, Pejovic V (2008) Treatment options in Alzheimer's disease: maximizing benefit, managing expectations. Dement Geriatr Cogn Disord 25:408–422

Fatemi SH, Yousefi MK, Kneeland RE, Liesch SB, Folsom TD, Thiras PD (2013) Antismoking and potential antipsychotic effects of varenicline in subjects with schizophrenia or schizoaffective disorder: a double-blind placebo and buroprion-controlled study. Schizophr Res 146:376–378

Felder CC, Bymaster FP, Ward J, DeLapp N (2000) Therapeutic opportunities for muscarinic receptors in the central nervous system. J Med Chem 43:4333–4353

Fernandes CC, Pinto-Duartes A, Ribiero JA, Sebastião AM (2008) Postsynaptic action of brain-derived neurotrophic factor attenuates alpha7 nicotinic acetylcholine receptor-mediated responses in hippocampal interneurons. J Neurosci 21:5611–5618

Gabis L, Wei Huang, Azizian A, DeVincent C, Tudorica A, Kesner-Baruch Y, Roche P, Pomeroy J (2008) 1H-magnetic resonance spectroscopy markers of cognitive and language ability in clinical subtypes of autism spectrum disorders. J Child Neurol 23:766–774

Garcia KL, Yu G, Nicolini C, Michalski B, Garzon DJ, Chiu VS, Tongiorgi E, Szatmari P, Fahnestock M (2012) Altered balance of proteolytic isoforms of pro-brain-derived neurotrophic factor in autism. J Neuropathol Exp Neurol 71:289–297

Gaynor CM, Handley SL (2001) Effects of nicotine on head-shakes and tryptophan metabolites. Psychopharmacol 153:327–333

Ghaleiha A, Ghyasvand M, Mohammadi MR, Farokhnia M, Yadegari N, Tabrizi M, Hajiaghaee R, Yekehtaz H, Akhondzadeh S (2013) Galantamine efficacy and tolerability as an augmentative therapy in autistic children: a randomized, double-blind, placebo-controlled trial. J Psychopharmacol 28:677–685

Gibbons RD, Mann JJ (2013) Varenicline, smoking cessation, and neuropsychiatric adverse events. Am J Psychiatry 170:1–8

Gillberg C, Billstedt E (2000) Autism and Asperger syndrome: coexistence with other clinical disorders. Acta Psychiat Scand 102:321–330

Gotti C, Zoli M, Clementi F (2006) Brain nicotinic acetylcholine receptors: native subtypes and their relevance. Trends Pharmacol Sci 27:482–491

Granon S, Faure P, Changeux JP (2003) Executive and social behaviors under nicotinic receptor regulation. Proc Natl Acad Sci USA 100:9596–9691

Handen BL, Johnson CR, McAuliffe-Bellin S, Murray PJ, Hardan AY (2011) Safety and efficacy of donepezil in children and adolescents with autism: neuropsychological measures. J Child Adolesc Psychopharmacol 21:43–50

Hardan AY, Handen BL (2002) A retrospective open trial of adjunctive donepezil in children and adolescents with autistic disorder. J Child Adolesc Psychopharmacol 12:237–241

Hardan AY, Minshew NJ, Melhem NM, Srihari S, Jo B, Bansal R, Leshavan MS, Stanley JA (2008) An MRI and proton spectroscopy study of the thalamus in children with autism. Psychiatry Res 163:97–105

Hashimoto K, Iwata Y, Nakamura K, Tsujii M, Tsuchiya K, Sekine Y, Suzuki K, Minabe Y, Takei N, Iyo M, Mori N (2006) Reduced serum levels of brain-derived neurotrophic factor in adult male patients with autism. Prog Neuropsychophramacol Biol Psychiatry 30:1529–1531

Hazlett HC, Poe MD, Lightbody AA, Styner M, MacFall JR, Reiss AL, Piven J (2012) Trajectories of early brain volume development in fragile X syndrome and autism. J Am Acad Child Adolesc Psychiatry 51:921–933

Heller JH, Spiridigliozzi GA, Dorraiswamy PM, Sullivan JA, Crissman BG, Kishnani PS (2004) Donepezil effects on language in children with Down syndrome: results of the first 22-week pilot clinical trial. Am J Med Genet 130A:325–356

Heller JH, Spiridigliozzi GA, Crissman BG, Sullivan JA, Eelis RL, Li JS, Doraiswamy PM, Krishnan KR, Kishnani PS (2006) Safety and efficacy of rivastigmine in adolescents with down syndrome: a preliminary 20-week, open-label study. J Child Adolesc Psychopharmacol 16:755–765

Heller JH, Spiridigliozzi GA, Crissman BG, McKillop JA, Yamamoto H, Kishnani PS (2010) Safety and efficacy of rivastigmine in adolescents with Down syndrome: long-term follow-up. J Child Adolesc Psychopharmacol 20:517–520

Herbert MR, Ziegler DA, Makris N, Filipek PA, Kemper TL, Normandin JJ, Sanders HA, Kennedy DN, Caviness VS Jr (2004) Localization of white matter volume increase in autism and developmental language disorder. Ann Neurol 55:530–540

Hertzman M (2003) Galantamine in the treatment of adult autism: a report of three clinical cases. Int J Psychiatry Medicine 33:395–398

Hillmer AT, Wooten DW, Slesarev MS, Ahlers EO, Barnhart TE, Murali D, Schneider ML, Mukherjee J, Christian BT (2012) PET imaging of α4β2* nicotinic acetylcholine receptors: quantitative analysis of 18F-nifene kinetics in the nonhuman primate. J Nucl Med 53:1471–1480

Hillmer AT, Wooten DW, Farhoud M, Barnhart TE, Mukherjee J, Christian BT (2013) The effects of lobeline on α4β2* nicotinic acetylcholine receptor binding and uptake of [18F]nifene in rats. J Neurosci Methods 214:163–169

Hohmann CF, Berger-Sweeney J (1998) Cholinergic regulation of cortical development and plasticity: new twists to an old story. Perspect Dev Neurobiol 5:401–425

Hohnadel E, Bouchard K, Terry AV Jr (2007) Galantamine and donepezil attenuate pharmacologically induced deficits in prepulse inhibition in rats. Neuropharmacology 52:542–551

Houghton PJ, Ren Y, Howes MJ (2006) Acetylcholinesterase inhibitors from plants and fungi. Nat Prod Rep 23:181–199

Howson AL, Batth S, Ilivitsky V, Boisjoli A, Jaworski M, Mahoney C, Knott VJ (2004) Clinical and attentional effects of acute nicotine treatment in Tourette's syndrome. Eur Psychiatry 19:102–112

Huh CYL, Danik M, Manseau F, Trudeau L-E, Williams S (2008) Chronic exposure to nerve growth factor increases acetylcholine and glutamate release from cholinergic neurons of the rat medial septum and diagonal band of Broca via Mechanisms mediated by p75NTR. J Neurosci 28:1404–1409

Jamain S, Betancur C, Quach H, Philippe A, Fellous M, Giros B, Gillberg C, Leboyer M, Bourgeron T, Study PARISP (2002) Linkage and association of the glutamate receptor 6 gene with autism. Mol Psychiatry 7:302–310

James SJ, Pauly M, Melnyk S, Stewart PA, Schmidt BL, Lemcke N, Reynolds AM, Molloy CA, Johnson C, Clemons T, Hyman SL (2011) Dietary choline intake by children with autism is below the recommended dietary reference intake (DRI) established by the IOM. International meeting for autism research: dietary choline intake by children with autism is below the recommended dietary reference intake (DRI) established by the IOM INSAR meeting, 11 May 2011

Kang K, Huang XF, Wang Q, Deng C (2009) Decreased density of serotonin 2A receptors in the superior temporal gyrus in schizophrenia—a postmortem study. Prog Neuropsychopharmacol Biol Psychiatry 33:867–871

Karvat G, Kimchi T (2013) Acetylcholine elevation relieves cognitive rigidity and social deficiency in a mouse model of autism. Neuropsychopharmacology 39:831–840

Katoh-Semba R, Wakako R, Komori T, Shigemi H, Miyazaki N, Ito H, Kumagai T, Tsuzuki M, Shigemi K, Yoshida F, Nakayama A (2007) Age-related changes in BDNF protein levels in human serum: differences between autism cases and normal controls. Int J Dev Neurosci 25:367–372

Kawai H, Zago W, Berg DK (2002) Nicotinic α7 receptor clusters on hippocampal GABAergic neurons: regulation by synaptic activity and neurotrophins. J Neurosci 22:7903–7912

Kawashima K, Fujii T, Moriwaki Y, Misawa H, Horiguchi K (2012) Reconciling neuronally and nonneuronally derived acetylcholine in the regulation of immune function. Ann NY Acad Sci 1261:7–17

Kennedy DO, Wake G, Savelev S, Tildesley NT, Perry EK, Wesnes KA, Scholey AB (2003) Modulation of mood and cognitive performance following acute administration of single doses of Melissa officinalis (Lemon balm) with human CNS nicotinic and muscarinic receptor-binding properties. Neuropsychopharmacology 28:1871–1881

Kishnani PS, Heller JH, Spiridigliozzi GA, Lott I, Escobar L, Richardson S, Zhang R, McRae T (2010) Donepezil for treatment of cognitive dysfunction in children with Down syndrome aged 10–17. Am J Med Genet A 152A:3028–3035

Koshimizu H, Leiter LM, Miyakawa T (2012) M4 muscarinic receptor knockout mice display abnormal social behavior and decreased prepulse inhibition. Mol Brain 5:10

Krasnoperova MG, Simashkova NV, Bashina VM (2004) Use of cholinomimetics in the treatment of endogeneous autism in children. Zh Nevrol Psikhiatr Im S S Korsakova 104:35–39

Kroker KS, Rast G, Rosenbrock H (2011) Differential effects of subtype-specific nicotinic acetylcholine receptor agonists on early and late hippocampal LTP. Eur J Pharmacol 671:26–32

Lamping KG, Wess J, Cui Y, Nuno DW, Faraci FM (2004) Muscarinic (M) receptors in coronary circulation: gene-targeted mice define the role of M2 and M3 receptors in response to acetylcholine. Arterioscler Thromb Vasc Biol 24:1253–1258

Lauder JM, Schambra UB (1999) Morphogenetic roles of acetylcholine. Environ Health Perspect 107 (suppl 1):65–69

Lavine N, Reuben M, Clarke PB (1997) A population of nicotinic receptors is associated with thalamocortical afferents in the adult rat: laminal and areal analysis. J Comp Neurol 380:175–190

Le Couteur A Bailey A Goode S Pickles A Robertson S Gottesman I Rutter M (1996) A broader phenotype of autism: the clinical spectrum in twins. J Child Psychol Psychiatry 37:785–801

Lee M, Martin-Ruiz CM, Graham AJ, Court JA, Jaros E, Perry RH, Iverson P, Bauman ML, Perry EK (2002) Nicotinic receptor abnormalities in the cerebellar cortex in autism. Brain 125:1483–1495

Levin ED, Caldwell DP (2006) Low-dose mecamylamine improves learning of rats in the radial-arm maze repeated acquisition procedure. Neurobiol Learn Mem 86:117–122

Levinson DF, Shi J, Wang K, Oh S, Riley B, Pulver AE, Wildenauer DB, Laurent C, Mowry BJ, Gejman PV, Owen MJ, Kendler KS, Nestadt G, Schwab SG, Mallet J, Nertney D, Sanders AR, Williams NM, Wormley B, Lasseter VK, Albus M, Godard-Bauché S, Alexander M, Duan J, O'Donovan MC, Walsh D, O'Neill A, Papadimitriou GN, Dikeos D, Maier W, Lerer B, Campion D, Cohen D, Jay M, Fanous A, Eichhammer P, Silverman JM, Norton N, Zhang N, Hakonarson H, Gao C, Citri A, Hansen M, Ripke S, Schizophrenia Psychiatric GWAS Consortium, Dudbridge F, Holmans PA (2012) Genome-wide association study of multiplex schizophrenia pedigrees. Am J Psychiatry 169:963–973

Levy RB, Reyes AD, Aoki C (2008) Chollinergic modulation of local pyramid-interneuron synapses exhibiting divergent short-term dynamics in rat sensory cortex. Brain Res 1215:97–104

Lewine JD, Andrews R, Chez M, Patil AA, Devinsky O, Smith M, Kanner A, Davis JT, Funke M, Jones G, Chong B, Provencal S, Weisend M, Lee RR, Orrison WW Jr (1999) Magnetoencephalographic patterns of epileptiform activity in children with regressive autism spectrum disorders. Pediatrics 104:405–418

Lippiello PM (2005) Nicotinic cholinergic antagonists: a novel approach for the treatment of autism. Med Hypotheses 66:985–990

Mancama D, Arranz MJ, Landau S, Kerwin R (2003) Reduced expression of the muscarinic 1 receptor cortical subtype in schizophrenia. Am J Med Genet B Neuropsychiatr Genet 119B:2–6
Mariani J (1982) Extent of multiple innervation of Purkinje cells by climbing fibres in the olivocerebellar system of weaver, reeler, and staggerer mutant mice. J Neurobiol 13:119–126
Martin-Ruiz CM, Lee M, Perry RH, Bauman ML, Court JA, Perry EK (2004) Molecular analysis of nicotinic receptor expression in autism. Brain Res Brain Mol Res 123:81–90
Massey KA, Zago WM, Berg DK (2006) BDNF up-regulates α7 nicotinic acetylcholine receptor levels on subpopulations of hippocampal interneurons. Mol Cell Neurosci 33:8
Matsui M, Motomura D, Karasawa H, Fujikawa T, Jiang J, Komiya Y, Takahashi S, Taketo MM (2000) Multiple functional defects in peripheral autonomic organs in mice lacking muscarinic acetylcholine receptor gene for the M3 subtype. Proc Natl Acad Sci USA 97:9579–9584
Metherte R (2004) Nicotinic acetylcholine receptors in sensory cortex. Learn Memory 11:50–59
Mills Schumann C Hamstra J Goodlin-Jones BL Lotspeich LJ Kwon H Buonocore MH Lammers CR Reiss AL Amaral DG (2004) The amygdala is enlarged in children but not adolescents with autism; the hippocampus is enlarged at all ages. J Neurosci 24:6392–6401
Mills JL, Hediger ML, Molloy CA, Chrousos GP, Manning-Courtney P, Yu KF, Brasington M, England LJ (2007) Elevated levels of growth-related hormones in autism and autism spectrum disorder. Clin Endocrinol 67:230–237
Minshew NJ, Sweeney JA, Bauman M (1997) Neurological aspects of autism. In: Cohen D, Volkmar F (eds) Handbook of autism and pervasive developmental disorders. Wiley, New York, pp 344–369
Möller JC, Eggert KM, Unger M, Odin P, Chaudhuri KR, Oertel WH (2008) Clinical risk-benefit assessment of dopamine agonists. Eur J Neurol 15(S2):15–23
Mowla A, Mosavinasab M, Haghshenas H, Borhani Haghighi A (2007) Does serotonin augmentation have any effect on cognition and activities of daily living in Alzheimer's disease? A double-blind, placebo-controlled clinical trial. J Clin Psychopharmacol 27:484–487
Mukaetova-Ladinska EB, Arnold H, Jaros E, Perry R, Perry E (2004) Depletion of MAP2 expression and laminar cytoarchitectonic changes in dorsolateral prefrontal cortex in adult autistic individuals. Neurobiol Appl Neurol 30:615–623
Mukaetova-Ladinska EB, Westwood J, Perry EK (2010) Cholinergic components in autism spectrum disorder. In: Blatt E (ed) The neurochemical basis autism. From Molecules to Minicolumns. Springer, Heidelberg, pp. 129–161
Mukaetova-Ladinska EB, Schaffer W, Bronnikova TV, Westwood J, Perry EK (2012) Cholinergic therapy for autistic spectrum disorder. In: White CJ, Tait JE (eds) Cholinesterase: production, uses and health effects. Nova Science, New York, pp 33–66
Mulley JC, Mefford HC (2011) Epilepsy and the new cytogenetics. Epilepsia 52:423–432
Murphy M, Bolton PF, Pickles A, Fombonne E, Piven J, Rutter M (2000) Personality traits of the relatives of autistic probands. Psychol Med 30:1411–1424
Nathanson NM (2008) Synthesis, trafficking, and localization of muscarinic acetylcholine receptors. Pharmacol Ther 119:33–43
Nelson KB, Grether JK, Croen LA, Dambrosia JM, Dickens BF, Hansen RL, Phillips TM (2000) Neuropeptides and neurotrophins in neonatal blood of children with autism, mental retardation and cerebral palsy. Neurology 54:A247
Nelson PG, Kuddo T, Song EY, Dambrosia JM, Kohler S, Satyanarayana G, Vandunk C, Grether JK, Nelson KB (2006) Selected neurotrophins, neuropeptides, and cytokines: developmental trajectory and concentrations in neonatal blood of children with autism or Down syndrome. Int J Dev Neurosci 24:73–80
Nicolson R, Craven-Thuss B, Smith J (2006) A prospective, open-label trial of galantamine in autistic disorder. J Child Adolesc Psychopharmacol 16:621–629
Niederhofer H, Staffen W, Mair A (2002) Galantamine may be effective in treating autistic disorder. BMJ 32:1422
Palma E, Conti L, Roseti C, Limatola C (2012) Novel approaches to study the involvement of α7-nAChR in human diseases. Curr Drug Targets 13:579–586

Palmen SMJC, Hulshoff Pol HE, Kemner C, Schnack HG, Durston S, Lahuis BE, Kahn RS, van Engeland H (2004) Increased gray-matter volume in medication-naive high-functioning children with autism spectrum disorder. Psychol Med 34:1–10

Pandey SK, Pan S, Kant R, Kuruvilla SA, Pan ML, Mukherjee J (2012) Synthesis and evaluation of 3-^{123}I-iodo-5-[2-(S)-3-pyrrolinylmethoxy]-pyridine (niodene) as a potential nicotinic α4β2 receptor imaging agent. Bioorg Med Chem Lett 22:7610–7614

Pennell PB, Burdette DE, Ross DA, Henry TR, Albin RL, Sackellares JC, Frey KA (1999) Muscarinic receptor loss and preservation of presynaptic cholinergic terminals in hippocampal sclerosis. Epilepsia 40:38–46

Pepeu G, Giovannini MG (2004) Changes in acetylcholine extracellular levels during cognitive processes. Learn Memory 11:21–27

Perry EK, Lee M, Martin-Ruiz CM, Court JA, Volsen SG, Merrit J, Folly E, Iversen PE, Bauman ML, Perry RH, Wenk GL (2001) Cholinergic activity in autism: abnormalities in the cerebral cortex and basal forebrain. Am J Psychiatry 158:1058–1066

Petersen AK, Ahmad A, Shafiq M, Brown-Kipphut B, Fong CT, Anwar Iqbal M (2013) Deletion 1q43 encompassing only CHRM3 in a patient with autistic disorder. Eur J Med Genet 56:118–122

Pickles A, Starr E, Kazak S, Bolton P, Papanikolaou K, Bailey A, Goodman R, Rutter M (2000) Variable expression of the autism broader phenotype: findings from extended pedigrees. J Child Psychol Psychiatry 41:491–502

Ragozzino ME, Artis S, Singh A, Twose TM, Beck JE, Messer WS Jr (2012) The selective M1 muscarinic cholinergic agonist CDD-0102A enhances working memory and cognitive flexibility. J Pharmacol Exp Ther 340:588–594

Ray MA, Graham AJ, Lee M, Perry RH, Court JA, Perry EK (2005) Neuronal nicotinic acetylcholine receptor subunits in autism: an immunohistochemical investigation in the thalamus. Neurobiol Dis 19:366–377

Ribeiz SR, Bassitt DP, Arrais JA, Avila R, Steffens DC, Bottino CM (2010) Cholinesterase inhibitors as adjunctive therapy in patients with schizophrenia and schizoaffective disorder: a review and meta-analysis of the literature. CNS Drugs 24:303–317

Riley B, Williamson M, Collier D, Wilkie H, Makoff A (2002) A 3-Mb map of a large Segmental duplication overlapping the alpha7-nicotinic acetylcholine receptor gene (CHRNA7) at human 15q13-q14. Genomics 79:197–209

Rötering S, Scheunemann M, Fischer S, Hiller A, Peters D, Deuther-Conrad W, Brust P (2013). Radiosynthesis and first evaluation in mice of [(18)F]NS14490 for molecular imaging of α7 nicotinic acetylcholine receptors. Bioorg Med Chem 21:2635–2642

Rubenstein JL, Merzenich MM (2003) Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav 2:255–267

Sadakata T, Shinoda Y, Oka M, Sekine Y, Sato Y, Saruta C, Miwa H, Tanaka M, Itohara S, Furuichi T (2012) Reduced axonal localization of a Caps2 splice variant impairs axonal release of BDNF and causes autistic-like behavior in mice. Proc Natl Acad Sci U S A 109:21104–21109

Scarr E, Sundram S, Keriakous D, Dean B (2007) Altered hippocampal muscarinic M4, but not M1, receptor expression from subjects with schizophrenia. Biol Psychiatry 61:1161–1170

Scholey AB, Tildesley NT, Ballard CG, Wesnes KA, Tasker A, Perry EK, Kennedy DO (2008) An extract of Salvia (sage) with anticholinesterase properties improves memory and attention in healthy older volunteers. Psychopharmacology (Berl) 198:127–139

Sheikh AM, Malik M, Wen G, Chauhan A, Chauhan V, Gong CX, Liu F, Brown WT, Li X (2010) BDNF-Akt-Bcl2 antiapoptotic signaling pathway is compromised in the brain of autistic subjects. J Neurosci Res 88:2641–2647

Shirey JK, Brady AE, Jones PJ, Davis AA, Bridges TM, Kennedy JP, Jadhav SB, Menon UN, Xiang Z, Watson ML, Christian EP, Doherty JJ, Quirk MC, Snyder DH, Lah JJ, Levey AI, Nicolle MM, Lindsley CW, Conn PJ (2009) A selective allosteric potentiator of the M1 muscarinic acetylcholine receptor increases activity of medial prefrontal cortical neurons and restores impairments in reversal learning. J Neurosci 29:14271–14286

Shuang M, Liu J, Jia MX, Yang JZ, Wu SP, Gong XH, Ling YS, Ruan Y, Yang XL, Zhang D (2004) Family-based association study between autism and glutamate receptor 6 gene in Chinese Han trios. Am J Med Genetics 131:48–50

Shytle RD, Silver AA, Philipp MK, McConville BJ, Sanberg PR (1998) Transdermal nicotine for Tourette's syndrome. Drug Dev Res 38:290–298

Spiridigliozzi GA, Heller JH, Crissman BG, Sullivan-Saarela JA, Eelis R, Dawson D, Li J, Kishnani PS (2007) Preliminary study of safety and efficacy of donepezil hydrochloride in children with Down syndrome: a clinical report series. Am J Med Genet A 143A:1408–1413

Srivastava RK, Agarwal M, Pundhir A (2011) Role of dopenezil in autism: its conductiveness in psychopharmacology. Case reports in psychiatry ID563204

Steinlein OK, Bertrand D (2008) Neuronal nicotinic acetylcholine receptors: from the genetic analysis to neurological diseases. Biochem Pharmacol 76:1175–1183

Stuart-Hamilton I, Griffith G, Totsika V, Nash S, Hastings RP, Felce D, Kerr M (2009) The circumstances and support needs of older people with Autism. Report for the Welsh Assembly Government, Welsh Assembly, Cardiff

Sylvester Vizi E, Rózsa B, Mayer A, Kiss JP, Zelles T, Lendvai B (2004) Further evidence for the functional role of nonsynaptic nicotinic acetylcholine receptors. Eur J Pharmacol 500:499–508.

Taly A, Corringer P-J, Guedin D, Lestage P, Changeux J-P (2009) Nicotinic receptors: allosteric transitions and therapeutic targets in the nervous system. Nat Rev Drug Discov 8:733–750

Tata AM, Cursi S, Biagioni S, Augusti-Tocco G (2003) Cholinergic modulation of neurofilament expression and neurite outgrowth in chick sensory neurons. J Neurosci Res 73:227–234

Tildesley NT, Kennedy DO, Perry EK, Ballard CG, Savelev S, Wesnes KA, Scholey AB (2003) Salvia lavandulaefolia (Spanish sage) enhances memory in healthy young volunteers. Pharmacol Biochem Behav 75:669–674

Tildesley NT, Kennedy DO, Perry EK, Ballard CG, Wesnes KA, Scholey AB (2005) Positive modulation of mood and cognitive performance following administration of acute doses of Salvia lavandulaefolia essential oil to healthy young volunteers. Physiol Behav 83:699–709

Torrao AS, Lindstrom JM, Britto LR (2003) Nicotine and alpha-bungarotoxin modify the dendritic growth of cholinoceptive neurons in the developing chick tectum. Brain Res Dev Brain Res 143:115–118

Vasconcelos MM, Brito AR, Domingues RC, da Cruz LC, Gasparetto EL, Werner J, Gonc Alves JP (2008) Proton magnetic resonance spectroscopy in school-aged autistic children. J Neuroimaging 18:288–295

Vilaró MT, Palacios JM, Mengod G (1990) Localization of m5 muscarinic receptor mRNA in rat brain examined by in situ hybridization histochemistry. Neurosci Letts 114:154–159

Walker BR, Diefenbach KS, Parikh TN (2007) Inhibition within the nucleus tractus solitarius (NTS) ameliorates environmental exploration deficits due to cerebellum lesions in an animal model for autism. Behav Brain Res 176:109–120

Wheless JW, Simos PG, Butler IJ (2002) Language dysfunction in epileptic conditions. Semin Pediatr Neurol 9:218–228

White SW, Oswald D, Ollendick T, ScahillL (2009) Anxiety in children and adolescents with autism spectrum disorders. Clin Psychol Rev 29:216–229

Wiker C, Schilstrom B, Sandback C, Wadenberg ML, Svensson TH (2008) Adjunctive galantamine, but not donepezil, enhances the antipsychotic-like effect of raclopride in rats. Int J Neuropsychopharmacol 14:1–6

Williams NM, Franke B, Mick E, Anney RJ, Freitag CM, Gill M, Thapar A, O'Donovan MC, Owen MJ, Holmans P, Kent L, Middleton F, Zhang-James Y, Liu L, Meyer J, Nguyen TT, Romanos J, Romanos M, Seitz C, Renner TJ, Walitza S, Warnke A, Palmason H, Buitelaar J, Rommelse N, Vasquez AA, Hawi Z, Langley K, Sergeant J, Steinhausen HC, Roeyers H, Biederman J, Zaharieva I, Hakonarson H, Elia J, Lionel AC, Crosbie J, Marshall CR, Schachar R, Scherer SW, Todorov A, Smalley SL, Loo S, Nelson S, Shtir C, Asherson P, Reif A, Lesch KP, Faraone SV (2012) Genome-wide analysis of copy number variants in attention deficit hyperactivity disorder: the role of rare variants and duplications at 15q13.3. Am J Psychiatry 169:195–204

Wonnacott, S, Sidhpura N, Balfour DJ (2005) Nicotine: from molecular mechanisms to behaviour. Curr Opinion Pharmacol 5:53–59

Yasui DH, Scoles HA, Horike S, Meguro-Horike M, Dunaway KW, Schroeder DI, Lasalle JM (2011) 15q11.2-13.3 chromatin analysis reveals epigenetic regulation of CHRNA7 with deficiencies in Rett and autism brain. Hum Mol Genet 20:4311–4323

Yoo JH, Valdovinos MG, Williams DC (2007) Relevance of donepezil in enhancing learning and memory in special populations: a review of the literature. J Autism Dev Disord 37:1883–1901

Zeisel SH (1986) Dietary influences on neurotransmission. Adv Pediatr 33:23–47

Chapter 16
Reelin, GABA, FMRP, and Autism

Timothy D. Folsom and S. Hossein Fatemi

Abstract Autism is a heterogeneous neurodevelopmental disorder. The etiology of autism remains unknown although both genetic and environmental factors are likely to be involved. These factors disrupt the course of normal brain development from the cellular to the gross anatomical levels. The Reelin, gamma-aminobutyric acid (GABA), and fragile X mental retardation protein (FMRP)—metabotropic glutamate receptor 5 (mGluR5) signaling systems play important roles during the development of the nervous system. Disruption of these pathways is likely to lead to altered synaptic transmission and, ultimately, the cognitive and behavioral deficits associated with autism. This chapter describes each of these signaling systems and summarizes the current evidence that link them to autism. Therapies that target molecules in these signaling systems may provide new means of treating the core symptoms of autism.

Keywords Reelin · GABA · FMRP · mGluR5 · Brain

Autism is a pervasive, debilitating, neurodevelopmental disorder characterized by three core symptoms: (1) abnormal social interaction; (2) impaired verbal and nonverbal communication; and (3) the presence of restrictive, repetitive or stereotyped behaviors (APA 2013). Currently, the prevalence of autism is rising in the United States with a rate of 14.7 per 1000 (1 in 68) for children aged 8 years old (CDC 2014). Individuals with autism often display a number of comorbidities including seizure disorder and intellectual impairment (Canitano 2007; Chakrabarti and Fombonne 2005). This chapter reviews the current evidence for dysfunction of the Reelin, GABAergic, and the FMRP-mGluR5 signaling pathways in autism. During development, the Reelin signaling system is instrumental in proper neuronal migration and brain lamination (Frotscher 1998; D'Arcangelo et al. 1995). In adults, Reelin is expressed in GABAergic interneurons (Pesold et al. 1998) and is involved in synapse formation and plasticity (reviewed by Stranahan et al. 2013).

S. H. Fatemi (✉)
Departments of Psychiatry and Neuroscience, University of Minnesota Medical School, 420 Delaware St SE, MMC 392, Minneapolis, MN 55455, USA
e-mail: fatem002@umn.edu

T. D. Folsom
Department of Psychiatry, University of Minnesota School of Medicine, Minneapolis, MN, USA

S. Hossein Fatemi (ed.), *The Molecular Basis of Autism,*
Contemporary Clinical Neuroscience, DOI 10.1007/978-1-4939-2190-4_16

GABA is the most common inhibitory neurotransmitter in the brain. Disruption of the excitatory/inhibitory balance in the brain resulting from perturbations to this system may lead to the presence of seizures, abnormal information processing, and cognitive dysfunction associated with autism. Fragile X syndrome (FXS) is one of the most common forms of mental retardation and is often comorbid with autism. Behavioral deficits such as social anxiety, gaze avoideance, hyperarousal to sensory stimuli are common to both autism and FXS. In this chapter, we review current data from gene association and postmortem studies which implicate these three signaling systems in the pathology of autism.

16.1 Reelin and Autism

Reelin is a secreted extracellular matrix glycoprotein (DeBergeyck et al. 1998) with multiple functions during brain development including mediating proper brain lamination (Boyle et al. 2011; Hamburgh 1963; Tissir and Goffinet 2003) and facilitation of neuronal cell migration (D'Arcangelo et al. 1995; Hadj-Sahraoui et al. 1996). In adults, Reelin signaling is involved in synapse formation and modulation of synaptic transmission (Beffert et al. 2005; Chen et al. 2005; Groc et al. 2007; Herz and Chen 2006; Qiu and Weeber 2007; Ventruti et al. 2011; Weeber et al. 2002). Full length Reelin has a molecular weight of 410 kDa, while several cleavage products including Reelin 330 kDa, Reelin 180 kDa, and other fragments including a 220 kDa as well as 100 kDa C-terminal entity can be identified using SDS-PAGE (Jossin 2008; Fatemi et al. 2005a,b; Ignatova et al. 2004; Lambert de Rouvroit et al. 1999; Smalheiser et al. 2000). Reeler mice, which lack Reelin expression, display deficits in long term potentiation (Marrone et al. 2006) as do heterozygous Reeler mice (HRM) which are characterized by an approximately 50 % reduction in normal levels of Reelin (Tueting et al. 2006; Qiu and Weeber, 2007). These mouse strains also display behavioral deficits relevant to autism including impaired executive function, increased anxiety and motor impulsivity, impaired fear-conditioned learning, and deficits in sensorimotor gating behavior as measured by prepulse inhibition (PPI) (Ammassari-Teule et al. 2009; Barr et al. 2008; Ognibene et al. 2007; Qiu and Weeber, 2007; Tueting et al. 1999). It should be noted, however, that other groups have found no difference in PPI between HRM and wild type mice (Barr et al. 2008; Podhorna and Didriksen 2004; Teixeira et al. 2011).

Reelin binds to three receptors: very low density lipoprotein receptor (VLDLR), apolipoprotein E receptor 2 (APOER2) (D'Arcangelo et al. 1999) and α3β1 integrin. Experiments involving Vldlr and Apoer2 knockout (KO) mice have demonstrated differing roles for each receptor with regard to neuronal migration with APOER2 enabling migration while Reelin binding to VLDLR may cease neuronal migration (Hack et al. 2007). Associated with APOER2 and VLDLR are ephrin B proteins (EFNBs) (Sentürk et al. 2011; Bouché et al. 2013). Reelin binding results in clustering of APOER2, VLDLR, and EFNBs and activation of FYN tyrosine kinase (Hiesberger et al. 1999; Strasser et al. 2004) and promoting the phosphorylation of

disabled 1 (DAB1), a cytoplasmic adaptor protein (Hiesberger et al. 1999; Sentürk et al. 2011). Loss of function of EFNBs results in reduced phosphorylation of DAB1 and impaired neuronal migration (Sentürk et al. 2011). Phosphorylation of DAB1 activates a kinase cascade including phosphatidylinositol-3-kinase (PI3K) and protein kinase B (PKB/AKT) ultimately leading to the phosphorylation and inhibition of glycogen synthase kinase 3-beta (GSK3β) (Beffert et al. 2002; Hiesberger et al. 1999). Mouse embryos that express a nonphosphorylated Dab1 mutation display deficits in migration of sympathetic preganglionic neurons that is similar to what is observed in the Reeler mouse (Yip et al. 2007a). DAB1 phosphorylation also leads to the recruitment of the lissencephaly 1 (LIS1) complex which is important in both neuronal migration and proper cortical lamination (Assadi et al. 2003). GSK3β phosphorylates tau, a microtubule stabilizing protein (Hiesberger et al. 1999; Kwok et al. 2005). Inhibition of GSK3β leads to reduced phosphorylation of tau and allows for altered microtubule dynamics promoting neuronal cell migration. Figure 16.1 summarizes the Reelin signaling system.

Reelin contributes to synapse formation and modulation of synaptic transmission via regulation of Ca^{2+} entry through N-methyl-d-aspartate (NMDA) receptors (Fig. 16.1) (Beffert et al. 2005; Chen et al. 2005; Groc et al. 2007; Herz and Chen 2006; Qiu and Weeber 2007; Ventruti et al. 2011; Weeber et al. 2002). Synaptic transmission in rodent models is impaired by mutations involving Apoer2 (Beffert et al. 2005, 2006) or loss of Dab1 (Trotter et al. 2013). Moreover, loss or transient downregulation of Dab1 results in impairments of associative learning, memory deficits, and impaired sensory motor gating as measured by prepulse inhibition, all of which are relevant to the pathology of autism (Teixeira et al. 2014; Trotter et al. 2013).

Based on its role in brain development and synaptic transmission, the gene that codes Reelin (RELN) has been investigated as an autism candidate gene. An initial discovery found that an increase in the number of GGC triplet repeats in the 5′ untranslated region immediately before the RELN initiation codon, conferred vulnerability to the development of autism (Persico et al. 2001). RELN alleles with at least 11 triplet repeats in this region were preferentially transmitted to subjects with autism (Lugli et al. 2003; Persico et al. 2001). Subsequent studies in various population samples have verified an association between this region and autism (Dutta et al. 2007; Kelemenova et al. 2010; Skaar et al. 2005; Zhang et al. 2002). However, several other studies found no such association (Bonora et al. 2003; Devlin et al. 2004; Krebs et al. 2002; Li et al. 2004).

A number of variants and single nucleotide polymorphisms (SNPs) of RELN have also been associated with autism in various populations including g.504742G >A in a Han Chinese sample (Tian 2012); rs2073559 in a Caucasian population sample (Ashley-Koch et al. 2007); rs736707 from intron 59 and rs362691 from exon 22 in a Caucasian population sample (Serajee et al. 2006); rs736707 in a Han Chinese population sample (Li et al. 2008), a finding that was recently confirmed in a South African population sample (Sharma et al. 2013). However, other groups have found no association between SNPs of RELN, including the ones listed above, and autism. A study of six RELN SNPs (rs727531, rs2072403, rs2072402, rs362691, rs362719, rs736707) in an Indian population sample failed to find a sig-

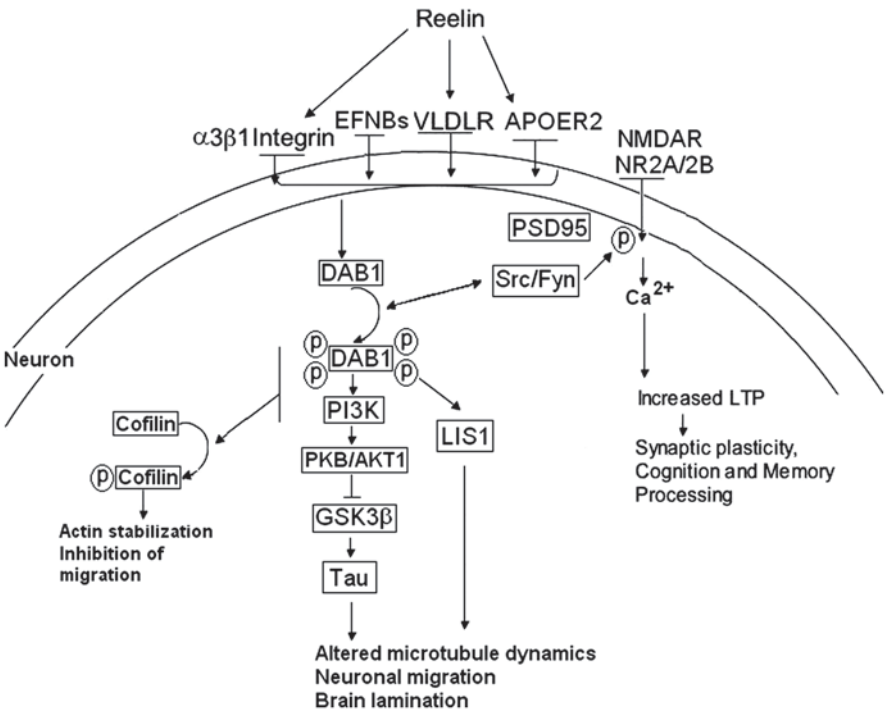


Fig. 16.1 The Reelin signaling cascade. Reelin binds to its extracellular receptors APOER2, VLDLR and α3β1 integrin resulting in clustering of the receptors and ephrin B proteins (ENFBs). This clustering recruits DAB1 proteins and activation of FYN kinase which phosphorylates DAB1. Phosphorylation of DAB1 leads to: (1) recruitment and activation of a kinase cascade involving PI3K and PKB/AKT1 which ultimately inhibits GSK3β; and (2) recruitment of LIS1 which promotes proper brain lamination. Inhibition of GSK3β results in dephosphorylation of tau which in turn destabilizes microtubule dynamics promoting neuronal migration. The binding of Reelin to its receptors and subsequent activation of FYN leads to phosphorylation of NMDA receptor subunits NR2A and NR2B. As a result, there is an inflow of calcium which induces long term potentiation (LTP) and synaptic plasticity. (Reprinted from Folsom and Fatemi 2013 Copyright (2013) with permission from Elsevier)

nificant association for any of the SNPs and autism (Dutta et al. 2008). In contrast to the finding by Li et al. (2008), a separate study failed to find an association between rs736707 and autism in a Han Chinese population sample and, moreover, found no association for rs2229864, rs362691, and rs2073559 and autism (He et al. 2011).

Reelin levels have been shown to be reduced in sera and in brains of subjects with autism (Fatemi et al. 2001, 2002, 2005a; Keller et al. 2000; Lugli et al. 2003). In sera from subjects with autism, reductions of Reelin 410 kDa have been observed (Fatemi et al. 2002; Keller et al. 2000). Lugli et al (2003) found that Reelin 330 kDa was significantly reduced by 25% in sera of subjects with 11 or more CGG triplet repeats, suggesting that transmission of long alleles resulted in reduced Reelin expression. In brain, Fatemi et al (2001) found reduced expression of Reelin 180 kDa in cerebellum of subjects with autism when compared with healthy controls. A follow up experiment found reduced expression of Reelin 410 kDa, Reelin 330 kDa, and Reelin 180 kDa in

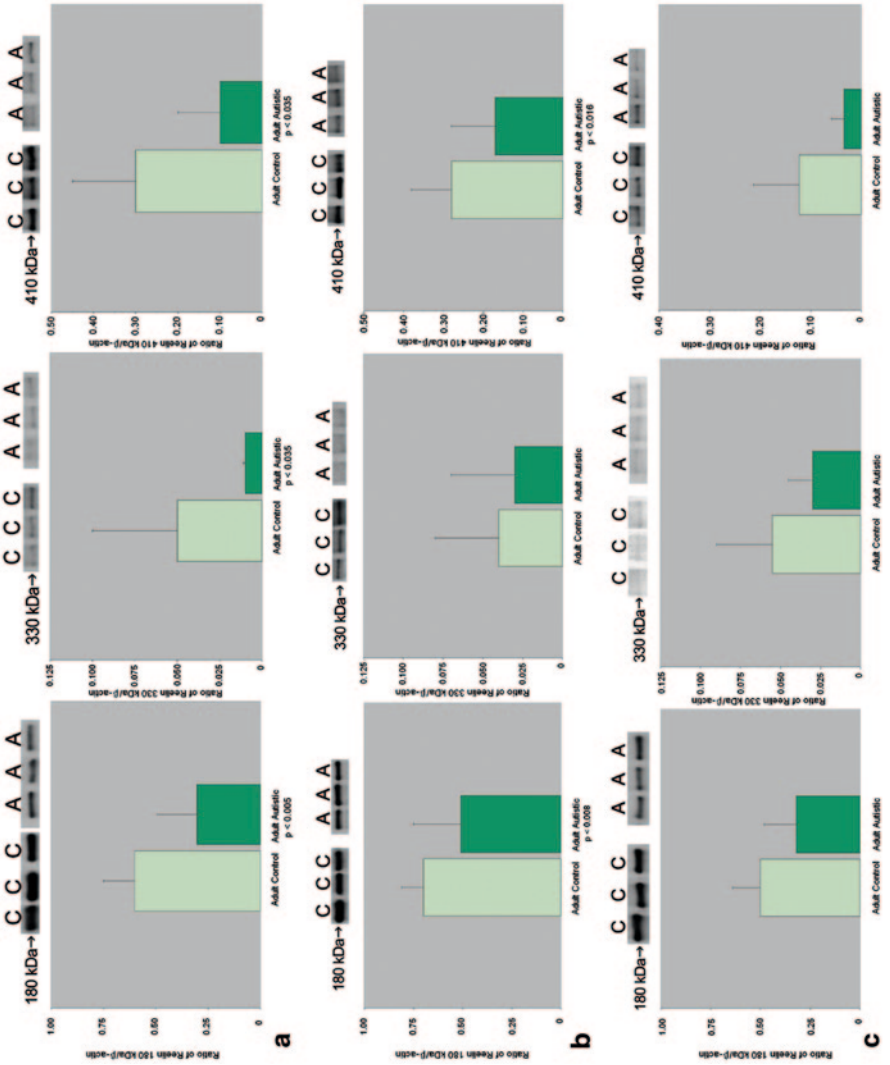


Fig. 16.2 The gel mobility of Reelin bands of 410, 330, and 180 kDa in BA9 (**a**), cerebellar (**b**), and BA40 (**c**) homogenates of representative control (*left histogram bar*) and autistic adults (*right histogram bar*). **a** In BA9, there were significant reductions of Reelin 180 kDa/β-actin ($p<0.005$), Reelin 330 kDa/β-actin ($p<0.035$) and Reelin 410 kDa/β-actin ($p<0.035$) versus control subjects. **b** In cerebella of subjects with autism, significant reductions in expression of Reelin 180 kDa/β-actin ($p<0.008$) and Reelin 410 kDa/β-actin ($p<0.016$) were observed versus control subjects. **c** In BA40, non-significant reductions were observed for full length reelin and the 330 kDa and 180 kDa fragments were observed. (Reprinted in a modified form Fatemi et al. 2005a. Copyright (2005) with permission from Elsevier)

prefrontal cortex of subjects with autism and reduction of Reelin 410 kDa and Reelin 180 kDa in cerebellum of subjects with autism, while there were no significant differences in parietal cortex of subjects with autism (Fig. 16.2) (Fatemi et al. 2005a). RELN

mRNA was similarly reduced in both BA9 and cerebellum of subjects with autism as was mRNA for DAB1, while mRNA for VLDLR was significantly upregulated in both areas (Fatemi et al. 2005a). In addition to autism, Reelin downregulation has been observed in subjects with schizophrenia and mood disorders (Eastwood and Harrison 2003, 2006; Fatemi et al. 2000, 2005b; Guidotti et al. 2000; Impagnatiello et al. 1998).

16.2 GABA and Autism

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the brain. GABA has many important roles in brain development including stimulating the proliferation of neural progenitor cells, migration and differentiation of neurites, and synapse formation (reviewed by Pizzarelli and Cherubini 2011). Approximately 20% of all central nervous system (CNS) neurons are GABAergic (Somogyi et al. 1998).

GABA is synthesized from glutamate by the enzyme glutamic acid decarboxylase. GABA is then transported to synaptic vesicles by the GABA transporter vGAT. When an action potential arrives at the nerve terminal, GABA is released into the synaptic cleft where it binds to its postsynaptic receptors. GABA receptors are either ionotropic ($GABA_A$) which are ligand-gated ion channels or metabotropic ($GABA_B$) which are G-protein coupled receptors. $GABA_A$ receptors are responsible for mediating the fast inhibitory action of GABA (Brandon et al. 2000). $GABA_A$ receptors are also the sites for clinical action of a number of drugs including benzodiazepines, barbiturates, and anesthetics. Thus far, 19 $GABA_A$ receptor subunits have been characterized (α1–α6, β1–β3, γ1– γ3, δ, ε, π, θ, and ρ1–ρ3) (Ma et al. 2005; Brandon et al. 2000) which combine to form the heteropentameric $GABA_A$ receptors. The most common $GABA_A$ receptors consist of two α subunits, two β subunits, and one γ subunit. $GABA_B$ receptors are heterodimeric, formed from two subunits: $GABA_B$ receptor 1 (GABBR1) and $GABA_B$ receptor 2 (GABBR2) (Jones et al. 1998). $GABA_B$ receptors require one GABBR1 and one GABBR2 subunits in order to be functional. $GABA_B$ receptors contribute to synaptic events in the mammalian brain presynaptically by facilitating the release of neurotransmitters, including dopamine, serotonin, glutamate and GABA (Steiniger and Kretschmer 2003; Takahashi et al. 2010; Waldmeier et al. 2008). Postsynaptically, stimulation of $GABA_B$ receptors results in the generation of slow, long-lasting, inhibitory potentials (Bowery 2000; Kuriyama et al. 2000).

Recent studies have investigated levels of GABA in brain and plasma of subjects with autism (Gaetz et al. 2013; Rojas et al. 2013; Russo 2013). Plasma levels of GABA have been shown to be significantly increased in children with autism and that high levels of GABA correlated significantly with increased hyperactivity and impulsivity, tip toeing severity, light sensitivity, and tactile sensitivity (Russo 2013). Rojas et al (2013) found that ratios of GABA to creatine (Cr) were significantly reduced when compared to controls in the perisylvian region of the left hemisphere as visualized by single-voxel, point resolved spectroscopy. Interestingly, unaffected siblings also displayed reduced GABA/Cr ratios when compared with controls

(Rojas et al. 2013). A second study, using the same procedure, found reduced GABA/Cr ratios in motor cortex and auditory cortex regions of interest (ROI) but not in the visual cortex ROI (Gaetz et al. 2013). The reduction of GABA levels in the brains of subjects with autism supports the hypothesis that the excitatory/inhibitory balance is disrupted in this population, which might help to explain the presence of seizure disorders (Tuchman and Rapin 2002) as well as cognitive and behavioral deficits associated with autism.

Glutamic acid decarboxylase 65 and 67 kDa proteins (GAD65/67) are the rate limiting enzymes responsible for the conversion of glutamate to GABA. GAD65 is significantly downregulated in cerebellum of subjects with autism and GAD67 is significantly reduced in parietal cortex of subjects with autism (Fatemi et al. 2002). More recently, significant reductions in GAD65 mRNA in the cerebellar dentate nuclei and significant reductions in GAD67 mRNA in Purkinje cells in cerebella from subjects with autism have been observed (Yip et al. 2007b 2009). Reductions in GAD65/67 could result in excessive glutamatergic (excitatory) and reduced GABAergic (inhibitory) signaling in important brain circuits that connect the cerebellum with the frontal cortex. The major deficiencies in levels of GAD 65 and 67 kDa proteins in two important brain areas in autism may subserve deficiency in the availability of GABA, thus affecting important biological functions including learning and motor activity. Additionally, decreased levels of GAD 65 and 67 kDa proteins could impact negatively on normal processing of visual, somatic, motor, and memory information processing, and could also explain the observations of increased blood, platelet, brain, and CSF glutamate levels in autistic patients (Moreno-Fuenmayor et al. 1996; Moreno et al. 1992; Shimmua et al. 2011; Shinohe et al. 2006).

A number of studies have now demonstrated that GABA receptors are reduced in brains of subjects with autism (Blatt et al. 2001; Samaco et al. 2005; Guptill et al. 2007; Fatemi et al. 2009a, b, 2010a, 2014; Oblak et al. 2010, 2011). Blatt et al. (2001) demonstrated a significant decrease in $GABA_A$ receptor binding sites (3H-muscimol-labeled binding sites) and benzodiazepine receptor binding sites (3H-flunitrazepam-labeled binding sites) in hippocampus of subjects with autism. Guptill et al. (2007), expanded on these experiments to demonstrate that the decrease in 3H-flunitrazepam-labeled benzodiazepine binding sites was due to a decrease in binding site number (B_{max}) rather than altered affinity to ligand binding (K_d). Reduced 3H-muscimol-labeled $GABA_A$ receptor binding sites have also been observed in the posterior cingulate cortex and fusiform gyrus (Oblak et al. 2011). Finally, ^{3}H-CGP54626-labeled $GABA_B$ receptor binding sites have also been observed to be reduced in the anterior and posterior cingulate cortex and fusiform gyrus of subjects with autism (Oblak et al. 2010).

Consistent with the results of binding assays, $GABA_A$ and $GABA_B$ receptor subunit proteins have been shown to be reduced in brains of subjects with autism (Fatemi et al. 2009a, b, 2010a, 2014). A comprehensive series of experiments have examined protein expression for GABA receptor subunits (16 $GABA_A$ and two $GABA_B$) in superior frontal cortex [Brodmann Area 9 (BA9)], parietal cortex (BA40), and cerebellum of subjects with autism vs. matched controls. In BA9, there were reduc-

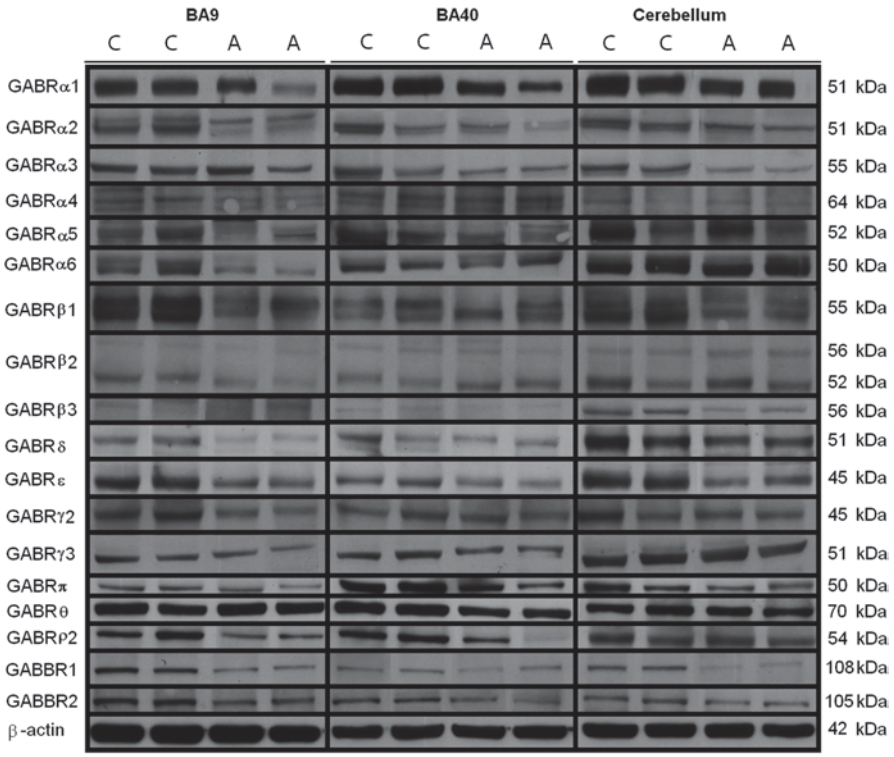


Fig. 16.3 Representative samples of GABRα1 (51 kDa), GABRα2 (51 kDa), GABRα3 (55 kDa), GABRα4 (64 kDa), GABRα5 (52 kDa), GABRα6 (50 kDa), GABRβ1 (55 kDa), GABRβ2 (56 kDa and 52 kDa), GABRβ3 (56 kDa), GABRδ (51 kDa), GABRε (45 kDa), GABRγ2 (45 kDa), GABRγ3 (51 kDa), GABRπ (50 kDa), GABRθ (70 kDa), GABRρ2 (54 kDa), GABBR1 (108 kDa), GABBR2 (105 kDa), and β-Actin (42 kDa) in BA9, BA40, and cerebellum of subjects with autism (**A**) and matched controls (**C**). [Reprinted in a modified from Fatemi et al. 2009a Copyright (2009) with permission from Springer Science+Business Media; from Fatemi et al. 2011b. Copyright (2011) with permission from Springer Science+Business Media; and from Fatemi et al. (2010a). Copyright (2010) with permission from Springer Science+Business Media]

tions in $GABA_A$ receptor alpha 1 (GABRα1), $GABA_A$ receptor alpha 4 (GABRα4), $GABA_A$ receptor alpha 5 (GABRα5), $GABA_A$ receptor alpha 6 (GABRα6), $GABA_A$ receptor beta 1 (GABRβ1), $GABA_A$ receptor beta 2 (GABRβ2), $GABA_A$ receptor delta (GABRδ), $GABA_A$ receptor epsilon (GABRε), $GABA_A$ receptor gamma 2 (GABRγ2), $GABA_A$ receptor rho 2 (GABRρ2), and GABAB receptor 1 (GABBR1) proteins in brains of subjects with autism (Fig. 16.3) (Fatemi et al. 2009a, b, 2010a, 2014). In BA40, we observed significant reductions in GABRα1, $GABA_A$ receptor alpha 2 (GABRα2), $GABA_A$ receptor alpha 3 (GABRα3), GABRα5, $GABA_A$ receptor beta 3 (GABRβ3), and GABBR1 proteins in brains of subjects with autism (Table 16.1) (Fig. 16.3) (Fatemi et al. 2009a, b, 2010a, 2014). GABRα1, GABRβ3, GABBR1, and $GABA_B$ receptor 2 (GABBR2) proteins were significantly decreased in cerebella obtained from subjects with autism vs. matched controls (Fig. 16.3)

Table 16.1 Summary of mRNA and protein findings for selected $GABA_A$ and $GABA_B$ receptor subunits in BA9, cerebellum, and BA40 of subjects with autism vs. matched controls

	BA9		Cerebellum		BA40	
	mRNA	Protein	mRNA	Protein	mRNA	Protein
GABRα1	–	↓	–	↓	–	↓
GABRα2	↓	–	↑	–	–	↓
GABRα3	↓	–	↑	–	↓	↓
GABRα4	↓	↓	↑	–	–	–
GABRα5	↓	↓	↑	–	–	↓
GABRα6	↑	↓	↓	–	–	–
GABRβ1	↓	↓	↑	–	–	–
GABRβ2	–	↓	↓	–	–	–
GABRβ3	↓	–	↑	↓	–	↓
GABRδ	–	↓	–	–	–	–
GABRε	–	↓	–	–	–	–
GABRγ2	–	↓	↑	–	–	–
GABRγ3	↓	–	↑	–	↑	–
GABRπ	–	–	–	–	–	–
GABRθ	↓	–	↑	–	–	–
GABRρ2	–	↓	–	–	–	–
GABBR1	–	↓	↓	↓	↑	↓
GABBR2	–	–	–	↓	–	–

(Fatemi et al. 2009a, b) (Table 16.1) (Fig. 16.3). Consistent with these findings, a previous report has also demonstrated reduction in GABRβ3 in cerebella of subjects with autism when compared with controls (Samaco et al. 2005). More GABA receptor subunits were reduced in BA9 than in BA40 or cerebellum. Gene expression changes have previously been demonstrated to be more robust in cerebral cortex of subjects with autism than in cerebellum, which is consistent with our findings (Voineagu et al. 2011). Altered protein expression for $GABA_A$ and $GABA_B$ receptor subunits have also been observed in the brains of subjects with schizophrenia and mood disorders (Fatemi et al. 2011a, 2013a, b, 2014).

mRNA species for the same $GABA_A$ and $GABA_B$ receptors were measured via qRT-PCR in BA9, BA40, and cerebellum of subjects with autism vs. controls using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin as housekeeping genes (Fatemi et al. 2010a, 2014). Increased mRNA was observed in BA9 of subjects with autism for GABRα6 while there were reduced mRNA species for GABRα2, GABRα3, GABRα4, GABRα5, GABRβ1, GABRβ3, GABRγ3, and $GABA_A$ receptor theta (GABRθ) (Table 16.1) (Fatemi et al. 2010a, 2014). In BA40, qRT-PCR revealed increased mRNA expression of GABRγ3 and GABBR1 and reduced mRNA expression for GABRα3 in subjects with autism (Table 16.1) (Fatemi et al. 2010a, 2014). qRT-PCR of cerebella from subjects with autism revealed significant upregulation of mRNA species for GABRα2, GABRα3, GABRα4, GABRα5, GABRβ1,

GABRβ3, GABRγ2, GABRγ3, and GABRθ (Table 16.1) (Fatemi et al. 2010a, 2014). In contrast, there were reduced mRNA species for GABRα6, GABRβ2, and GABBR1 in cerebella of subjects with autism (Fatemi et al. 2010a, 2014). While mRNA expression changes were variable in each brain region, protein expression was consistently downregulated.

Gene association studies for GABA receptor subunit genes and autism have been equivocal. An analysis of 12 $GABA_A$ and two $GABA_B$ receptor subunit genes found that GABRA4 (which codes for GABRα4), potentially through interaction with GABRB1 (which codes for GABRβ1) was associated with autism (Ma et al. 2005), a finding that was subsequently confirmed (Collins et al. 2006). Several studies have implicated the 15q11.2–q13 region, which includes genes for GABRβ3, GABRα5, and GABRγ3 (GABRB3, GABRA5, GABRG3) with autism (Hogart et al. 2007; Maddox et al. 1999; McCauley et al. 2004). GABRB3 has been associated with autism in a Korean population sample (Kim et al. 2006). SNPs of GABRA5 and GABRB3 have been shown to be nominally associated with autism (McCauley et al. 2004) as have SNPs of GABRG3 (Menold et al. 2001). However, a study using a Japanese population sample failed to find significant associations between GABRB3, GABRA5, or GABRG3 and autism (Tochigi et al. 2007). Moreover, others have similarly found no association for these genes and autism (Ma et al. 2005; Kelemenova et al. 2010). Recently, truncating mutations of the genes that code for GABRα3 and GABRθ (GABRA3 and GABRQ) have shown an association with autism spectrum disorders (Piton et al. 2013). Taken together, these results suggest that association between GABA receptor genes and autism may be population specific.

Reduction in $GABA_A$ and $GABA_B$ receptor subunits may help explain comorbid seizure disorder and cognitive deficits present in subjects with autism. Seizure disorder was present in many of the medical histories of subjects with autism used in our postmortem studies (Fatemi et al. 2009a, b, 2010a, 2014). However, when analyzed as a confound, we did not find an impact of seizure disorder on our results (Fatemi et al. 2009a, b, 2010a, 2014). Animal models of seizure disorder have shown reduced expression of GABBR1 and GABBR2 in brain (Han et al. 2006; Princivalle et al. 2003; Straessle et al. 2003). GABBR1 KO mice display seizure disorder and memory deficits (Prosser et al. 2001; Schuler et al. 2001). Epileptiform activity interferes with cognition by causing disturbances of vigilance, shifting attention, and language difficulties (Binnie 1993) phenomena that often occur in children with autism and epilepsy. It has been hypothesized that the regression of language skills in children between the ages of two and three with autism may be due to epileptiform activity (Canitano 2007).

16.3 FMRP and Autism

Autism and fragile X syndrome (FXS) share many commonalities including intellectual disability, presence of seizures, learning difficulties, social deficits, anxiety, decreased attention, poor eye contact, delayed language acquisition and disorders

of expression. Previous reports have shown the presence of autistic behavior in 15–47% of patients with FXS (Bailey et al. 1998; Hatton et al. 2006; Kau et al. 2004; Kauffman et al. 2004). Individuals with diagnoses of both autism and FXS display greater severity of symptoms (Kau et al. 2004; Philofsky et al. 2004) and greater impairment in nonverbal cognition and expressive language (Philofsky et al. 2004). Boys with autism and FXS also show more cognitive impairment, abnormal behavior, and less adaptive behavior when compared to those with FXS alone (Kau et al. 2004). Interestingly, autistic symptoms have been shown to improve with age in subjects with FXS (McDuffie et al. 2010). In addition to phenotypic overlap, recently identified biological substrates have been proposed that create intriguing avenues of scientific interest.

The fragile X mental retardation 1 (FMR1) gene is located to the X chromosome. Mutations in this gene, resulting in loss of function, are almost entirely responsible for the development of FXS. Under normal circumstances there are anywhere from 5-55 5′ CGG repeats in the untranlated portion of the gene (Fu et al. 1991). Individuals with 56–200 repeats are often found in FXS families but do not display clinical symptoms of FXS and are considered to have the FMR1 premutation (Bardoni et al. 2001). When more than 200 5′ CGG repeats are present, there is extensive methylation of the 5′ region, including the promoter of FMR1 resulting in transcriptional silencing of FMR1 and the presence of clinical symptoms of FXS (Pieretti et al. 1991). FMRP binds to approximately 5% of all mRNAs expressed in brain (Darnell and Klann 2013) potentially controlling a large number of important processes. It has been hypothesized that reduction in FMRP expression leads to unregulated protein synthesis, induced by group 1 metabotropic glutamate receptors (mGluRs), which in turn is responsible for the multiple physical and cognitive pathologies of FXS (Bear et al. 2004; Dölen and Bear 2008).

Boys who have the FMR1 premutation, especially if they present as clinical probands, are more likely to have a comorbid diagnosis of autism than nonprobands or control siblings who lack the premutation (Chonchaiya et al. 2012; Farzin et al. 2006). Additionally, these probands displayed increased rates of seizure disorder (Chonchaiya et al. 2012) and attention deficit/hyperactivity disorder (Farzin et al. 2006). Carriers of the FMR1 premutation have also been shown to have reduced amygdala volume, reduced activation of the right amygdala during an emotion matching task, and higher ratings of autism spectrum symptoms (Hessl et al. 2011). The authors found that while reduced FMRP and increased FMR1 mRNA were associated with reduced activation, reduced FMRP expression was identified statistically as the primary factor associated with reduced amygdala activation (Hessl et al. 2011).

The FMR1 gene may be a candidate gene for autism. The Xq27-q28 region, which includes FMR1 and methyl CpG binding protein 2 (MECP2), has shown some association with increased risk of autism (Vincent et al. 2005). A rare point mutation of FMR1 (A to C substitution at nucleotide 879 in exon 9) may contribute to autism and mental retardation in Japanese patients (Shinahara et al. 2004). An intronic variant of FMR1 (IVS10+14C-T) showed no evidence of increasing the risk of autism (Vincent et al. 2004). There may also be structural differences in the FMR1 gene in subjects with autism vs. controls. An investigation of CGG repeat

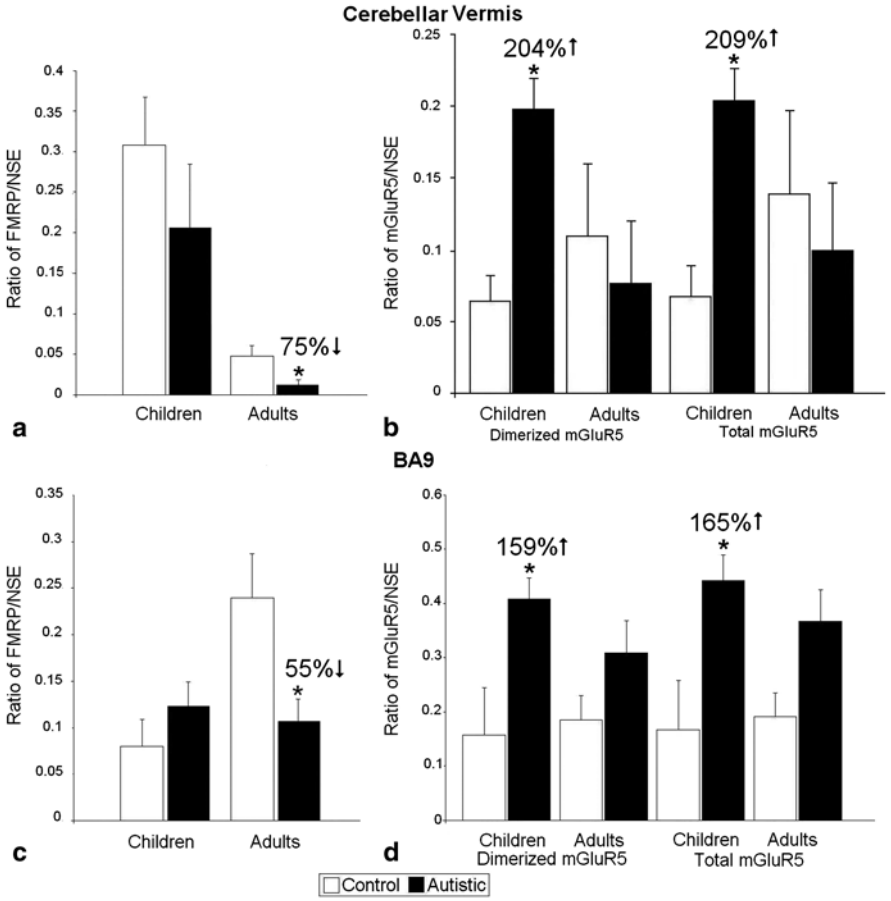


Fig. 16.4 Expression of FMRP and mGluR5 in brains from subjects with autism vs. matched controls. **a)** Ratio of FMRP/NSE in cerebellar vermis. **b)** Ratio of mGluR5/NSE in cerebellar vermis. **c)** Ratio of FMRP/NSE in BA9. **d)** Ratio of mGluR5/NSE in BA9. Ratios are expressed as mean ± standard error *, $p < 0.05$. [Reprinted in a modified form Fatemi et al. 2011b Copyright (2011) with permission from John Wiley and Sons and from Molecular Autism; Fatemi and Folsom 2011. Copyright (2011) with permission from BioMed Central]

length and AGG interruption of the FMR1 gene in subjects with infantile autism vs. healthy controls found that in the case of infantile autism, there were less AGG interruptions (Poon et al. 1998). This pattern is similar to what is found in subjects with FXS, although none of the study subjects had a comorbid diagnosis of FXS (Poon et al. 1998).

A recent series of experiments involving postmortem brain tissue have shown dysregulation of FMRP, mGluR5, and downstream targets of FMRP-mGluR5 signaling in cerebellar vermis and PFC in subjects with autism (Fatemi and Folsom 2011; Fatemi et al. 2011b, 2013c; Rustan et al. 2013). Levels of FMRP were reduced in PFC and cerebellar vermis of adults with autism while there were no

differences in these brain regions in children with autism (Fig. 16.4) (Fatemi and Folsom 2011; Fatemi et al. 2011b). Phosphorylated FMRP (p-FMRP) is reduced in cerebellar vermis of adults and children with autism and in PFC of adults with autism (Rustan et al. 2013). Dephosphorylation of FMRP leads to its ubiquitination and subsequent degradation, a process that may be the result of overactive mGluR5 signaling (Nalavadi et al. 2012). Indeed, in the same tissues, increased expression of mGluR5 was observed in PFC and cerebellar vermis of children with autism (Fig. 16.4) (Fatemi and Folsom 2011; Fatemi et al. 2011b). Interestingly, none of the subjects with autism had a comorbid diagnosis of FXS (Fatemi and Folsom 2011; Fatemi et al. 2011b, 2013c). Moreover, similar studies performed in subjects with schizophrenia and mood disorders found reduced expression of FMRP and mGluR5 in lateral cerebellum of subjects with schizophrenia, bipolar disorder, and major depression and in BA9 of subjects with schizophrenia and bipolar disorder (Fatemi et al. 2010b, 2013a, b). These results indicate that dysfunction in FMRP and mGluR5 expression may be common to multiple psychiatric disorders.

FMRP is a negative regulator of mGluR5. It has been proposed that in the absence of FMRP, mGluR5-dependent protein synthesis goes unchecked, resulting in the anatomical and physical deficits associated with FXS (Bear 2005; Bear et al. 2004; Dölen and Bear 2008). Evidence from FMR1 KO mice support this theory including: (1) increased long-term depression (LTD) (Huber et al. 2002); (2) an increased number of immature dendritc spines (Grossman et al. 2006); and (3) increased epileptiform activity (Yan et al. 2005), all of which are present in individuals with FXS (reviewed by Garber et al. 2008). Importantly, use of allosteric inhibitors of mGluR5 such as such as 2-methyl-6-(phenylethynyl)-pyridine (MPEP), or lowering of the concentration of mGluR5 reverse structural and behavioral deficits in FMR1 KO mice including the number of dendritic spines, deficits in prepulse inhibition, and presence of seizure that are also present in autism (de Vrij et al. 2008; Dölen et al. 2007; Westmark et al. 2009; Yan et al. 2005; Yuskaitis et al. 2010). The use of allosteric modulators of mGluR5 as well as other treatments for FXS and autism has been an ongoing line of research (Berry-Kravis et al. 2011; Hagerman et al. 2012; Li et al. 2013).

Four downstream targets of FMRP-mGluR5 signaling have also been investigated: homer 1, amyloid beta A4 precursor protein (APP), ras-related C3 botulinum toxin substrate 1 (RAC1), and striatal-enriched protein tyrosine phosphatase (STEP) (Fatemi et al. 2013c). These proteins are involved in synapse formation and neural plasticity (APP); regulation of N-methyl-D-aspartate (NMDA) receptor function (STEP); synaptogenesis, receptor trafficking, and involvement in dopaminergic and glutamatergic signaling (homer 1); and modulation of dendritic spine morphology and density (RAC1), all of which are relevant to autism (Goebel-Goody et al. 2012; Nakayama et al. 2000; Priller et al. 2006; Szumlinski et al. 2006; Turner et al. 2003). Protein levels of RAC1, APP 120 kDa and APP 88 kDa were upregulated in BA9 of children with autism (Fatemi et al. 2013c). In BA9 of adults with autism, there was increased protein expression of RAC1 and STEP 46 kDa while there was reduced expression of homer 1 (Fatemi et al. 2013c). In cerebellar vermis of adults with autism there was significantly increased RAC1 protein expression, while there was

significantly reduced expression of APP 120 kDa, STEP 66 kDa, STEP 27 kDa, and homer 1 (Fatemi et al. 2013c). In contrast, there were no changes observed in cerebellar vermis of children with autism (Fatemi et al. 2013c).

The reduced expression of FMRP in subjects with autism may help explain reduced expression of GABA receptor subunits and Reelin. In animal models of FXS, expression of $GABA_A$ receptor subunits have been shown to be reduced or eliminated by the loss of function of the FRM1 gene and consequent loss of FMRP (D'Hulst et al. 2006; El Idrissi et al. 2005, Gantois et al. 2006). El Idrissi et al. (2005) found reduced expression of the $GABA_A$ β subunit in cortex, hippocampus, brainstem, and diencephalon of fragile X (FraX) mice. Gantois et al (2006) found reduced GABRδ mRNA in hippocampus and neocortex of Fmr1 knockout (KO) mice. A separate study found reduced mRNA for GABRα1, GABRα3, GABRα4, GABRβ1, GABRβ2, GABRγ1, and GABRγ2 in cortex, but not cerebellum of Fmr1 KO mice (D'Hulst et al. 2006).

Reelin has also been identified as a downstream target of FMRP (Darnell et al. 2011). Altered expression of FMRP in subjects with autism may impact levels of Reelin as well.

16.4 Conclusions

Autism is a heterogeneous disorder in which multiple signaling systems are impacted. The Reelin, GABAergic, and FMRP-mGluR5 signaling systems separately, and perhaps synergistically, contribute to the pathology of autism. Dysfunction of the Reelin signaling system may result in abnormalities in brain morphology associated with autism as well as dysfunctional synaptic transmission. GABAergic system dysfunction could result in cognitive impairments as well as seizure disorder. Deficits in the FMRP-mGluR5 signaling system contribute to intellectual impairment, altered neuronal structure and seizure disorder. Reelin is known to regulate GABAergic and glutamatergic neurotransmission (Tissir and Goffinet 2003; Marrone et al. 2006). FMRP regulates $GABA_A$ receptor expression (D'Hulst et al. 2006; El Idrissi et al. 2005, Gantois et al. 2006) and targets Reelin (Darnell et al. 2011), potentially regulating its expression as well. Dysfunction in one system may lead to dysfunction in other systems. Interplay between these systems may result in multiple abnormal phenotypes associated with autism. These systems also identify targets for therapeutic intervention which may ameliorate multiple symptoms of autism.

Acknowledgments Grant support from NICHD (R01HD052074), NIMH (R01MH086000), the Bernstein Endowed Chair in Adult Psychiatry, and the Ewald Bipolar Disease Research Fund to SHF is gratefully acknowledged. Dr. Fatemi currently holds United States patents for Reelin as a diagnostic marker of autism (7341844) and as a diagnostic marker for schizophrenia, bipolar disorder, and major depression (7682805).

References

American Psychiatric Association (APA) (2013) Diagnostic and statistical manual of mental disorders, 5th edn. American Psychiatric Publishing, Washington, DC

Ammassari-Teule M, Sgobio C, Biamonte F, Marrone C, Mercuri NB, Keller F (2009) Reelin haploinsufficiency reduces the density of PV + neurons in circumscribed regions of the striatum and selectively alters striatal-based behaviors. Psychopharmacology (Berl.) 204:511–521

Ashley-Koch AE, Jaworski J, Ma de Q, Mei H, Ritchie MD, Skaar DA, Robert Delong G, Worley G, Abramson RK, Wright HH, Cuccaro ML, Gilbert JR, Martin ER, Pericak-Vance MA (2007) Investigation of potential gene–gene interactions between APOE and RELN contributing to autism risk. Psychatr Genet 17:221–226

Assadi AH, Zhang G, Beffert U, McNeil RS, Renfro AL, Niu S, Quattrocchi CC, Antalffy BA, Sheldon M, Armstrong DD, Wynshaw-Boris A, Herz J, D'Arcangelo G, Clark GD (2003) Interaction of Reelin signaling and Lis1 in brain development. Nat Genet 35:270–276

Bailey DB, Mesibov GB, Hatton DD, Clark RD, Roberts JE, Mayhew L (1998) Autistic behavior in young boys with fragile x syndrome. J Autism Dev Disord 28:499–507

Bardoni B, Schenck A, Mandel J-L (2001) The fragile X mental retardation protein. Brain Res Bull 56:375–382

Barr AM, Fish KN, Markou A, Honer WG (2008) Heterozygous reeler mice exhibit alterations in sensorimotor gating but not presynaptic proteins. Eur J Neurosci 27:2568–2574

Bear MF (2005) Therapeutic implications of the mGluR theory of fragile X mental retardation. Genes Brain Behav 4:393–398

Bear MF, Huber KM, Warren ST (2004) The mGluR theory of Fragile X mental retardation. Trends Neurosci 27:370–377

Beffert U, Morfini G, Bock HH, Reyna H, Brady ST, Herz J (2002) Reelin-mediated signalling locally regulates protein kinase B/Akt and glycogen synthase kinase 3β. J Biol Chem 51:49958–49964

Beffert U, Weeber EJ, Durudas A, Qiu S, Masiulis I, Sweatt JD, Li WP, Adelmann G, Frotscher M, Hammer RE, Herz J (2005). Modulation of synaptic plasticity and memory by Reelin involves differential splicing of the lipoprotein receptor Apoer2. Neuron 47:567–579

Beffert U, Nematollah Farsian F, Masiulis I, Hammer RE, Yoon SO, Giehl KM, Herz J (2006) ApoE receptor 2 controls neuronal survival in the adult brain. Curr Biol 16:2446-2452

Berry-Kravis E, Knox A, Hervey C (2011) Targeted treatments for fragile X syndrome. J Neurodev Disord 3:193–210

Binnie CD (1993) Significance and management of transitory cognitive impairment due to subclinical EEG discharges in children. Brain Dev 15:23–30

Blatt GJ, Fitzgerald CM, Guptill JT, Booker AB, Kemper TL, Bauman ML (2001) Density and distribution of hippocampal neurotransmitter receptors in autism: an autoradiographic study. J Autism Dev Disord 31:537–544

Bonora E, Beyer KS, Lamb JA, Parr JR, Klauck SM, Benner A, Paolucci M, Abbott A, Ragoussis I, Poustka A, Bailey AJ, Monaco AP (2003) International molecular genetic study of autism (IMGSAC). Analysis of reelin as a candidate gene for autism. Mol Psychiatry 10:885–892

Bouché E, Romero-Ortega MI, Henkemeyer M, Catchpole T, Leemhuis J, Frotscher M, May P, Herz J, Bock HH (2013) Reelin induces EphB activation. Cell Res 23:473–490

Bowery NG (2000) $GABA_B$ receptors structure and function. In: Martin DL, Olsen RW (eds) GABA in the nervous system: the view at fifty years. Lippincott, Williams and Wilkins, Philadelphia, pp 233–244

Boyle MP, Bernard A, Thompson CL, Ng L, Boe A, Mortrud M, Hawrylycz MJ, Jones AR, Hevner RF, Lein ES (2011) Cell-type-specific consequences of Reelin deficiency in the mouse neocortex, hippocampus, and amygdala. J Comp Neurol 519:2061–2089

Brandon NJ, Smart TG, Moss SJ (2000) Regulation of $GABA_A$ receptors by protein phosphorylation. In: Martin DL, Olsen RW (eds) GABA in the nervous system: the view at fifty years. Lippincott, Williams and Wilkins, Philadelphia, pp 191–206

Canitano R (2007) Epilepsy in autism spectrum disorders. Eur Child Adolesc Psychiatry 16:61–66
Centers for Disease Control and Prevention (CDC) (2014) Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States. MMWR Surveill Summ 63:1–21
Chakrabarti S, Fombonne E (2005) Pervasive development disorders in preschool children: confirmation of high prevalence. Am J Psychiatry 162:1133–1341
Chen Y, Beffert U, Ertunc M, Tang TS, Kavalali ET, Bezprozvanny I, Herz J (2005) Reelin modulates NMDA receptor activity in cortical neurons. J Neurosci 25:8209–8216
Chonchaiya W, Au J, Schneider A, Hessl D, Harris SW, Laird M, Mu Y, Tassone F, Nguyen DV, Hagerman RJ (2012). Increased prevalence of seizures in boys who were probands with the FMR1 premutation and co-morbid autism spectrum disorder. Hum Genet 131:581–589
Collins AL, Ma D, Whitehead PL, Martin ER, Wright HH, Abramson RK, Hussman JP, Haines JL, Cuccaro ML, Gilbert JR, Pericak-Vance MA (2006) Investigation of autism and GABA receptor subunit genes in multiple ethnic groups. Neurogenetics 7:167–174
D'Arcangelo G, Miao GG, Soares HD, Morgan JI, Curran T (1995) A protein related to extracellular matrix proteins detected in the mouse mutant reeler. Nature 374:719–723
D'Arcangelo G, Homayouni R, Keshvara L, Rice DS, Sheldon M, Curran T (1999) Reelin is a ligand for lipoprotein receptors. Neuron 24:471–479
D'Hulst C, De Geest N, Reeve SP, Van Dam D, De Deyn PP, Hassan BA, Kooy RF (2006) Decreased expression of the $GABA_A$ receptor in fragile X syndrome. Brain Res 1121:238–245
Darnell JC, Klann E (2013) The translation of translational control by FMRP: therapeutic targets for FXS. Nat Neurosci 16:1530–1536
Darnell JC, Van Driesche SJ, Zhang C, Hung KY, Mele A, Fraser CE, Stone EF, Chen C, Fak JJ, Chi SW, Licatalosi DD, Richter JD, Darnell RB (2011) FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. Cell 146:247–261
de Vrij FM Levenga J van der Linde HC Koekkoek SK De Zeeuw CI Nelson DL Oostra BA Willemsen R (2008) Rescue of behavioral phenotype and neuronal protrusion morphology in Fmr1 KO mice. Neurobiol Dis 31:127–132
DeBergeyck V, Naerhuyzen B, Goffinet AM, deRouvroit C (1998) A panel of monoclonal antibodies against Reelin, the extracellular matrix protein defective in reeler mutant mice. J Neurosci Meth 82:17–24
Devlin B, Bennett P, Dawson G, Figlewicz DA, Grigorenko EL, McMahon W, Minshew N, Pauls D, Smith M, Spence MA, Rodier PM, Stodgell C, Schellenberg GD, CPEA Genetics Network (2004) Alleles of a Reelin CGG repeat do not convey liability to autism in a sample from the CPEA network. Am J Med Genet 126B:46–50
Dölen G, Bear MF (2008) Role for metabotropic glutamate receptor 5 (mGluR5) in the pathogenesis of fragile X syndrome. J Physiol 586:1503-1508
Dölen G, Osterweil E, Shankaranarayana Rao BS, Smith GB, Auerbach D, Chattarji S, Bear MF (2007) Correction of fragile X syndrome in mice. Neuron 56:955–962
Dutta S, Guhathakurta S, Sinha S, Chatterjee A, Ahmed S, Ghosh S, Gangopadhyay PK, Singh M, Usha R (2007) Reelin gene polymorphisms in the Indian population: a possible paternal 5'UTR-CGG-repeat-allele effect on autism. Am J Med Genet B Neuropsychiatr Genet 144B:106–112
Dutta S, Sinha S, Ghosh S, Chatterjee A, Ahmed S, Usha R (2008) Genetic analysis of Reelin gene (RELN) SNPs: no association with autism spectrum disorder in the Indian population. Neurosci Lett 441:56–60
Eastwood SL, Harrison PJ (2003) Interstitial white matter neurons express less Reelin and are abnormally distributed in schizophrenia: towards an integration of molecular and morphologic aspects of the neurodevelopmental hypothesis. Mol Psychiatry 8:821–831
Eastwood SL, Harrison PJ (2006) Cellular basis for reduced cortical Reelin expression in schizophrenia. Am J Psychiatry 163:540–542
El Idrissi A Ding XH Scalia J Trenkner E Brown WT Dobkin C (2005) Decreased $GABA_A$ receptor expression in the seizure-prone fragile X mouse. Neurosci Lett 377:141–146

Farzin F, Perry H, Hessl D, Loesch D, Cohen J, Bacalman S, Gane L, Tassone F, Hagerman P, Hagerman R (2006) Autism spectrum disorders and attention-deficit/hyperactivity disorder in boys with the fragile X premutation. J Dev Behav Pediatr 27:S137–S144

Fatemi SH, Folsom TD (2011) Dysregulation of fragile X mental retardation protein and metabotropic glutamate receptor 5 in superior frontal cortex of subjects with autism: a postmortem brain study. Mol Autism 2:6

Fatemi SH, Folsom TD (2015) GABA receptor subunit distribution and FMRP-mGluR5 signaling abnormalities in the cerebellum of subjects with schizophrenia, mood disorders, and autism. Schiz Res, in press

Fatemi SH, Earle JA, McMenomy T (2000) Reduction in Reelin immunoreactivity in hippocampus of subjects with schizophrenia, bipolar disorder, and major depression. Mol Psychiatry 5:654–663

Fatemi SH, Stary JM, Halt AR, Realmuto G (2001) Dysregulation of Reelin and Bcl-2 proteins in autistic cerebellum. J Autism Dev Disord 6:529–535

Fatemi SH, Stary JM, Egan EA (2002) Reduced blood levels of Reelin as a vulnerability factor in pathophysiology of autistic disorder. Cell Mol Neurobiol 22:139–152

Fatemi SH, Snow AV, Stary JM, Araghi-Niknam M, Brooks AI, Pearce DA, Reutiman TJ, Lee S (2005a) Reelin signaling is impaired in autism. Biol Psychiatry 57:777–787

Fatemi SH, Stary JM, Earle JA, Araghi-Niknam M, Egan E (2005b) GABAergic dysfunction in schizophrenia and mood disorders as reflected by decreased levels of glutamic acid decarboxylase 65 and 67 kDa and Reelin proteins in cerebellum. Schizophr Res 72:109–122

Fatemi SH, Reutiman TJ, Folsom TD, Thuras PD (2009a) GABA(A) Receptor downregulation in brains of subjects with autism. J Autism Dev Disord 39:223–230

Fatemi SH, Folsom TD, Reutiman TJ, Thuras PD (2009b) Expression of GABA(B) receptors is altered in brains of Subjects with autism. Cerebellum 8:64–69

Fatemi SH, Reutiman TJ, Folsom TD, Rooney RJ, Patel DH, Thuras PD (2010a) mRNA and protein levels for GABA(A) alpha 4, alpha 5, beta 1, and GABA(B)R1 receptors are altered in brains from subjects with autism. J Autism Dev Disord 40:743–750

Fatemi SH, Kneeland RE, Liesch SB, Folsom TD (2010b) Fragile X mental retardation protein levels are decreased in major psychiatric disorders. Schizophr Res 124:246–247

Fatemi SH, Folsom TD, Thuras PD (2011a) Deficits in GABA(B) receptor system in schizophrenia and mood disorders: a postmortem study. Schizophr Res 128:37–43

Fatemi SH, Folsom TD, Kneeland RE, Liesch SB (2011b) Metabotropic glutamate receptor 5 upregulation in children with autism is associated with underexpression of both Fragile X mental retardation protein and $GABA_A$ receptor beta 3 in adults with autism. Anat Rec 294:1635–1645

Fatemi SH, Folsom TD, Rooney RJ, Thuras PD (2013a) mRNA and protein expression for novel $GABA_A$ receptors θ and ρ2 are altered in schizophrenia and mood disorders; relevance to FMRP-mGluR5 signaling pathway. Transl Psychiatry 3:e271

Fatemi SH, Folsom TD, Rooney RJ, Thuras PD (2013b) Expression of $GABA_A$ α2-, β1- and ε-receptors are altered significantly in the lateral cerebellum of subjects with schizophrenia, major depression and bipolar disorder. Transl Psychiatry 3:e303

Fatemi SH, Folsom TD, Kneeland RE, Yousefi M, Liesch S, Thuras PD (2013c) Impairment of fragile X mental retardation protein-metabotropic glutamate receptor 5 signaling and its downstream cognates ras-related C3 botulinum toxin substrate 1, amyloid beta A4 precursor protein, striatal-enriched protein tyrosine phosphatase, and homer 1, in autism: a postmortem study in cerebellar vermis and superior frontal cortex. Mol Autism 4:21

Fatemi SH, Reutiman TJ, Folsom TD, Rustan OG, Rooney RJ, Thuras PD (2014) Downregulation of $GABA_A$ receptor protein subunits α6, β2, ε, γ2, and ρ2 in superior frontal cortex of subjects with autism. J Autism Dev Disord 44(8):1833–1845

Folsom TD, Fatemi SH (2013) The involvement of Reelin in neurodevelopmental disorders. Neuropharmacology 68:122–135

Frotscher M (1998) Cajal-Retzius cells, Reelin, and the formation of layers. Curr Opin Neurobiol 8:570–575

Fu YH, Kuhl DP, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, Verkerk AJ, Holden JJ, Fenwick RG Jr, Warren ST et al (1991) Variation in the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. Cell 67:1047–1058

Gaetz W, Bloy L, Wang DJ, Port RG, Blaskey L, Levy SE, Roberts TP (2013) GABA estimation in the brains of children on the autism spectrum: measurement precision and regional cortical variation. Neuroimage 86:1–9

Gantois I, Vandescompele J, Speleman F, Reyniers E, D'Hooge R, Severijnen LA, Willemsen R, Tassone F, Kooy RF (2006) Expression profiling suggests underexpression of the $GABA_A$ receptor subunit delta in the fragile X knockout mouse model. Neurobiol Dis 21:346–357

Garber KB, Visootsak J, Warren ST, Oostra BA, Nelson DL, Caskey CT (2008) Fragile X syndrome. Eur J Hum Genet 16:666–672

Goebel-Goody SM, Baum M, Paspalas CD, Fernandez SM, Carty NC, Kurup P, Lombroso PJ (2012) Therapeutic implications for striatal-enriched protein tyrosine phosphatase (STEP) in neuropsychiatric disorders. Pharmacol Rev 64:65–87

Groc L, Choquet D, Stephenson FA, Verrier D, Manzoni OJ, Chavis P (2007) NMDA receptor surface trafficking and synaptic subunit composition are developmentally regulated by the extracellular matrix protein Reelin. J Neurosci 27:10165–10175

Grossman AW, Elisseou NM, McKinney BC, Greenough WT (2006) Hippocampal pyramidal cells in adult Fmr1 knockout mice exhibit an immature-appearing profile of dendritic spines. Brain Res. 1084:158–164

Guidotti A, Auta J, Davis JM, Di-Giorgi-Gerevini V, Dwivedi Y, Grayson DR, Impagnatiello F, Pandey G, Pesold C, Sharma R, Uzunov D, Costa E (2000) Decrease in Reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a postmortem brain study. Arch Gen Psychiatry 57:1061–1069

Guptill JT, Booker AB, Gibbs TT, Kemper TL, Bauman ML, Blatt GJ (2007) [3H]-flunitrazepam-labeled benzodiazepine binding sites in the hippocampal formation in autism: a multiple concentration autoradiographic study. J Autism Dev Disord 37:911–920

Hack I, Hellwig S, Junghans D, Brunne B, Bock HH, Zhao S, Frotscher M (2007) Divergent roles of ApoER2 and Vldlr in the migration of cortical neurons. Development 134:3883–3891

Hadj-Sahraoui N, Frédéric F, Delhaye-Bouchaud N, Mariani J (1996) Gender effect on Purkinje cell loss in the cerebellum of the heterozygous reeler mouse. J Neurogenet 11:45–58

Hagerman R, Lauterborn J, Au J, Berry-Kravis E (2012) Fragile X syndrome and targeted treatment trials. Results Probl Cell Differ 54:297–335

Hamburgh M (1963) Analysis of the postnatal developmental effects of "reeler," a neurological mutation in mice. A study in developmental genetics. Dev Biol 19:165–185

Han Y, Qin J, Bu DF, Chang XZ, Yang ZX (2006) Successive alterations of hippocampal gamma-aminobutyric acid B receptor subunits in a rat model of febrile seizure. Life Sci 78:2944–2952

Hatton DD, Sideris J, Skinner M, Bailey DB Jr, Roberts J, Mirrett P (2006) Autistic behavior in children with fragile X syndrome: prevalence, stability, and the impact of FMRP. Am J Med Genet A 140A:1804–1813

He Y, Xun G, Xia K, Hu Z, Lv L, Deng Z, Zhao J (2011) No significant association between RELN polymorphisms and autism in case-control and family-based association study in Chinese Han population. Psychiatry Res 187:462–464

Herz J, Chen Y (2006) Reelin, lipoprotein receptors and synaptic plasticity. Nat Rev Neurosci 7:850–859

Hessl D, Wang JM, Schneider A, Koldewyn K, Le L, Iwahashi C, Cheung K, Tassone F, Hagerman PJ, Rivera SM (2011) Decreased fragile X mental retardation protein expression underlies amygdala dysfunction in carriers of the fragile X premutation. Biol Psychiatry 70:859–865

Hiesberger T, Trommsdorff M, Howell BW, Goffinet A, Mumby MC, Cooper JA, Herz J (1999) Direct biding of Reelin to VLDL receptor and ApoE receptor 2 induces tyroene phosphorylation of disabled-1 and modulates tau phosphorylation. Neuron 24:481–489

Hogart A, Nagarajan RP, Patzel KA, Yasui DH, Lasalle JM (2007) 15q11–13 $GABA_A$ receptor genes are normally biallelically expressed in brain yet are subject to epigenetic dysregulation in autism-spectrum disorders. Hum Mol Genet 16:691–703

Huber KM, Gallagher SM, Warren ST, Bear MF (2002) Altered synaptic plasticity in a mouse model of fragile X mental retardation. Proc Natl Acad Sci U S A 99:7746–7750

Ignatova N, Sindic CJM, Goffinet AM (2004) Characterization of the various farms of the Reelin protein in the cerebrospinal fluid of normal subjects and in neurological diseases. Neurobiol Dis 15:326–330

Impagnatiello F, Guidotti AR, Pesold C, Dwivedi Y, Caruncho H, Pisu MG, Uzunov DP, Smalheiser NR, Davis JM, Pandey GN, Pappas GD, Tueting T, Sharma RP, Costa E (1998) A decrease of Reelin expression as a putative vulnerability factor in schizophrenia. Proc Nat Acad Sci U S A 95:15718–15723

Jones KA, Borowsky B, Tamm JA, Craig DA, Durkin MM, Dai M, Yao WJ, Johnson M, Gunwaldsen C, Huang LY, Tang C, Shen Q, Salon JA, Morse K, Laz T, Smith KE, Nagarathnam D, Noble SA, Branchek TA, Gerald C (1998) GABA(B) receptors function as a heteromeric assembly of the subunits GABA(B)R1 and GABA(B)R2. Nature 396:674–679

Jossin Y (2008) Chemistry of Reelin. In: Fatemi SH (ed) Reelin glycoprotein: structure, biology and roles in health and disease. Springer, New York, pp 37–48

Kau AS, Tierney E, Bukelis I, Stump MH, Kates WR, Trescher WH, Gray RM, Cox C, Capone GT, Stanard P (2004) Social behavior profile in young males with fragile X syndrome: characteristics and specificity. Am J Med Genet A 126A:9–17

Kauffman WE, Cortell R, Kau A, bukelis I, Tierney E, Gray R, Cox C, Capone G, Stanard P (2004) Autism spectrum disorder in fragile X syndrome: communication, social interaction, and specific behaviors. Am J Med Genet Part A 129A:225–234

Kelemenova S, Schmidtova E, Ficek A, Celec P, Kubranska A, Ostatnikova D (2010) Polymorphisms of candidate genes in Slovak autism patients. Psychiatr Genet 20:137–139

Keller F, Persico AM, Zelante L, Gasperial P, D'Agruma N, Maiorano N, Totaro A, Militerni R, Bravaccio C, Wassink TH, Schneider C, Melmed R, Trillo S, Montecchi F, Palermo M, Pascucci T, Puglisi-Allegra S, Reichelt K-L, Conciatori M, Marino R, Baldi A, Quattrocchi CC (2000). Reelin gene alleles and haplotypes are associated with autistic disorder. Soc Neurosci 26:77(31.11)

Kim SA, Kim JH, Park M, Cho IH, Yoo HJ (2006) Association of GABRB3 polymorphisms with autism in Korean trios. Neuropsychobiology 54:160–165

Krebs MO, Betancur C, Leroy S, Bourdel MC, Gillberg C, Leboyer M (2002) Paris Autism Research International Sibpair (PARIS) study. Absence of association between a polymorphic GGC repeat in the 5' untranslated region of the reelin gene and autism. Mol Psychiatry 7:801–804

Kuriyama K, Hirouchi M, Kimura H (2000) Neurochemical and molecular pharmacological aspects of the GABA(B) receptor. Neurochem Res 25:1233–1239

Kwok JB, Hallupp M, Loy CT, Chan DK, Woo J, Mellick GD, Buchanan DD, Silburn PA, Halliday GM, Schofield PR (2005) GSK3B polymorphisms alter transcription and splicing in Parkinson's disease. Ann Neurol 58:829–839

Lambert de Rouvroit C de Bergeyck V Cortvrindt C Bar I Eeckhout Y Goffinet AM (1999) Reelin, the extracellular matrix protein deficient in reeler mutant mice, is processed by a metalloproteinase. Exp Neurol 156:214–217

Li J, Nguyen L, Gleason C, Lotspeich L, Spiker D, Risch N, Myers RM (2004) Lack of evidence for an association between WNT2 and RELN polymorphisms and autism. Am J Med Genet 126B:51–57

Li H, Li Y, Shao J, Li R, Qin Y, Xie C, Zhao Z (2008) The association analysis of RELN and GRM8 genes with autistic spectrum disorder in Chinese Han population. Am J Med Genet B Neuropsychiatr Genet 147B:194–200

Li G, Jørgensen M, Campbell BM (2013) Metabotropic glutamate receptor 5-negative allosteric modulators for the treatment of psychiatric and neurological disorders (2009–July 2013). Pharm Pat Anal 2:767–802

Lugli G, Krueger JM, Davis JM, Persico AM, Keller F, Smalheiser NR (2003) Methodological factors influencing measurement and processing of plasma reelin in humans. BMC Biochem 4:9

Ma DQ, Whitehead PL, Menold MM, Martin ER, Ashley-Koch AE, Mei H, Ritchie MD, Delong GR, Abramson RK, Wright HH, Cuccaro ML, Hussman JP, Gilbert JR, Pericak-Vance MA (2005) Identification of significant association and gene-gene interaction of GABA receptor subunit genes in autism. Am J Hum Genet 77:377–388

Maddox LO, Menold MM, Bass MP, Rogala AR, Pericak-Vance MA, Vance JM, Gilbert JR (1999) Autistic disorder and chromosome 15q11-q13: construction and analysis of a BAC/PAC contig. Genomics 62:325–331

Marrone MC, Marinelli S, Biamonte F, Keller F, Sgobio CA, Ammassari-Teule M, Bernardi G, Mercuri NB (2006) Altered cortico-striatal synaptic plasticity and related behavioural impairments in reeler mice. Eur J Neurosci 24:2061–2070

McCauley JL, Olson LM, Delahanty R, Amin T, Nurmi EL, Organ EL, Jacobs MM, Folstein SE, Haines JL, Sutcliffe JS (2004) A linkage disequilibrium map of the 1-Mb 15q12 GABA(A) receptor subunit cluster and association to autism. Am J Med Genet B Neuropsychiatr Genet 131B:51–59

McDuffie A, Abbeduto L, Lewis P, Kover S, Kim JS, Weber A, Brown WT (2010) Autism spectrum disorder in children and adolescents with fragile X syndrome: within-syndrome differences and age-related changes. Am J Intellect Dev Disabil 115:307–326

Menold MM, Shao Y, Wolpert CM, Donnelly SL, Raiford KL, Martin ER, Ravan SA, Abramson RK, Wright HH, Delong GR, Cuccaro ML, Pericak-Vance MA, Gilbert JR (2001) Association analysis of chromosome 15 $GABA_A$ receptor subunit genes in autistic disorder. J Neurogenet 15:245–259

Moreno H, Borjas L, Arrieta A, Salz L, Prassad A, Estevez J, Bonilla E (1992) Clinical heterogeneity of the autistic syndrome: a study of 60 families. Invest Clin 33:13–31

Moreno-Fuenmayor H, Borjas L, Arrieta A, Valera V, Socorro-Candanoza L (1996) Plasma excitatory amino acids in autism. Invest Clin 37:113–128

Nakayama AY, Harms MB, Luo L (2000) Small GTPases Rac and Rho in the maintenance of dendritic spines and branches in hippocampal pyramidal neurons. J Neurosci 20:5329–5338

Nalavadi VC, Muddashetty RS, Gross C, Bassell GJ (2012) Dephosphorylation-induced ubiquitination and degradation of FMRP in dendrites: a role in immediate early mGluR-stimulated translation. J Neurosci 32:2582–2587

Oblak AL, Gibbs TT, Blatt GJ (2010) Decreased GABA(B) receptors in the cingulate cortex and fusiform gyrus in autism. J Neurochem 114:1414–1423

Oblak AL, Gibbs TT, Blatt, GJ (2011) Reduced GABA(A) receptors and benzodiazepine binding sites in the posterior cingulate cortex and fusiform gyrus in autism. Brain Res 1380:218–228

Ognibene E, Adriani W, Granstrem O, Pieretti S, Laviola G (2007) Impulsivity-anxiety-related behavior and profiles of morphine-induced analgesia in heterozygous reeler mice. Brain Res 113:173–180

Persico AM, D'Agruma L, Maiorano N, Totaro A, Militerni R, Bravaccio C, Wassink TH, Schneider C, Melmed R, Trillo S, Montecchi F, Palermo M, Pascucci T, Puglisi-Allegra S, Reichelt KL, Conciatori M, Marino R, Quattrocchi CC, Baldi A, Zelante L, Gasparini P, Keller F, Collaborative Linkage Study of Autism (2001) Reelin gene alleles and haplotypes as a factor predisposing to autistic disorder. Mol Psychiatry 6:150–159

Pesold C, Impagnatiello F, Pisu MG, Uzunov DP, Costa E, Guidotti A, Caruncho HJ (1998) Reelin is preferentially expressed in neurons synthesizing gamma-aminobutyric acid in cortex and hippocampus of adult rats. Proc Nat Acad Sci U S A 95:3221–3226

Philofsky A, Hepburn SL, Hayes A, Hagerman RJ, Rogers SJ (2004) Linguistic and cognitive functioning and autism symptoms in young children with fragile X syndrome. Am J Ment Retard 109:208–218

Pieretti M, Zhang FP, Fu YH, Warren ST, Oostra BA, Caskey CT, Nelson DL (1991) Absence of expression of FMR-1 gene in fragile X syndrome. Cell 66:817–822

Piton A, Jouan L, Rochefort D, Dobrzeniecka S, Lachapelle K, Dion PA, Gauthier J, Rouleau GA (2013) Analysis of the effects of rare variants on splicing identifies alterations in GABA(A) receptor genes in autism spectrum disorder individuals. Eur J Hum Genet 21:749–756

Pizzarelli R, Cherubini E (2011) Alterations of GABAergic signaling in autism spectrum disorders. Neural Plast 2011:297153

Podhorna J, Didriksen M (2004) The heterozygous reeler mouse: behavioural phenotype. Behav Brain Res 153:43–54

Poon PM, chen QL, Lai KY, Wong CK, Pang CP (1998) CGG repeat interruptions in the FMR1 gene in patients with infantile autism. Clin Chem Lab Med 36:649–653

Priller C, Bauer T, Mitteregger G, Krebs B, Kretzschmar HA, Herms J (2006) Synapse formation and function is modulated by the amyloid precursor protein. J Neurosci 26:7212–7221

Princivalle AP, Richards DA, Duncan JS, Spreafico R, Bowery NG (2003) Modification of GABA(B1) and GABA(B2) receptor subunits in the somatosensory cerebral cortex and thalamus of rats with absence seizures (GAERS). Epilepsy Res 55:39–51

Prosser HM, Gill CH, Hirst WD, Grau E, Robbins M, Calver A, Soffin EM, Farmer CE, Lanneau C, Gray J, Schenck E, Warmerdam BS, Clapham C, Reavill C, Rogers DC, Stean T, Upton N, Humphreys K, Randall A, Geppert M, Davies CH, Pangalos MN (2001) Epileptogenesis and enhanced prepulse inhibition in GABA(B1)-deficient mice. Mol Cell Neurosci 17:1059–1070

Qiu S, Weeber SJ (2007) Reelin signaling facilitates maturation of CA1 glutamatergic synapses. J Neurophysiol 97:2312–2321

Rojas DC, Singel D, Steinmetz S, Hepburn S, Brown MS (2013) Decreased left perisylvian GABA concentration in children with autism and unaffected siblings. Neuroimage 86:28–34

Russo AJ (2013) Correlation between hepatocyte growth factor (HGF) and Gamma-aminobutyric acid (GABA) plasma levels in autistic children. Biomark Insights 8:69–75

Rustan OG, Folsom TD, Yousefi MK, Fatemi SH (2013) Phosphorylated fragile X mental retardation protein at serine 499, is reduced in cerebellar vermis and superior frontal cortex of subjects with autism: implications for fragile X mental retardation protein -metabotropic glutamate receptor 5 signaling. Mol Autism 4:41

Samaco RC, Hogart A, LaSalle JM (2005) Epigenetic overlap in autism-spectrum neurodevelopmental disorders: MECP2 deficiency causes reduced expression of UBE3A and GABRB3. Hum Mol Genet 14:483–492

Schuler V, Lüscher C, Blanchet C, Klix N, Sansig G, Klebs K, Schmutz M, Heid J, Gentry C, Urban L, Fox A, Spooren W, Jaton AL, Vigouret J, Pozza M, Kelly PH, Mosbacher J, Froestl W, Käslin E, Korn R, Bischoff S, Kaupmann K, van der Putten H, Bettler B (2001) Epilepsy, hyperalgesia, impaired memory, and loss of pre- and postsynaptic GABA(B) responses in mice lacking GABA(B(1)). Neuron 31:47–58

Sentürk A, Pfennig S, Weiss A, Burk K, Acker-Palmer A (2011) Ephrin Bs are essential components of the Reelin pathway to regulate neuronal migration. Nature 472:356-360

Serajee FJ, Zhong H, Mahbubul Huq AH (2006) Association of Reelin gene polymorphisms with autism. Genomics 87:75–83

Sharma JR, Arieff Z, Gameeldien H, Davids M, Kaur M, van der Merwe L (2013) Association analysis of two single-nucleotide polymorphisms of the RELN gene with autism in the South African population. Genet Test Mol Biomarkers 17:93–98

Shimmua C, Suda S, Tsuchiya KJ, Hashimoto K, Ohno K, Matsuzaki H, Iwata K, Matsumoto K, Wakuda T, Kameno Y, Suzuki K, Tsujii M, Nakamura K, Takei N, Mori N (2011) Alteration of plasma glutamate and glutamine levels in children with high-functioning autism. PLoS One 6:e25340

Shinahara K, Saijo T, Mori K, Kuroda Y (2004) Single-strand conformation polymorphism analysis of the FMR1 gene in autistic and mentally retarded children in Japan. J Med Invest 51:52–58

Shinohe A, Hashimoto K, Nakamura K, Tsujii M, Iwata Y, Tsuchiya KJ, Sekine Y, Suda S, Suzuki K, Sugihara G, Matsuzaki H, Minabe Y, Sugiyama T, Kawai M, Iyo M, Takei N, Mori N (2006) Increased serum levels of glutamate in adult patients with autism. Prog Neuropsychopharmacol Biol Psychiatry 30:1472–1477

Skaar DA, Shao Y, Haines JL, Stenger JE, Jaworski J, Martin ER, DeLong GR, Moore JH, McCauley JL, Sutcliffe JS, Ashley-Koch AE, Cuccaro ML, Folstein SE, Gilbert JR, Pericak-Vance MA (2005) Analysis of the RELN gene as a genetic risk factor for autism. Mol Psychiatry 10:563–571

Smalheiser NR, Costa E, Guidotti A, Impagnatiello F, Auta J, Lacor P, Kriho V, Pappas GD (2000) Expression of Reelin in adult mammalian blood, liver, pituitary pars intermedia, and adrenal chromaffin cells. Proc Nat Acad Sci USA 97:1281–1286

Somogyi P, Tamás G, Lujan R, Buhl EH (1998) Salient features of synaptic organization in the cerebral cortex. Brain Res Brain Res Rev 26:113–135

Steiniger B, Kretschmer BD (2003) Glutamate and GABA modulate dopamine in the pedunculopontine tegmental nucleus. Exp Brain Res 149:422–430

Straessle A, Loup F, Arabadzisz D, Ohning GV, Fritschy JM (2003) Rapid and long-term alterations of hippocampal $GABA_B$ receptors in a mouse model of temporal lobe epilepsy. Eur J Neurosci 18:2213–2226

Stranahan AM, Erion JR, Wosiski-Kuhn M (2013) Reelin signaling in development, maintenance, and plasticity of neural networks. Ageing Res Rev 12:815–822

Strasser V, Fasching D, Hauser C, Mayer H, Bock HH, Hiesberger T, Herz J, Weeber EJ, Sweatt JD, Pramatarova A, Howell B, Schneider WJ, Nimpf J (2004) Receptor clustering is involved in Reelin signaling. Mol Cell Biol 24:1378–1386

Szumlinski KK, Kalivas PW, Worley PF (2006) Homer proteins: implications for neuropsychiatric disorders. Curr Opin Neurobiol 16:251–257

Takahashi A, Shimamoto A, Boyson CO, DeBold JF, Miczek KA (2010) GABA(B) receptor modulation of serotonin neurons in the dorsal raphe nucleus and escalation of aggression in mice. J Neurosci 30:11771–11780

Teixeira CM, Martín ED, Sahún I, Masachs N, Pujadas L, Corvelo A, Bosch C, Rossi D, Martinez A, Maldonado R, Dierssen M, Soriano E (2011) Overexpression of Reelin prevents the manifestation of behavioral phenotypes related to schizophrenia and bipolar disorder. Neuropsychopharmacology 36:2395–2405

Teixeira CM, Masachs N, Muhaisen A, Bosch C, Pérez-Martínez J, Howell B, Soriano E (2014) Transient downregulation of Dab1 protein levels during development leads to behavioral and structural deficits: relevance for psychiatric disorders. Neuropsychopharmacology 39:556–568

Tian P (2012) RELN gene polymorphisms and susceptibility to autism in Chinese Han population. Neurol India 60:581–584

Tissir F, Goffinet AM (2003) Reelin and brain development. Nature Rev Neurosci 4:496–505

Tochigi M, Kato C, Koishi S, Kawakubo Y, Yamamoto K, Matsumoto H, Hashimoto O, Kim SY, Watanabe K, Kano Y, Nanba E, Kato N, Sasaki T (2007) No evidence for significant association between GABA receptor genes in chromosome 15q11–q13 and autism in a Japanese population. J Hum Genet 52:985–989

Trotter J, Lee GH, Kazdoba TM, Crowell B, Domogauer J, Mahoney HM, Franco SJ, Müller U, Weeber EJ, D'Arcangelo G (2013) Dab1 is required for synaptic plasticity and associative learning. J Neurosci 33:15652–15668

Tuchman R, Rapin I (2002) Epilepsy in autism. Lancet Neurol 1:352–358

Tueting P, Costa E, Dwivedi Y, Guidotti A, Impagnatiello F, Manev R, Pesold C (1999). The phenotypic characteristics of heterozygous reeler mouse. NeuroReport 10:1327–1334

Tueting P, Doueiri MS, Guidotti A, Davis JM, Costa E (2006) Reelin down-regulation in mice and psychosis endophenotypes. Neurosci Biobehav Rev 30:1065–1077

Turner PR, O'Connor K, Tate WP, Abraham WC (2003) Roles of amyloid precursor protein and its fragments in regulating neural activity, plasticity and memory. Prog Neurobiol 70:1–32

Ventruti A, Kazdoba TM, Niu S, D'Arcangelo G (2011) Reelin deficiency causes specific defects in the molecular composition of the synapses in the adult brain. Neuroscience 189:32–42

Vincent JB, Thevarkunnel S, Kolozsvari D, Paterson AD, Roberts W, Scherer SW (2004) Association and transmission analysis of the FMR1 IVS10 + 14C-T variant in autism. Am J Med Genet B Neuropsychiatr Genet 125B:54–56

Vincent JB, Melmer G, Bolton PF, Hodgkinson S, Holmes D, Curtis D, Gurling HM (2005) Genetic linkage analysis of the X chromosome in autism, with emphasis on the fragile X region. Psychiatr Genet 15:83–90

Voineagu I, Wang X, Johnston P, Lowe JK, Tian Y, Horvath S, Mill J, Cantor RM, Blencowe BJ, Geschwind DH (2011) Transcriptomic analysis of autistic brain reveals convergent molecular pathology. Nature 474:380–384

Waldmeier PC, Kaupmann K, Urwyler S (2008) Roles of $GABA_B$ receptor subtypes in presynaptic and auto- and heteroreceptor function regulating GABA and glutamate release. J Neural Transm 115:1401–1411

Weeber EJ, Beffert U, Jones C, Christian JM, Forster E, Sweatt JD, Herz J (2002) Reelin and ApoE receptors cooperate to enhance hippocampal synaptic plasticity and learning. J Biol Chem 277:39944–39952

Westmark CJ, Westmark PR, Malter JS (2009) MPEP reduces seizure severity in Fmr-1 KO mice over expressing human Abeta. Int J Clin Exp Pathol 3:56–68

Yan QJ, Rammal M, Tranfaglia M, Bauchwitz RP (2005) Suppression of two major fragile X syndrome mouse model phenotypes by the mGluR5 antagonist MPEP. Neuropharmacology 49:1053–1066

Yip YP, Kronstadt-O'Brien P, Capriotti C, Cooper JA, Yip JW (2007a) Migration of sympathetic preganglionic neurons in the spinal cord is regulated by Reelin-dependent Dab1 tyrosine phosphorylation and CrkL. J Comp Neurol 502:635–643

Yip J, Soghomonian JJ, Blatt GJ (2007b) Decreased GAD67 mRNA levels in cerebellar Purkinje cells in autism: pathophysiological implications. Acta Neuropathologica 113:559–568

Yip J, Soghomonian JJ, Blatt GJ (2009) Decreased GAD65 mRNA levels in select subpopulations of neurons in the cerebellar dentate nuclei in autism: an in situ hybridization study. Autism Res 2:50–59

Yuskaitis CJ, Mines MA, King MK, Sweatt JD, Miller CA, Jope RS (2010) Lithium ameliorates altered glycogen synthase kinase-3 and behavior in a mouse model of fragile X syndrome. Biochem Pharmacol 79:632–646

Zhang H, Liu X, Zhang C, Mundo E, Macciardi F, Grayson DR, Guidotti AR, Holden JJ (2002) Reelin gene alleles and susceptibility to autism spectrum disorders. Mol Psychiatry 7:1012–1017

Chapter 17

The Role of Neurexins and Neuroligins in Autism

Amy C. Reichelt and James Dachtler

Abstract Autistic spectrum disorder (ASD) is a common, chronic psychiatric disorder for which the current generation of therapeutics are limited in their success at alleviating the neurobehaviors. While it is well established that both genetic and environmental factors contribute to the disorder, there is a lack of understanding about how ASD alters multiple domains of brain function. Identifying genes that are associated with ASD, and then relating how these genetic alterations affect brain structure and function is important to furthering our ability to design treatment and prevention strategies. Recent genome screening using copy number variant (CNV) analysis has identified deletions and duplications within the neurexin and neuroligin genes in patients with ASDs, highlighting their potential importance in ASD research. Neurexins and neuroligins are synaptic cell adhesion molecules and are found at the presynapse and postsynapse, respectively, of both excitatory and inhibitory cells. Neuroligins and leucine-rich repeat transmembranes bind to neurexins and convey a role in synaptic function and maintenance. However, little is known about how alterations within the genes encoding these proteins disrupt biological processes. Here we discuss the functional role of neurexins and neuroligins, the genetic evidence for their involvement in ASD and studies with transgenic mice to elucidate the consequences of these mutations.

Keywords Autism · neuroligin · neurexin · NRXN · NLGN · development · synaptic transmission

Autism spectrum disorder (ASD) is a heterogeneous grouping of neurodevelopmental disorders characterized by impairment in social interaction, verbal communication and repetitive/stereotypic behaviors. ASD is comorbid with a number of genetic diseases, such as Fragile X, Rett and Angelman Syndrome. These disorders

J. Dachtler (✉)
The Leeds Psychosis Research Group, School of Biomedical Sciences, University of Leeds, Leeds LS2 9JT, UK
e-mail: j.dachtler@leeds.ac.uk

A. C. Reichelt
Department of Psychology, School of Medical Sciences, University of New South Wales, Kensington, Sydney, NSW 2052, Australia

S. Hossein Fatemi (ed.), *The Molecular Basis of Autism,*
Contemporary Clinical Neuroscience, DOI 10.1007/978-1-4939-2190-4_17

have been successfully modeled in transgenic mice and have aided understanding of the molecular changes underlying autism. Recent advances in defining the molecular and cellular pathology of ASD point to altered patterns of neuronal connectivity in the developing brain as the neurobiological basis of these disorders. The recorded incidence of ASD has increased over the last 20 years by approximately 170% (Betancur 2011); however there is no agreed consensus of the underlying cause of the sudden increased prevalence (Fombonne 2003). The cost of ASD is borne by families of affected children involved in the caregiving and must financially support the behavioral and therapeutic interventions to ameliorate the social, communication and behavioral deficits associated with the disorder.

Autism is defined as a "spectrum disorder" because of its phenotypic variability but can be classified in three groups; autism, Asperger's syndrome and pervasive developmental disorder not otherwise specified (PDD-NOS). Heritability estimates using twins and family studies are approximately 90%, underlying the clear genetic basis of ASD (Bailey et al. 1995). Despite this, the underlying genetic mechanisms are far from clear. Human genetic studies about autism indicate that the disruption of synaptic components, particularly cell-adhesion molecules (CAMs), may contribute to the ASD phenotype (Arons et al. 2012; Aldinger et al. 2011). CAMs play a crucial function in synaptic development by initiating contact between pre- and postsynaptic cells, maintaining adhesion, and anchoring scaffolding proteins that assemble the essential components of a synapse. CAMs also determine the function of synapses, thus influencing the balance of excitatory and inhibitory (E/I) synapses formed within the brain.

Autism is generally diagnosed within the first three years of life, a time period during which intense experience-dependent circuit refinement takes place. Functional imaging studies in ASD have indicated that cortical dysfunction arises both from long-range "underconnectivity" between cortical areas and from short-range "overconnectivity", especially in frontal and temporal cortex (Geschwind and Levitt 2007; Just et al. 2007; Scott-Van Zeeland et al. 2010; Courchesne and Pierce 2005; Frith 2004). Hyperconnectivity of neuronal circuits due to increased synaptic protein synthesis is thought to cause ASDs and therefore cell adhesion molecules involved in synaptic stabilisation have been indicated as possible underlying pathologies. This may result in hyperconnectivity within cortical circuits, contributing to an excessive E/I ratio leading to disturbances in cortical signaling and sensory representations (Rubenstein and Merzenich 2003).

Over the last few years, genetic studies of large cohorts of patients with distinct brain disorders have been analyzed for CNVs (Merikangas et al. 2009), of which deletions eliminating exons of the neurexin-1 gene (*NRXN1*) have been implicated. Deletions affecting *NRXN1* have been found in cohorts of patients affected with a range of neurodevelopmental or neuropsychiatric disorders such as ASD, schizophrenia and mental retardation. Neurexins are therefore important in synaptic formation and function. Another family of cell-adhesion molecules, the neuroligins, are essential for the formation of functional neural synapses (Jamain et al. 2003; Talebizadeh et al. 2004). Neurexins and neuroligins align to form a trans-synaptic complex involved in the stabilization of synapses as shown in Fig. 17.1. In addition,

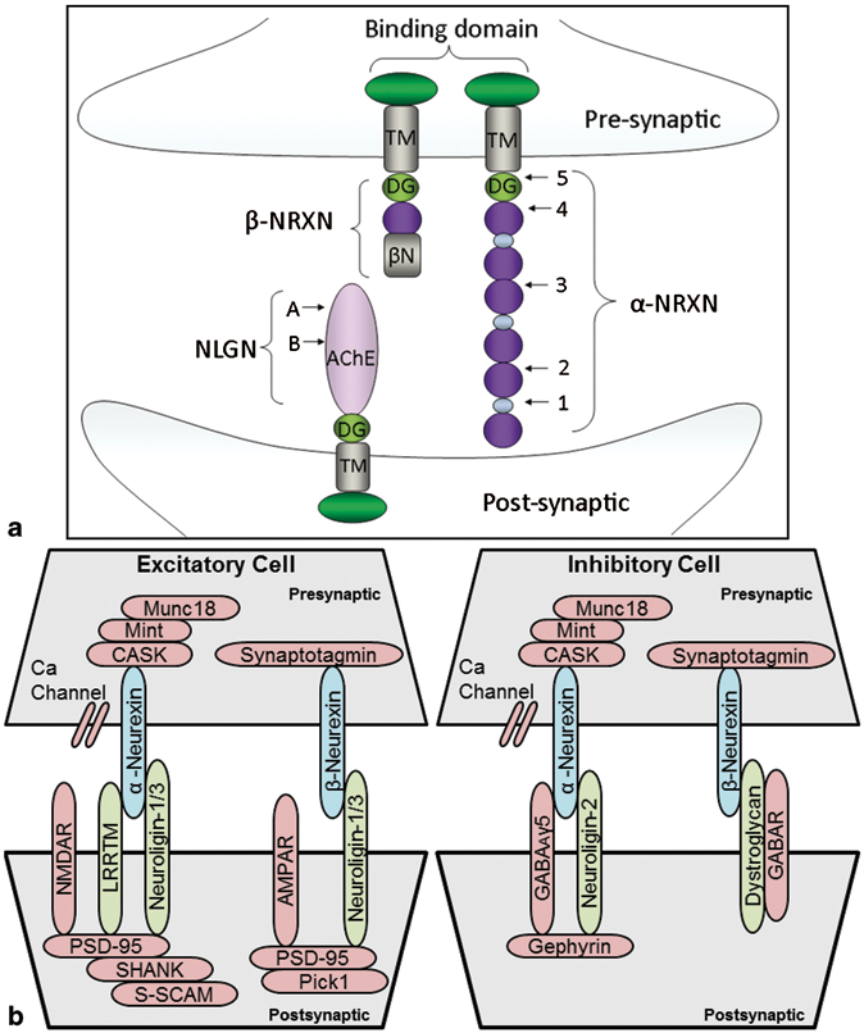


Fig. 17.1 **a**) Structure of a synapse showing interaction between pre-synaptic neurexins (α- and β-NRXN) and postsynaptic neuroligin (NLGN) allowing the formation of a trans-synaptic adhesion complex, detailing transmembrane proteins (TM) and dystroglycan binding region (DG) that terminate in PDZ-domain-binding sites (Binding domains). An upstream promoter is used to generate the larger α-neurexins and a downstream promoter generates the smaller β-neurexins. Thus, β-neurexins can be thought of as truncated α-neurexins that have a short β-specific leader (βN). α-Neurexins are composed of LNS domains (purple circles) and EGF-like domains (blue circles). Five sites of alternative splicing (*1–5*) are shown, which selectively regulate ligand interactions. Neuroligins contain an extracellular acetylcholinesterase (AChE)-homologous domain containing one or two sites of alternative splicing (A, plus B in the case of NLGN1). **b**) Examples of some of the binding partners of the neurexins depending upon whether the synapse is excitatory or inhibitory

leucine-rich repeat transmembranes (LRRTMs), a CAM similar to neuroligins, also interacts with NRXNs and disruptions within the LRRTM family of genes have been associated with ASD (Sousa et al. 2010) and schizophrenia (Francks et al. 2007). Different variants of neurexins (for example α or β-neurexins) are expressed at both inhibitory and excitatory synapses and through their linkage to a variety of both presynaptic and postsynaptic proteins (Fig. 17.1b), have a role in the mediation of neuronal synapse function (Sect. 17.2.1).

17.1 Neurexins, Neuroligins and Synaptic Function

The efficacy of synaptic transmission depends upon the release of transmitter from the presynaptic and the postsynaptic receptors that can receive the signal. On a local level, changes to this efficacy are termed synaptic plasticity, and it is well established that the alterations to proteins in either the presynapse or postsynapse can be deleterious to plasticity (Dachtler et al. 2011). On a neural network level, this synaptic efficacy reflects experience-dependent plasticity, whereby specific neural populations become more synchronous during task-related behavior. It is now known using fMRI techniques that ASD sufferers have deficiencies in this type of task-dependent neural synchrony (Gotts et al. 2012; Just et al. 2004).

Within the synapse, neurexins interact with postsynaptic neuroligins, LRRTMs and SHANK to regulate activity-dependent synaptogenesis and synapse maintenance (Scheiffele et al. 2000; Craig and Kang 2007). It is this dysfunction of neurexin and neuroligin proteins at the synapse that is hypothesized to disrupt the E/I balance during neurodevelopment and leads to aberrant connectivity both within and between cortical regions, leading to ASD phenotypes (LeBlanc and Fagiolini 2011; Qiu et al. 2011). We will explore how neurexins and neuroligins function, and discuss recent genetic evidence of mutations linking them to ASD and how these mutations are being modeled in mice.

17.1.1 Neurexin and Neuroligin Function

The mammalian genome contains three neurexin genes (*NRXN1*, *NRXN2* and *NRXN3*), each encoding an α- and a β-neurexin from independent promoters (Ichtchenko et al. 1996). α-Neurexins show evolutionary conservation throughout vertebrates, while β-neurexins do not (Tabuchi and Sudhof 2002; Li et al. 2007). α-Neurexins are larger than β-neurexins; the longer α-neurexins have six LNS (laminin/neurexin/sex hormone binding globulin) domains with three intercalated epidermal growth factor (EGF)-like domains, whereas β-neurexins have a single LNS domain as shown in Fig. 17.1a. The human protein molecular weights of the neurexin family (excluding various splice variants) are: NRXN1α-161.883 kDa; NRXN1β-46.861 kDa; NRXN2α-184.982 kDa; NRXN2β-70.927 kDa; NRXN3α-

180.599 kDa and NRXN3β-69.305 kDa. Neuroligins bind to both α- and β-neurexins, binding involving the sixth LNS domain of α-neurexins, which corresponds to the only LNS domain of β-neurexins (Comoletti et al. 2006).

In vertebrates, neurexins are synthesised throughout the brain in all excitatory and inhibitory neurons (Ichtchenko et al. 1995, 1996; Ullrich et al. 1995). While different α- and β-neurexins can be co-expressed in the same class of neuron, the exact expression is dependent upon the brain region of interest [for example, CA3 pyramidal cells of the hippocampus express all 6 neurexins (α- and β-neurexin-1–3), while CA1 pyramidal cells lack neurexin-1β and 3α] (Ullrich et al. 1995). However, each type of neurexin was found to be differentially distributed between different classes of neurons (Ichtchenko et al. 1996).

The functional role of the different classes of neurexins remains poorly understood, however strides have been made in recent years to elucidate their role. Part of the confusion is due to the complexity of the different variants of both neurexins and their postsynaptic partners (see Fig. 17.1) (Comoletti et al. 2006). There are five conserved splice sites within the NRXN genes, of which splice sites 1–4 involve short sequences (<30 residues) while others (i.e. splice site 5 of *NRXN3*) insert >100 residues that include multiple variants with in-frame stop codons (Ullrich et al. 1995; Ushkaryov and Sudhof 1993). It has been estimated that due to splicing, there could be anywhere from 600 to 3000 distinct neurexins (Ullrich et al. 1995). Neuroligins on the other hand, are only alternatively spliced at one canonical site (termed splice site A) except for neuroligin-1, which is also spliced at splice site B (Ichtchenko et al. 1995; Boucard et al. 2005). These of course will alter the binding characteristics. For example, α and β-neurexins can exist with or without an insert at S4. Neurexin-1α with or without the S4 insert binds to neuroligin-2 and -3 and but only binds to neuroligin-1 that lacks the B insert (Boucard et al. 2005).

Neurexin-1α and neurexin-1β intracellularly bind CASK, Mint, Munc18, syntenin and synaptotagmin (Hata et al. 1996; Biederer and Sudhof 2000; Grootjans et al. 2000), highlighting their potential importance for neurotransmitter release. It is likely that this direct action of neurexins is in part due to calcium influx through N and P/Q calcium channels (Missler et al. 2003; Zhang et al. 2005). There is now strong evidence that α-neurexins act through both excitatory and inhibitory cells, conveying a broader functional importance than first appreciated.

Manipulations of neurexins have been shown to alter the constituents of the postsynaptic terminal. Cells transfected with all three neurexins (both α and β) induce clustering of gephyrin (although there was stronger clustering by β-neurexins) only through neuroligin-2 and not neuroligin-1, 3 or 4, while α-neurexins failed to induce any clustering of PSD-95 (although β-neurexins did; Kang et al. 2008). The α-neurexins were selective in inducing clustering of the $GABA_A$ receptor γ5 but not α5 (Kang et al. 2008). Given that α-neurexins specifically induced clusters of gephyrin and $GABA_A$ receptor γ5 through neuroligin-2 (which is restricted to inhibitory synapses), it is plausible that α-neurexins mediate inhibitory function. Further evidence comes from hippocampal neurons transfected with neurexin-1α which resulted in a suppression of inhibitory postsynaptic currents (IPSCs) that was only counteracted when co-expressed with neuroligin-2 but not -1, confirming the theory

that neurexins mediate IPSCs by sequestration of neuroligin-2 (Zhang et al. 2010). This furthers the evidence of the selectivity of neuroligins; neuroligin-2 deletion has been shown to cause drastic reductions in unitary IPSCs from fast-spiking inhibitory interneurons (Gibson et al. 2009). In addition, a method of visually tagging neurexin-1α [pH-sensitive pHluorin (SEP)-tagged construct] that was expressed in parvalbumin positive (PV) interneurons during development found that neurexin-1α was localised diffusely throughout the axon and motile filopodia (Fu and Huang 2010). Neurexin-1α is freely diffusible and is involved in rapid exchange across presynaptic boutons, while the neurexin-1β turn-over rate suggests higher binding affinity to its postsynaptic neuroligin partner (Fu and Huang 2010). Theoretically, neurexin-1α could act to initially detect a postsynaptic binding site, while neurexin-1β stabilises the connection and promotes activity-dependent strengthening (Fu and Huang 2010).

There are five known neuroligins which are encoded by separate genes, which are localized in the human genome at 3q26 (*NLGN1*), 17p13 (*NLGN2*), Xq13 (*NLGN3*), Xp22.3 (*NLGN4* or *NLGN4X*), and Yq11.2 (*NLGN4Y*), respectively. The human protein molecular weights of the neuroligins are: NLGN1-93.835 kDa; NLGN2-90.820 kDa; NLGN3-93.895 kDa; NLGN4X-91.915 kDa and NLGN4Y-92.021 kDa. Functionally, neuroligins are essential for life as a triple knockout mice of neuroligin-1, 2 and 3 die shortly after birth because of respiratory failure; this is presumably due to reduced glutamatergic and GABAergic/glycinergic transmission in the brainstem (Varoqueaux et al. 2006). These mice were generated by the deletion of exon sequences covering the translation start site and several hundred base pairs of the 5′ coding sequence, which resulted in a lack of expression of either full length or truncated variants of any neuroligin (Varoqueaux et al. 2006). As previously suggested, the type of neuroligin isoform dictates whether it will be located in an excitatory or an inhibitory cell; neuroligin-2 is predominantly restricted to GABAergic inhibitory synapses, neuroligin-1 favors glutamatergic excitatory synapses while neuroligin-3 appears present at both (Varoqueaux et al. 2004; Song et al. 1999; Budreck and Scheiffele 2007). Neuroligin-1 overexpression increases excitatory responses and potentiates NMDA/AMPA receptor ratios, while neuroligin-2 overexpression enhances inhibitory synaptic responses (or vice versa when deleted) (Chubykin et al. 2007). However, the presence of neuroligins do not seem necessary for synapse establishment. Deletion of neuroligin-1, 2 and 3 or activity blockade using antagonists of NMDA receptors or CaMKII do not alter overall synaptogenesis or synaptic morphology (Chubykin et al. 2007; Varoqueaux et al. 2006), suggesting a role in activity-dependent function. Within the postsynaptic terminal, neuroligins are able to interact with PSD-95, gephyrin, Shank3, Pick1, and S-SCAM (for review see Craig and Kang (2007)). Recent evidence highlights the functional importance of these interactions as Shank3 through its neurexin/neuroligin complex can infer widespread changes to pre and postsynaptic protein composition and regulate AMPA and NMDA receptor signaling (Arons et al. 2012).

In addition to neuroligins, dystroglycan binds either to the common LNS domain of both α and β-neurexins or to the second LNS domain of α-neurexins (Sugita et al. 2001). Dystroglycan has been shown to be important for normal neuronal function as a knockout mouse model exhibits impaired synaptic plasticity (Moore et al. 2002). Despite this and its localisation to a subset of GABAergic cells, its functional

link to neurexins remains unclear. Dystroglycan binding to neurexins had no effect upon the neurexin suppression of IPSCs (Zhang et al. 2010), nor when using synaptic imaging was there evidence that either α or β-neurexins could induce clustering of dystroglycan (Kang et al. 2008). Taken together, further work is required to fully understand the role of neurexin and dystroglycan binding.

Despite the evidence that α-neurexins act through the inhibitory system, there are examples where alterations to α-neurexins modulate glutamatergic function. Whilst there were no reductions in protein levels of NMDA receptors or GluA1 receptors of newborn triple α-neurexin knockout mice compared to wild-types, the triple knockout mice had reduced NMDA receptor currents, suggesting that the normal function of NMDA receptors requires α-neurexins (Kattenstroth et al. 2004). In addition, neurexin-1α knockout mice have reduced mini excitatory but not inhibitory postsynaptic current frequency and weaker excitatory synaptic strength (Etherton et al. 2009).

Recent discoveries have identified a new postsynaptic molecule through which neurexins can act, named LRRTMs, which are also localised to the glutamatergic network. α-Neurexins are able to bind to LRRTM1 and LRRTM2, but only when the neurexins lack the S4 insert (Siddiqui et al. 2010). When this binding occurs, PSD-95 is corecruited along with LRRTM2. Interestingly, using shRNA to knockdown LRRTM2, a 40% reduction in hippocampal excitatory synapse density was found with no effects upon inhibitory synapse density, along with deficits in excitatory synaptic transmission (de Wit et al. 2009). This is perhaps not surprising given that GluA1, GluA2 and GluN1 all interact with LRRTM2 (de Wit et al. 2009). Similar reductions in AMPA receptor mediated transmission have been noted by LRRTM1 and 2 knockdown during early development (Soler-Llavina et al. 2011). However, there is evidence that LRRTM knockdown in more mature brain does not cause significant alterations to synaptic transmission (Soler-Llavina et al. 2011).

17.2 NRXN1 Mutations in Humans

The neurexins are a family of pre-synaptic cell adhesion proteins encoded by paralogous genes (*NRXN1-3*). Neurexins exist as two proteins; a longer α and a shorter β isoform, encoded by separate promoters. *NRXN1* is located on chromosome 2p16.3 of which α and β neurexins have distinct extracellular domains. The promoter for neurexin-1α lies upstream of exon 1, while the promoter for neurexin-1β is located in the intron downstream of exon 17 (Rowen et al. 2002).

17.2.1 Neurexins in Autistic Spectrum Disorder

17.2.1.1 NRXN1 Mutations and Autism

Since most of the ASD-associated deletions in *NRXN1* are predicted to affect the expression of neurexin-1α, but leave neurexin-1β coding sequences intact, elucidating

the function of the neurexin-1α form is particularly pertinent to understanding the pathogenesis of disease. We have previously undertaken a review of the literature outlining *NRXN1* deletions and their association with ASD [see Reichelt et al. (2012)]. Here we will briefly recap these findings and supplement them with the latest data.

The original description of *NRXN1* alterations and its link to ASD can be found in Friedman et al. (2006). The authors discovered a de novo deletion affecting the promoter and exons 1–5 of neurexin-1α in a 7-year old boy. The child suffered autistic traits, cognitive impairment, vertebral anomalies and mild facial dysmorphism (Friedman et al. 2006). Further screening by the Autism Genome Project Consortium of 1181 families (where a minimum of two family members suffered with ASD) found an additional de novo deletion (300 kb in size) affecting two female siblings that displayed typical autism and mental retardation (Autism Genome Project et al. 2007). Glessner et al. (2009) also screened 859 sufferers of ASD and confirmed inherited deletions within *NRXN1*, as did Marshall et al. (2008), although the latter also found *NRXN1* deletions within their control population. A comprehensive review of 12 subjects with exonic *NRXN1* deletions was undertaken by Ching et al. (2010) linking the size and location of deletion to the patients clinical symptomatology.

Current evidence suggests that ASD is associated with heterozygous de novo or inherited deletions or mutations; there is only one documented example of someone being homozygous for *NRXN1*. Zweier et al. (2009) discovered compound heterozygosity for two mutations in *NRXN1*; a deletion spanning exons 1–4 from the healthy mother and a stop mutation in exon 15 on the second allele from the healthy father. The female suffered with Pitt-Hopkins-like syndrome-2, autistic traits, mental retardation, hyperbreathing and developmental regression (Zweier et al. 2009).

Recent publications have furthered the evidence that *NRXN1* mutations are associated with the development of ASD, solidifying their importance and the need for further research to understand their impact upon biological pathways. Gauthier et al. (2011), who within this important study also found a deletion in *NRXN2*, noted a rare heterozygous S14L missense mutation within *NRXN1* in a patient diagnosed with ASD that was inherited from his mother. This particular missense mutation is of growing interest as it has been found in four ASD sufferers, but not in 1201 controls (Gauthier et al. 2011). Similar screening work has been conducted using a Chinese Han population. Out of 313 patients suffering with autism, 7 missense mutations, 3 deletions and 12 synonymous mutations were found (Liu et al. 2012). However, when compared to 500 controls, only one single nucleotide polymorphism (SNP), the P300P (rs2303298), was significantly associated with autism (Liu et al. 2012). The functional result of these missense mutations has seldom been investigated in patients beyond what clinical symptoms (mostly neurobehavioral) they display.

Voineskos et al. (2011) studied people with SNPs (rs1045881 and rs858932) within *NRXN1* which were demonstrated to be predictive of reduced frontal white matter and thalamic volume visualized through MRI scanning and sensorimotor

function. Interestingly, these SNPs were found in healthy volunteers, raising the hypothesis that those and similar mutations within *NRXN1* can explain the aberrant neural development that can lead to ASD.

In addition to the reports of SNPs within *NRXN1*, further examples of CNVs have been described. Schaaf et al. (2012) employed array comparative genomic hybridization in 8051 patients and found 24 had intragenic deletions of *NRXN1*. Deletions varied in size from 17 to 913 kb, which resulted in the deletion of between 2 and 13 exons, more frequently affecting the N-terminal domain of neurexin-1 (and hence *NRXN1α*) (Schaaf et al. 2012). However, in four cases the deletions did affect both *NRXN1α* and *β*. The authors provide interesting analysis of the clinical symptomology of those with these deletions; 93% had intellectual disability, 59% infantile hypotonia and 56% had ASD (Schaaf et al. 2012). Of those that had inherited the CNVs, 89% of their parents also displayed learning issues and/or neuropsychiatric disorders (Schaaf et al. 2012). Dabell et al. (2013) undertook an even greater screening study; out of 30,065 patients, 35 had exonal deletions within *NRXN1*, the majority clustering over the 5′ region of the gene and affecting the first four coding exons, which would disrupt neurexin-1α. Of those patients, 43% were diagnosed with ASD while 16% had epilepsy (Dabell et al. 2013). Again, Curran et al. (2013) found deletions within *NRXN1* that predominantly affected neurexin-1α in their screening efforts, although the patients had a broader range of neuropsychiatric phenotypes.

Whilst the majority of mutations associated with ASD have been discovered within neurexin-1α, neurexin-1β has gained recent prominence. The coding exons of 86 patients diagnosed with autism and mental retardation were sequenced and compared to 200 ethnically matched controls. Four novel point mutations were discovered, two being within exon 18 (c. −3G>T and p.MET1) affecting the initiation codon and are specific to *NRXN1β* (Camacho-Garcia et al. 2012). The other two point mutations were located within exon 24 at positions 1124 and 1132 and are common to both neurexin-1α and β (Camacho-Garcia et al. 2012). All four patients harboring these inherited mutations had autism and profound mental retardation, some comorbid with other phenotypes such as seizures. Family members were also screened, although inheritance was variable and they exhibited broad neurobehavioral phenotypes that were not uniformly autism (Camacho-Garcia et al. 2012).

While polymorphisms and CNVs are now well established as causative factors of developing ASD, it is interesting to note that it is often comorbid with other neurobehavioral phenotype(s) such as developmental delay, learning difficulties or epilepsy (Schaaf et al. 2012; Curran et al. 2013). Hence, the genetic alterations alone are unlikely to fully explain why one may develop ASD. Exciting research is currently furthering our understanding of how gene/gene and gene/environment interactions influence the etiology of neuropsychiatric disorders, and it will be of great interest to pursue these questions in relation to neurexins. Further work is also required to understand how these alterations disrupt the biological pathways through which neurexins mediate their synaptic function.

17.2.1.2 NRXN2 and 3 Mutations in Autism

Exciting evidence has come to light that dispels earlier theories that ASD was only linked to mutations within *NRXN1*. A 2011 study found a deletion located in exon 12 of *NRXN2* in a boy of European ancestry with an ADOS-G score of 21 (autism cut-off = 12), inherited from his father who had severe language delay (Gauthier et al. 2011). The mutation removes LNS6 of neurexin-2, and a cell binding assay shows deficient binding to neuroligin-2 and LRRTM2 (Gauthier et al. 2011). This was in addition to ten disease-cohort specific missense variants in *NRXN2* and *NRXN3* from sporadic cases (Gauthier et al. 2011). Furthermore, a 21-year old patient with speech problems, autistic traits and dysmorphic facial features was found to have a 0.57 Mb deletion in chromosome 11q13.1, which contained *NRXN2* (Mohrmann et al. 2011). Neurexin-3 has also been recently associated with ASD. Four patients diagnosed with ASD (of varying severity) had either inherited or possessed de novo microdeletions at chromosome 14q24.3-31.1 (Vaags et al. 2012). These first insights into how neurexin-2 and 3 mutations can result in ASD highlights the importance of understanding how all neurexins support cellular function and behavior.

17.2.1.3 Neurexin Mutations and Other Disorders

Although alterations to the NRXN1 gene are well established with ASD, it is arguable that associations with schizophrenia (SZ) are equally well proven. The first evidence for the involvement of *NRXN1* in SZ came in 2008 when Kirov et al. (2008) found a heterozygous deletion of the promoter and exon 1 of neurexin-1α in schizophrenic siblings. Walsh et al. (2008) noted deletions that eliminated exons that were common to both neurexin-1α and β. Further examples are discussed in Reichelt et al. (2012).

More recently a de novo heterozygous frameshift mutation occurring in *NRXN1*, affecting both NRXN1α and NRXN1β, from a female diagnosed with disorganised SZ was discovered (Gauthier et al. 2011). In cell binding assays, this mutation resulted in a functional deficiency in surface trafficking and binding of NRXN1 to LRRTM2 and neuroligin-2 (Gauthier et al. 2011), highlighting the effects that *NRXN1* mutations can have upon the excitatory and inhibitory networks. It was estimated in 2009 that 0.19% of schizophrenia cases (17/8789) are associated with *NRXN1* deletions (Kirov et al. 2009).

Despite the strong links to ASD and SZ, *NRXN1* mutations have been linked to other disorders. A screen of idiopathic generalised epilepsy sufferers found 0.3% had exon disrupting deletions within *NRXN1*, all within the promoter and/or the first exons (Moller et al. 2013). In addition, alterations in *NRXN3* have now been associated with SZ and other neuropsychiatric disorders. Within a Chinese Han population, SNPs within *NRXN3* were associated with SZ (rs7157669; rs724373 and rs7154021) (Hu et al. 2013). Other SNPs within *NRXN3* have been linked to borderline personality disorder (Panagopoulos et al. 2013). Taken together, these

examples highlight the importance of the neurexins upon normal neuropsychiatric function and the broad range of disorders when mutations occur.

17.3 Neuroligin Mutations in Humans

Deletions or point mutations in neuroligin genes are found in patients with ASD or mental retardations. The observation that ASD is 4 times more likely to occur in boys than in girls (Lintas and Persico 2009; Baron-Cohen et al. 2005) and that three out of five neuroligin genes (*NLGN3-4X&Y*) are located on the sex chromosomes may provide a rationale for the prevalence of ASD in boys.

17.3.1 Neuroligins in Autistic Spectrum Disorder

The postsynaptic adhesion molecule neuroligin-3 is localized at both excitatory and inhibitory synapses (Budreck and Scheiffele 2007). Less is known about the function of neuroligin-4, partly because the murine *Nlgn4* differs dramatically from *NLGN4* in other species and even between different mouse strains, limiting research opportunities (Bolliger et al. 2008). Of the mouse neuroligin-4, tagging has shown that, like other neuroligins, it is postsynaptic and is transported into dendritic spines (Bolliger et al. 2008). Like neuroligin-2, mouse neuroligin-4 appears specific to the inhibitory network. NLGN4 is widely expressed in the brain, however they are found associated with gephyrin and not PSD-95 (Hoon et al. 2011).

A study of Swedish families with autistic children identified a point mutation (R451C) replacing arginine with a cysteine in the extracellular portion of neuroligin-3 in two brothers, one with typical autism and the other with Asperger's syndrome (Jamain et al. 2003). In addition this study also identified a frameshift mutation in neuroligin-4 in another set of autistic brothers (Varoqueaux et al. 2006). Further missense mutations within *NLGN4X* have been demonstrated in a Portuguese cohort of ASD patients (Yan et al. 2005), a Greek patient with mild autism (Pampanos et al. 2009), a R87W mutation in two brothers with ASD (Zhang et al. 2009) as well as a frameshift mutation in a French family suffering a range of psychiatric disorders including autism (Laumonnier et al. 2004). A missense mutation has also been found within *NLGN4Y* (Yan et al. 2005). In addition, CNVs within *NLGN4* of autistic patients have also been reported (Marshall et al. 2008). Talebizadeh et al. (2006) discovered a novel truncated form of *NLGN3* within autistic and control samples, in addition to a de novo truncated form of *NLGN4X* only found in an autistic female, leading to the suggestion that mutations altering the expressing of these truncated proteins can modulate the ASD phenotype. Recent screening of *NLGN3* and *NLGN4X* in 144 male ASD patients has found variants in the highly conserved 3' untranslated region (UTR) and intronic variants at conserved transcription factor binding sites (Steinberg et al. 2012). Two intronic variants in

NLGN3 were located at transcription sites and segregated with autism, but none of the 3' UTR variants (one in *NLGN3* and two in *NLGN4X*) significantly affected neuroligin-3 or -4 expression (Steinberg et al. 2012).

Mutations within neuroligin-1 have also been found. Glessner et al. (2009) found a duplication of CNVs within *NLGN1* in autistic patients compared to controls, while Ylisaukko-oja et al. (2005) reported a mutation (rs1488545) within a Finnish autistic cohort. A 6.5-year old female with developmental delay and a range of neurobehavioral phenotypes was found to harbor an inherited 2.1 Mb deletion (3q26.31–3q26.32) that included *NAALADL2* and the 3′ end of *NLGN1* (Millson et al. 2012). Alterations to neuroligin-2 may also have links to neurodevelopment. A patient with mental retardation, afebrile seizure and dysmorphic features had a de novo 790–830 kb duplication on chromosome 17p13.1, of which one gene that was affected was *NLGN2*, providing the first evidence of neuroligin-2 and genetic disorders (Belligni et al. 2012).

Neuroligin production falls under the control of translation factors and mutations of translation factors upstream (i.e. mTOR/rapamycin) and downstream (translation factors) are likely to alter the expression of neuroligins at the synapse. Gkogkas et al. (2013) indicated that that deletion of the gene [4E-binding protein 2 (*4E-BP2*)] encoding the translational repressor eukaryotic translation initiation factor 4E (eIF4E), a key factor for protein translation initiation of which mutations have been associated with autism, disrupted synaptic transmission and produced ASD-related behavioral deficits in mice, that could be rescued by deletion of *NLGN1*. Hence, alterations in the neuroligins, either directly or in proteins that affect neuroligin expression, can produce phenotypes that reflect ASD.

However, it is important to note that analysis of *NLGN1*, *NLGN3*, *NLGN4X*, and *NLNG4Y* suggests that neuroligin mutations represent only rare causes of autism (Ylisaukko-oja et al. 2005).

17.4 Behavioral Phenotypes of Neurexin and Neuroligin Mouse Models

17.4.1 Neurexin Mutations

Given that mutations and microdeletions have been strongly associated with ASD, transgenic mouse models have been utilized to understand the impact of these mutations upon behavioral phenotypes. This has been made possible with the development of mice that have a microdeletion within the promoter and exon 1 of *Nrxn1α* (Geppert et al. 1998).

Homozygous *Nrxn1α* mice have been tested in a range of behavioral paradigms. While homozygous deletions of *NRXN1* have only been documented once (Zweier et al. 2009) and heterozygous deletions are more commonly associated with ASD, knockout mice may provide a clearer behavioral phenotype from which to inform

future research. As such, 2–6 month old *Nrxn1α* knockout mice and their wild-type littermates were tested and found to have reduced prepulse inhibition (PPI), indicating sensorimotor gating deficiencies, increased grooming behaviors and impaired nest building (Etherton et al. 2009). The sensorimotor defects have been demonstrated in ASD patients (Perry et al. 2007), although it is a rare phenotype of ASDs, while the increased grooming and altered nest building are suggested to represent increased stereotyped patterns of behavior and a lack of social care, respectively, which are core features of ASD. Despite this, these mice were unaffected in social behaviors with other mice and had 'normal' spatial learning in the watermaze (Etherton et al. 2009). The lack of sociability deficits in *Nrxn1α* knockout mice, the major behavioral domain of ASD, would suggest that these mice only partially recapitulate aspects of an ASD-like phenotype. However, female *Nrxn1α* knockout mothers exhibit less care for their litter, regardless of genotype (Geppert et al. 1998). When compared to their normal performance in gross measures of sociability, *Nrxn1α* knockout may only produce a very specific form of social impairment.

Further behavioral characterization has been performed in heterozygous *Nrxn1α* mice. The use of heterozygous neurexin-1 mice is of interest, given that this type of deletion is associated with ASDs. Heterozygous male *Nrxn1α* knockout mice were hyperactive when exposed to a novel arena, although they habituated with repeated exposures, while female heterozygous mice were similar to wild-types (Laarakker et al. 2012). Male heterozygous *Nrxn1α* knockout mice also show long-term object discrimination memory, but only during the first 5 min of the 10 min test phase; the discrimination for the latter 5 min was significantly impaired (Laarakker et al. 2012). Sex-dependent behavioral effects of *Nrxn1α* deletions would corroborate the male biased expression of autism, although as yet the underlying mechanism remains unresolved. The lack of characterization of *Nrxn1α* knockout mice, especially in tests of cognition, highlights that further work is required to fully understand the roles of neurexin-1. As yet there has been no behavioral characterization of neurexin-2 or -3 mice, and given the recent association of *NRXN2* and *3* with ASD, we look forward to research that addresses this.

17.4.2 Neuroligin Mutations

The importance of understanding the behavioral consequences of neuroligin mutations is two-fold. First, genetic screens of ASD patients have found deletions and mutations within the neuroligin genes, so modeling these in mice to determine their function is crucial. Second, neuroligins bind to neurexins, and since neurexin mutations are also linked to ASD, behavioral research will help to clarify the functional links between neurexins and neuroligins.

Nlgn1 knock-out mice display deficits in spatial learning and memory in the Morris water maze which was hypothesized to be related to impaired hippocampal long-term potentiation (Blundell et al. 2010). These mice exhibit a dramatic increase in repetitive, stereotyped grooming behavior and impaired nest building,

characterized as ASD-relevant abnormalities. However, minimal social deficits were observed and anxiety-like behaviors, locomotor activity and PPI were normal in these mice (Blundell et al. 2010).

As discussed previously, dysfunction of the eIF4E repressor by *4E-BP2* knockout increased translation of neuroligin-1, impacting upon inhibitory and excitatory synaptic transmission (Gkogkas et al. 2013). The *4E-BP2* knockout mice displayed ASD-related behaviors including reduced sociability and social memory, altered communication, and increased repetitive behaviors (Gkogkas et al. 2013).

Although there is little evidence for the association of *NLGN2* mutations with ASDs, *Nlgn2* knockout mice have nevertheless been behaviorally characterized. These mice expressed an anxiety phenotype, with deceased time spent in the center of an open field arena and anxiety-like behavior in the dark/light box, although their other social behaviors were normal (Blundell et al. 2009).

The *Nlgn3* (neuroligin-3 knock in) mouse carries the R451C mutation in the NLGN3 gene reported in a family affected with ASD (Jamain et al. 2003). In addition to altered synaptic transmission, these mice showed reduced social interactions with a novel mouse while also having enhanced spatial learning in the Morris water maze (Tabuchi et al. 2007). However, two studies have been unable to replicate these results. Chadman et al. (2008) found no differences in sociability across several different paradigms in *Nlgn3* (R451C) mice, while Karvat and Kimchi (2012) also found no difference in social interaction or stereotyped behaviors. *Nlgn3* knockout mice have shown phenotypes relevant to ASD, including impaired social memory and olfaction, hyperactivity in an open field and reduced fear memory (Radyushkin et al. 2009). Finally, *Nlgn4* knockout mice also exhibit reduced sociability and social memory in a three chambered arena, in addition to reduced ultrasonic vocalisations upon exposure to a novel mouse (Jamain et al. 2008).

17.5 Conclusions

Research over the previous 15 years has found that presynaptic neurexins and their postsynaptic binding partners are prime candidates responsible for the organization of synaptic proteins and strengthening of neural transmission. However, the intricacy of how the neurexin/neuroligin/LRRTM complexes mediate their function is more complicated that first envisaged. Given that mutations in *NRXN*, *NLGN* and *LRRTM* have now been associated with autism and other neuropsychiatric disorders, the importance of determining the functional roles of these CAMs is vital. Mouse models containing microdeletions within these genes have shown neuropsychiatric-like behaviors and offer useful tools for future investigations. However, further fundamental research is needed; little is known about how these CAMs function *in vivo* or in the adult animal, partly since the literature is dominated by cell culture experiments which can limit their interpretations. Studies examining the possible rescue of aberrant behavioral or synaptic transmission phenotypes in

knockout mouse models by pharmaceutical intervention are vital to the challenge of developing therapies for patients afflicted with these genetic alterations.

References

Aldinger KA, Plummer JT, Qiu S, Levitt P (2011) SnapShot: genetics of autism. Neuron 72:418

Arons MH, Thynne CJ, Grabrucker AM, Li D, Schoen M, Cheyne JE, Boeckers TM, Montgomery JM, Garner CC (2012) Autism-associated mutations in ProSAP2/Shank3 impair synaptic transmission and neurexin-neuroligin-mediated transsynaptic signaling. J Neurosci 32:14966–14978

Autism Genome Project C, Szatmari P, Paterson AD, Zwaigenbaum L, Roberts W, Brian J, Liu XQ, Vincent JB, Skaug JL, Thompson AP, Senman L, Feuk L, Qian C, Bryson SE, Jones MB, Marshall CR, Scherer SW, Vieland VJ, Bartlett C, Mangin LV, Goedken R, Segre A, Pericak-Vance MA, Cuccaro ML, Gilbert JR, Wright HH, Abramson RK, Betancur C, Bourgeron T, Gillberg C, Leboyer M, Buxbaum JD, Davis KL, Hollander E, Silverman JM, Hallmayer J, Lotspeich L, Sutcliffe JS, Haines JL, Folstein SE, Piven J, Wassink TH, Sheffield V, Geschwind DH, Bucan M, Brown WT, Cantor RM, Constantino JN, Gilliam TC, Herbert M, Lajonchere C, Ledbetter DH, Lese-Martin C, Miller J, Nelson S, Samango-Sprouse CA, Spence S, State M, Tanzi RE, Coon H, Dawson G, Devlin B, Estes A, Flodman P, Klei L, McMahon WM, Minshew N, Munson J, Korvatska E, Rodier PM, Schellenberg GD, Smith M, Spence MA, Stodgell C, Tepper PG, Wijsman EM, Yu CE, Roge B, Mantoulan C, Wittemeyer K, Poustka A, Felder B, Klauck SM, Schuster C, Poustka F, Bolte S, Feineis-Matthews S, Herbrecht E, Schmotzer G, Tsiantis J, Papanikolaou K, Maestrini E, Bacchelli E, Blasi F, Carone S, Toma C, Van Engeland H, de Jonge M, Kemner C, Koop F, Langemeijer M, Hijmans C, Staal WG, Baird G, Bolton PF, Rutter ML, Weisblatt E, Green J, Aldred C, Wilkinson JA, Pickles A, Le Couteur A, Berney T, McConachie H, Bailey AJ, Francis K, Honeyman G, Hutchinson A, Parr JR, Wallace S, Monaco AP, Barnby G, Kobayashi K, Lamb JA, Sousa I, Sykes N, Cook EH, Guter SJ, Leventhal BL, Salt J, Lord C, Corsello C, Hus V, Weeks DE, Volkmar F, Tauber M, Fombonne E, Shih A, Meyer KJ (2007) Mapping autism risk loci using genetic linkage and chromosomal rearrangements. Nat Genet 39:319–328

Bailey A, Le Couteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E, Rutter M (1995) Autism as a strongly genetic disorder: evidence from a British twin study. Psychol Med 25:63–77

Baron-Cohen S, Knickmeyer RC, Belmonte MK (2005) Sex differences in the brain: implications for explaining autism. Science 310:819–823

Belligni EF, Di Gregorio E, Biamino E, Calcia A, Molinatto C, Talarico F, Ferrero GB, Brusco A, Silengo MC (2012) 790 Kb microduplication in chromosome band 17p13.1 associated with intellectual disability, afebrile seizures, dysmorphic features, diabetes, and hypothyroidism. Eur J Med Genet 55:222–224

Betancur C (2011) Etiological heterogeneity in autism spectrum disorders: more than 100 genetic and genomic disorders and still counting. Brain Res 1380:42–77

Biederer T, Sudhof TC (2000) Mints as adaptors. Direct binding to neurexins and recruitment of munc18. J Biol Chem 275:39803–39806

Blundell J, Tabuchi K, Bolliger MF, Blaiss CA, Brose N, Liu X, Sudhof TC, Powell CM (2009) Increased anxiety-like behavior in mice lacking the inhibitory synapse cell adhesion molecule neuroligin 2. Genes Brain Behav 8:114–126

Blundell J, Blaiss CA, Etherton MR, Espinosa F, Tabuchi K, Walz C, Bolliger MF, Sudhof TC, Powell CM (2010) Neuroligin-1 deletion results in impaired spatial memory and increased repetitive behavior. J Neurosci 30:2115–2129

Bolliger MF, Pei J, Maxeiner S, Boucard AA, Grishin NV, Sudhof TC (2008) Unusually rapid evolution of Neuroligin-4 in mice. Proc Nat Acad Sci U S A 105:6421–6426

Boucard AA, Chubykin AA, Comoletti D, Taylor P, Sudhof TC (2005) A splice code for trans-synaptic cell adhesion mediated by binding of neuroligin 1 to alpha- and beta-neurexins. Neuron 48:229–236

Budreck EC, Scheiffele P (2007) Neuroligin-3 is a neuronal adhesion protein at GABAergic and glutamatergic synapses. Eur J Neurosci 26:1738–1748

Camacho-Garcia RJ, Planelles MI, Margalef M, Pecero ML, Martinez-Leal R, Aguilera F, Vilella E, Martinez-Mir A, Scholl FG (2012) Mutations affecting synaptic levels of neurexin-1beta in autism and mental retardation. Neurobiol Dis 47:135–143

Chadman KK, Gong S, Scattoni ML, Boltuck SE, Gandhy SU, Heintz N, Crawley JN (2008) Minimal aberrant behavioral phenotypes of neuroligin-3 R451C knockin mice. Autism Res 1:147–158

Ching MS, Shen Y, Tan WH, Jeste SS, Morrow EM, Chen X, Mukaddes NM, Yoo SY, Hanson E, Hundley R, Austin C, Becker RE, Berry GT, Driscoll K, Engle EC, Friedman S, Gusella JF, Hisama FM, Irons MB, Lafiosca T, LeClair E, Miller DT, Neessen M, Picker JD, Rappaport L, Rooney CM, Sarco DP, Stoler JM, Walsh CA, Wolff RR, Zhang T, Nasir RH, Wu BL, Children's Hospital Boston Genotype Phenotype Study G (2010) Deletions of NRXN1 (neurexin-1) predispose to a wide spectrum of developmental disorders. Am J Med Genet B Neuropsychiatri Genet 153B:937–947

Chubykin AA, Atasoy D, Etherton MR, Brose N, Kavalali ET, Gibson JR, Sudhof TC (2007) Activity-dependent validation of excitatory versus inhibitory synapses by neuroligin-1 versus neuroligin-2. Neuron 54:919–931

Comoletti D, Flynn RE, Boucard AA, Demeler B, Schirf V, Shi J, Jennings LL, Newlin HR, Sudhof TC, Taylor P (2006) Gene selection, alternative splicing, and post-translational processing regulate neuroligin selectivity for beta-neurexins. Biochemistry 45:12816–12827

Courchesne E, Pierce K (2005) Why the frontal cortex in autism might be talking only to itself: local over-connectivity but long-distance disconnection. Curr Opin Neurobiol 15:225–230

Craig AM, Kang Y (2007) Neurexin–neuroligin signaling in synapse development. Curr Opin Neurobiol 17:43–52

Curran S, Ahn J, Grayton H, Collier D, Ogilvie C (2013) NRXN1 deletions identified by array comparative genome hybridisation in a clinical case series—further understanding of the relevance of NRXN1 to neurodevelopmental disorders. J Mol Psychiatry 1:1–7

Dabell MP, Rosenfeld JA, Bader P, Escobar LF, El-Khechen D, Vallee SE, Dinulos MB, Curry C, Fisher J, Tervo R, Hannibal MC, Siefkas K, Wyatt PR, Hughes L, Smith R, Ellingwood S, Lacassie Y, Stroud T, Farrell SA, Sanchez-Lara PA, Randolph LM, Niyazov D, Stevens CA, Schoonveld C, Skidmore D, MacKay S, Miles JH, Moodley M, Huillet A, Neill NJ, Ellison JW, Ballif BC, Shaffer LG (2013) Investigation of NRXN1 deletions: clinical and molecular characterization. Am J Med Genet A 161:717–731

Dachtler J, Hardingham NR, Glazewski S, Wright NF, Blain EJ, Fox K (2011) Experience-dependent plasticity acts via GluR1 and a novel neuronal nitric oxide synthase-dependent synaptic mechanism in adult cortex. J Neurosci 31:11220–11230

de Wit J, Sylwestrak E, O'Sullivan ML, Otto S, Tiglio K, Savas JN, Yates JR 3rd, Comoletti D, Taylor P, Ghosh A (2009) LRRTM2 interacts with Neurexin1 and regulates excitatory synapse formation. Neuron 64:799–806

Etherton MR, Blaiss CA, Powell CM, Sudhof TC (2009) Mouse neurexin-1alpha deletion causes correlated electrophysiological and behavioral changes consistent with cognitive impairments. Proc Nat Acad Sci U S A 106:17998–18003

Fombonne E (2003) Epidemiological surveys of autism and other pervasive developmental disorders: an update. J Autism Dev Disord 33:365–382

Francks C, Maegawa S, Lauren J, Abrahams BS, Velayos-Baeza A, Medland SE, Colella S, Groszer M, McAuley EZ, Caffrey TM, Timmusk T, Pruunsild P, Koppel I, Lind PA, Matsumoto-Itaba N, Nicod J, Xiong L, Joober R, Enard W, Krinsky B, Nanba E, Richardson AJ, Riley BP, Martin NG, Strittmatter SM, Moller HJ, Rujescu D, St Clair D, Muglia P, Roos JL, Fisher SE, Wade-Martins R, Rouleau GA, Stein JF, Karayiorgou M, Geschwind DH, Ragoussis J, Kendler

KS, Airaksinen MS, Oshimura M, DeLisi LE, Monaco AP (2007) LRRTM1 on chromosome 2p12 is a maternally suppressed gene that is associated paternally with handedness and schizophrenia. Mol Psychiatry 12:1129–1139

Friedman JM, Baross A, Delaney AD, Ally A, Arbour L, Armstrong L, Asano J, Bailey DK, Barber S, Birch P, Brown-John M, Cao M, Chan S, Charest DL, Farnoud N, Fernandes N, Flibotte S, Go A, Gibson WT, Holt RA, Jones SJ, Kennedy GC, Krzywinski M, Langlois S, Li HI, McGillivray BC, Nayar T, Pugh TJ, Rajcan-Separovic E, Schein JE, Schnerch A, Siddiqui A, Van Allen MI, Wilson G, Yong SL, Zahir F, Eydoux P, Marra MA (2006) Oligonucleotide microarray analysis of genomic imbalance in children with mental retardation. Am J Hum Genet 79(3):500–513

Frith C (2004) Is autism a disconnection disorder? Lancet Neurol 3:577

Fu Y, Huang ZJ (2010) Differential dynamics and activity-dependent regulation of alpha- and beta-neurexins at developing GABAergic synapses. Proc Natl Acad Sci U S A 107:22699–22704

Gauthier J, Siddiqui TJ, Huashan P, Yokomaku D, Hamdan FF, Champagne N, Lapointe M, Spiegelman D, Noreau A, Lafreniere RG, Fathalli F, Joober R, Krebs MO, DeLisi LE, Mottron L, Fombonne E, Michaud JL, Drapeau P, Carbonetto S, Craig AM, Rouleau GA (2011) Truncating mutations in NRXN2 and NRXN1 in autism spectrum disorders and schizophrenia. Hum Genet 130:563–573

Geppert M, Khvotchev M, Krasnoperov V, Goda Y, Missler M, Hammer RE, Ichtchenko K, Petrenko AG, Sudhof TC (1998) Neurexin I alpha is a major alpha-latrotoxin receptor that cooperates in alpha-latrotoxin action. J Biol Chem 273:1705–1710

Geschwind DH, Levitt P (2007) Autism spectrum disorders: developmental disconnection syndromes. Curr Opin Neurobiol 17:103–111

Gibson JR, Huber KM, Sudhof TC (2009) Neuroligin-2 deletion selectively decreases inhibitory synaptic transmission originating from fast-spiking but not from somatostatin-positive interneurons. J Neurosci 29:13883–13897

Gkogkas CG, Khoutorsky A, Ran I, Rampakakis E, Nevarko T, Weatherill DB, Vasuta C, Yee S, Truitt M, Dallaire P, Major F, Lasko P, Ruggero D, Nader K, Lacaille JC, Sonenberg N (2013) Autism-related deficits via dysregulated eIF4E-dependent translational control. Nature 493:371–377

Glessner JT, Wang K, Cai G, Korvatska O, Kim CE, Wood S, Zhang H, Estes A, Brune CW, Bradfield JP, Imielinski M, Frackelton EC, Reichert J, Crawford EL, Munson J, Sleiman PM, Chiavacci R, Annaiah K, Thomas K, Hou C, Glaberson W, Flory J, Otieno F, Garris M, Soorya L, Klei L, Piven J, Meyer KJ, Anagnostou E, Sakurai T, Game RM, Rudd DS, Zurawiecki D, McDougle CJ, Davis LK, Miller J, Posey DJ, Michaels S, Kolevzon A, Silverman JM, Bernier R, Levy SE, Schultz RT, Dawson G, Owley T, McMahon WM, Wassink TH, Sweeney JA, Nurnberger JI, Coon H, Sutcliffe JS, Minshew NJ, Grant SF, Bucan M, Cook EH, Buxbaum JD, Devlin B, Schellenberg GD, Hakonarson H (2009) Autism genome-wide copy number variation reveals ubiquitin and neuronal genes. Nature 459:569–573

Gotts SJ, Simmons WK, Milbury LA, Wallace GL, Cox RW, Martin A (2012) Fractionation of social brain circuits in autism spectrum disorders. Brain 135:2711–2725

Grootjans JJ, Reekmans G, Ceulemans H, David G (2000) Syntenin-syndecan binding requires syndecan-synteny and the co-operation of both PDZ domains of syntenin. J Biol Chem 275:19933–19941

Hata Y, Butz S, Sudhof TC (1996) CASK: a novel dlg/PSD95 homolog with an N-terminal calmodulin-dependent protein kinase domain identified by interaction with neurexins. J Neurosci 16:2488–2494

Hoon M, Soykan T, Falkenburger B, Hammer M, Patrizi A, Schmidt KF, Sassoe-Pognetto M, Lowel S, Moser T, Taschenberger H, Brose N, Varoqueaux F (2011) Neuroligin-4 is localized to glycinergic postsynapses and regulates inhibition in the retina. Proc Natl Acad Sci U S A 108:3053–3058

Hu X, Zhang J, Jin C, Mi W, Wang F, Ma W, Ma C, Yang Y, Li W, Zhang H, Du B, Li K, Liu C, Wang L, Lu T, Zhang H, Lv L, Zhang D, Yue W (2013) Association study of NRXN3

polymorphisms with schizophrenia and risperidone-induced bodyweight gain in Chinese Han population. Prog Neuropsychopharmacol Biol Psychiatry 43:197–202
Ichtchenko K, Hata Y, Nguyen T, Ullrich B, Missler M, Moomaw C, Sudhof TC (1995) Neuroligin 1: a splice site-specific ligand for beta-neurexins. Cell 81:435–443
Ichtchenko K, Nguyen T, Sudhof TC (1996) Structures, alternative splicing, and neurexin binding of multiple neuroligins. J Biol Chem 271:2676–2682
Jamain S, Quach H, Betancur C, Rastam M, Colineaux C, Gillberg IC, Soderstrom H, Giros B, Leboyer M, Gillberg C, Bourgeron T (2003) Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. Nat Genet 34:27–29
Jamain S, Radyushkin K, Hammerschmidt K, Granon S, Boretius S, Varoqueaux F, Ramanantsoa N, Gallego J, Ronnenberg A, Winter D, Frahm J, Fischer J, Bourgeron T, Ehrenreich H, Brose N (2008) Reduced social interaction and ultrasonic communication in a mouse model of monogenic heritable autism. Proc Natl Acad Sci U S A 105:1710–1715
Just MA, Cherkassky VL, Keller TA, Minshew NJ (2004) Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. Brain 127:1811–1821
Just MA, Cherkassky VL, Keller TA, Kana RK, Minshew NJ (2007) Functional and anatomical cortical underconnectivity in autism: evidence from an FMRI study of an executive function task and corpus callosum morphometry. Cereb Cortex 17:951–961
Kang Y, Zhang X, Dobie F, Wu H, Craig AM (2008) Induction of GABAergic postsynaptic differentiation by alpha-neurexins. J Biol Chem 283:2323–2334
Karvat G, Kimchi T (2012) Systematic autistic-like behavioral phenotyping of 4 mouse strains using a novel wheel-running assay. Behav Brain Res 233:405–414
Kattenstroth G, Tantalaki E, Sudhof TC, Gottmann K, Missler M (2004) Postsynaptic N-methyl-D-aspartate receptor function requires alpha-neurexins. Proc Natl Acad Sci U S A 101:2607–2612
Kirov G, Gumus D, Chen W, Norton N, Georgieva L, Sari M, O'Donovan MC, Erdogan F, Owen MJ, Ropers HH, Ullmann R (2008) Comparative genome hybridization suggests a role for NRXN1 and APBA2 in schizophrenia. Hum Mol Genet 17:458–465
Kirov G, Rujescu D, Ingason A, Collier DA, O'Donovan MC, Owen MJ (2009) Neurexin 1 (NRXN1) deletions in schizophrenia. Schizophr Bull 35:851–854
Laarakker MC, Reinders NR, Bruining H, Ophoff RA, Kas MJ (2012) Sex-dependent novelty response in neurexin-1alpha mutant mice. PLoS One 7:e31503
Laumonnier F, Bonnet-Brilhault F, Gomot M, Blanc R, David A, Moizard MP, Raynaud M, Ronce N, Lemonnier E, Calvas P, Laudier B, Chelly J, Fryns JP, Ropers HH, Hamel BC, Andres C, Barthelemy C, Moraine C, Briault S (2004) X-linked mental retardation and autism are associated with a mutation in the NLGN4 gene, a member of the neuroligin family. Am J Hum Genet 74:552–557
LeBlanc JJ, Fagiolini M (2011) Autism: a "critical period" disorder? Neural Plast 2011:921680
Li J, Ashley J, Budnik V, Bhat MA (2007) Crucial role of Drosophila neurexin in proper active zone apposition to postsynaptic densities, synaptic growth, and synaptic transmission. Neuron 55:741–755
Lintas C, Persico AM (2009) Autistic phenotypes and genetic testing: state-of-the-art for the clinical geneticist. J Med Genet 46:1–8
Liu Y, Hu Z, Xun G, Peng Y, Lu L, Xu X, Xiong Z, Xia L, Liu D, Li W, Zhao J, Xia K (2012) Mutation analysis of the NRXN1 gene in a Chinese autism cohort. J Psychiatr Res 46:630–634
Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, Skaug J, Shago M, Moessner R, Pinto D, Ren Y, Thiruvahindrapduram B, Fiebig A, Schreiber S, Friedman J, Ketelaars CE, Vos YJ, Ficicioglu C, Kirkpatrick S, Nicolson R, Sloman L, Summers A, Gibbons CA, Teebi A, Chitayat D, Weksberg R, Thompson A, Vardy C, Crosbie V, Luscombe S, Baatjes R, Zwaigenbaum L, Roberts W, Fernandez B, Szatmari P, Scherer SW (2008) Structural variation of chromosomes in autism spectrum disorder. Am J Hum Genet 82:477–488
Merikangas AK, Corvin AP, Gallagher L (2009) Copy-number variants in neurodevelopmental disorders: promises and challenges. Trends Genet 25:536–544

Millson A, Lagrave D, Willis MJ, Rowe LR, Lyon E, South ST (2012) Chromosomal loss of 3q26.3–3q26.32, involving a partial neuroligin 1 deletion, identified by genomic microarray in a child with microcephaly, seizure disorder, and severe intellectual disability. Am J Med Genet A 158A:159–165

Missler M, Zhang W, Rohlmann A, Kattenstroth G, Hammer RE, Gottmann K, Sudhof TC (2003) Alpha-neurexins couple Ca^{2+} channels to synaptic vesicle exocytosis. Nature 423:939–948

Mohrmann I, Gillessen-Kaesbach G, Siebert R, Caliebe A, Hellenbroich Y (2011) A de novo 0.57 Mb microdeletion in chromosome 11q13.1 in a patient with speech problems, autistic traits, dysmorphic features and multiple endocrine neoplasia type 1. Eur J Med Genet 54:e461–464

Moller RS, Weber YG, Klitten LL, Trucks H, Muhle H, Kunz WS, Mefford HC, Franke A, Kautza M, Wolf P, Dennig D, Schreiber S, Ruckert IM, Wichmann HE, Ernst JP, Schurmann C, Grabe HJ, Tommerup N, Stephani U, Lerche H, Hjalgrim H, Helbig I, Sander T, Consortium E (2013) Exon-disrupting deletions of NRXN1 in idiopathic generalized epilepsy. Epilepsia 54:256–264

Moore SA, Saito F, Chen J, Michele DE, Henry MD, Messing A, Cohn RD, Ross-Barta SE, Westra S, Williamson RA, Hoshi T, Campbell KP (2002) Deletion of brain dystroglycan recapitulates aspects of congenital muscular dystrophy. Nature 418:422–425

Pampanos A, Volaki K, Kanavakis E, Papandreou O, Youroukos S, Thomaidis L, Karkelis S, Tzetis M, Kitsiou-Tzeli S (2009) A substitution involving the NLGN4 gene associated with autistic behavior in the Greek population. Genet Test Mol Biomarkers 13:611–615

Panagopoulos VN, Trull TJ, Glowinski AL, Lynskey MT, Heath AC, Agrawal A, Henders AK, Wallace L, Todorov AA, Madden PA, Moore E, Degenhardt L, Martin NG, Montgomery GW, Nelson EC (2013) Examining the association of NRXN3 SNPs with borderline personality disorder phenotypes in heroin dependent cases and socio-economically disadvantaged controls. Drug Alcohol Depend 128:187–193

Perry W, Minassian A, Lopez B, Maron L, Lincoln A (2007) Sensorimotor gating deficits in adults with autism. Biol Psychiatry 61:482–486

Qiu S, Anderson CT, Levitt P, Shepherd GM (2011) Circuit-specific intracortical hyperconnectivity in mice with deletion of the autism-associated Met receptor tyrosine kinase. J Neurosci 31:5855–5864

Radyushkin K, Hammerschmidt K, Boretius S, Varoqueaux F, El-Kordi A, Ronnenberg A, Winter D, Frahm J, Fischer J, Brose N, Ehrenreich H (2009) Neuroligin-3-deficient mice: model of a monogenic heritable form of autism with an olfactory deficit. Genes Brain Behav 8:416–425

Reichelt AC, Rodgers RJ, Clapcote SJ (2012) The role of neurexins in schizophrenia and autistic spectrum disorder. Neuropharmacology 62:1519–1526

Rowen L, Young J, Birditt B, Kaur A, Madan A, Philipps DL, Qin S, Minx P, Wilson RK, Hood L, Graveley BR (2002) Analysis of the human neurexin genes: alternative splicing and the generation of protein diversity. Genomics 79:587–597

Rubenstein JL, Merzenich MM (2003) Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav 2:255–267

Schaaf CP, Boone PM, Sampath S, Williams C, Bader PI, Mueller JM, Shchelochkov OA, Brown CW, Crawford HP, Phalen JA, Tartaglia NR, Evans P, Campbell WM, Tsai AC, Parsley L, Grayson SW, Scheuerle A, Luzzi CD, Thomas SK, Eng PA, Kang SH, Patel A, Stankiewicz P, Cheung SW (2012) Phenotypic spectrum and genotype-phenotype correlations of NRXN1 exon deletions. Eur J Hum Genet 20:1240–1247

Scheiffele P, Fan J, Choih J, Fetter R, Serafini T (2000) Neuroligin expressed in nonneuronal cells triggers presynaptic development in contacting axons. Cell 101:657–669

Scott-Van Zeeland AA Abrahams BS Alvarez-Retuerto AI Sonnenblick LI Rudie JD Ghahremani D Mumford JA Poldrack RA Dapretto M Geschwind DH Bookheimer SY (2010) Altered functional connectivity in frontal lobe circuits is associated with variation in the autism risk gene CNTNAP2. Sci Transl Med 2:56ra80

Siddiqui TJ, Pancaroglu R, Kang Y, Rooyakkers A, Craig AM (2010) LRRTMs and neuroligins bind neurexins with a differential code to cooperate in glutamate synapse development. J Neurosci 30:7495–7506

Soler-Llavina GJ, Fuccillo MV, Ko J, Sudhof TC, Malenka RC (2011) The neurexin ligands, neuroligins and leucine-rich repeat transmembrane proteins, perform convergent and divergent synaptic functions in vivo. Proc Natl Acad Sci U S A 108:16502–16509

Song JY, Ichtchenko K, Sudhof TC, Brose N (1999) Neuroligin 1 is a postsynaptic cell-adhesion molecule of excitatory synapses. Proc Natl Acad Sci U S A 96:1100–1105

Sousa I, Clark TG, Holt R, Pagnamenta AT, Mulder EJ, Minderaa RB, Bailey AJ, Battaglia A, Klauck SM, Poustka F, Monaco AP, International Molecular Genetic Study of Autism C (2010) Polymorphisms in leucine-rich repeat genes are associated with autism spectrum disorder susceptibility in populations of European ancestry. Mol Autism 1:7

Steinberg KM, Ramachandran D, Patel VC, Shetty AC, Cutler DJ, Zwick ME (2012) Identification of rare X-linked neuroligin variants by massively parallel sequencing in males with autism spectrum disorder. Mol Autism 3:8

Sugita S, Saito F, Tang J, Satz J, Campbell K, Sudhof TC (2001) A stoichiometric complex of neurexins and dystroglycan in brain. J Cell Biol 154:435–445

Tabuchi K, Sudhof TC (2002) Structure and evolution of neurexin genes: insight into the mechanism of alternative splicing. Genomics 79:849–859

Tabuchi K, Blundell J, Etherton MR, Hammer RE, Liu X, Powell CM, Sudhof TC (2007) A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. Science 318:71–76

Talebizadeh Z, Bittel DC, Veatch OJ, Butler MG, Takahashi TN, Miles JH (2004) Do known mutations in neuroligin genes (NLGN3 and NLGN4) cause autism? J Autism Dev Disord 34:735–736

Talebizadeh Z, Lam DY, Theodoro MF, Bittel DC, Lushington GH, Butler MG (2006) Novel splice isoforms for NLGN3 and NLGN4 with possible implications in autism. J Med Genet 43:e21

Ullrich B, Ushkaryov YA, Sudhof TC (1995) Cartography of neurexins: more than 1000 isoforms generated by alternative splicing and expressed in distinct subsets of neurons. Neuron 14:497–507

Ushkaryov YA, Sudhof TC (1993) Neurexin III alpha: extensive alternative splicing generates membrane-bound and soluble forms. Proc Natl Acad Sci U S A 90:6410–6414

Vaags AK, Lionel AC, Sato D, Goodenberger M, Stein QP, Curran S, Ogilvie C, Ahn JW, Drmic I, Senman L, Chrysler C, Thompson A, Russell C, Prasad A, Walker S, Pinto D, Marshall CR, Stavropoulos DJ, Zwaigenbaum L, Fernandez BA, Fombonne E, Bolton PF, Collier DA, Hodge JC, Roberts W, Szatmari P, Scherer SW (2012) Rare deletions at the neurexin 3 locus in autism spectrum disorder. Am J Hum Genet 90:133–141

Varoqueaux F, Jamain S, Brose N (2004) Neuroligin 2 is exclusively localized to inhibitory synapses. Eur J Cell Biol 83:449–456

Varoqueaux F, Aramuni G, Rawson RL, Mohrmann R, Missler M, Gottmann K, Zhang W, Sudhof TC, Brose N (2006) Neuroligins determine synapse maturation and function. Neuron 51:741–754

Voineskos AN, Lett TA, Lerch JP, Tiwari AK, Ameis SH, Rajji TK, Muller DJ, Mulsant BH, Kennedy JL (2011) Neurexin-1 and frontal lobe white matter: an overlapping intermediate phenotype for schizophrenia and autism spectrum disorders. PLoS One 6:e20982

Walsh T, McClellan JM, McCarthy SE, Addington AM, Pierce SB, Cooper GM, Nord AS, Kusenda M, Malhotra D, Bhandari A, Stray SM, Rippey CF, Roccanova P, Makarov V, Lakshmi B, Findling RL, Sikich L, Stromberg T, Merriman B, Gogtay N, Butler P, Eckstrand K, Noory L, Gochman P, Long R, Chen Z, Davis S, Baker C, Eichler EE, Meltzer PS, Nelson SF, Singleton AB, Lee MK, Rapoport JL, King MC, Sebat J (2008) Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. Science 320:539–543

Yan J, Oliveira G, Coutinho A, Yang C, Feng J, Katz C, Sram J, Bockholt A, Jones IR, Craddock N, Cook EH, Jr., Vicente A, Sommer SS (2005) Analysis of the neuroligin 3 and 4 genes in autism and other neuropsychiatric patients. Mol Psychiatry 10:329–332

Ylisaukko-oja T, Rehnstrom K, Auranen M, Vanhala R, Alen R, Kempas E, Ellonen P, Turunen JA, Makkonen I, Riikonen R, Nieminen-von Wendt T, von Wendt L, Peltonen L, Jarvela I (2005) Analysis of four neuroligin genes as candidates for autism. Eur J Hum Genet 13:1285–1292

Zhang W, Rohlmann A, Sargsyan V, Aramuni G, Hammer RE, Sudhof TC, Missler M (2005) Extracellular domains of alpha-neurexins participate in regulating synaptic transmission by selectively affecting N- and P/Q-type Ca^{2+} channels. J Neurosci 25(17):4330–4342

Zhang C, Milunsky JM, Newton S, Ko J, Zhao G, Maher TA, Tager-Flusberg H, Bolliger MF, Carter AS, Boucard AA, Powell CM, Südhof TC (2009) A neuroligin-4 missense mutation associated with autism impairs neuroligin-4 folding and endoplasmic reticulum export. J Neurosci 29:10843–10854

Zhang C, Atasoy D, Arac D, Yang X, Fucillo MV, Robison AJ, Ko J, Brunger AT, Sudhof TC (2010) Neurexins physically and functionally interact with GABA(A) receptors. Neuron 66:403–416

Zweier C, de Jong EK, Zweier M, Orrico A, Ousager LB, Collins AL, Bijlsma EK, Oortveld MA, Ekici AB, Reis A, Schenck A, Rauch A (2009) CNTNAP2 and NRXN1 are mutated in autosomal-recessive Pitt-Hopkins-like mental retardation and determine the level of a common synaptic protein in Drosophila. Am J Hum Genet 85:655–666

Chapter 18

Neurotrophins, Their Receptors and Autism: Ligand vs. Receptor Abnormalities

Elizabeth M. Sajdel-Sulkowska

Abstract The role of the neurotrophins in supporting brain growth, development and maintenance is increasingly recognized in health and disease. On the other hand, the role of their receptors is just unfolding, as is, the hypothesis of the ligand-receptor homeostasis. Many toxins, toxicants, and infectious agents affect both the ligands and their receptors, disrupting this homeostasis and affecting many developmental processes. Accumulating evidence points to the abnormalities in both the neurotrophins and their receptors in autism. This chapter highlights some of the recent findings regarding neurotrophin/receptor abnormalities in autism

Keywords Brain development · Brain derived neurotrophic factor (BDNF) · Nerve growth factor (NGF) · Neurotropin-3 (NT-3) · Neurotrophin 4/5 (NT-4/5)

18.1 Role of Neurotrophins During Development

Abnormal brain growth and development in autism is suggested by imaging and head circumference studies (Courchesne et al. 2001) and stereological Purkinje cell analysis (Whitney et al. 2008). It is logical to assume that etiology of autism is related to disrupted brain development, and abnormal course of various developmental events. Many of these processes, such as cell proliferation, migration, differentiation and synaptogenesis are dependent on neurotrophins (McAllister et al. 1999).

The term neurotrophin generally refers to six structurally related factors: brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), neurotrophin-5 (NT-5) and neurotrophin-6 (NT-6). During brain development individual cell populations in different brain regions undergo chronological transition from cell proliferation, cell migration, differentiation, synaptogenesis and elimination of cell excess; neurotrophins play impor-

E. M. Sajdel-Sulkowska (✉)
Department of Psychiatry, Harvard Medical School and Brigham Womens Hospital, 02115 Boston, MA, USA
e-mail: esulkowska@rics.bwh.harvard.edu

S. Hossein Fatemi (ed.), *The Molecular Basis of Autism,*
Contemporary Clinical Neuroscience, DOI 10.1007/978-1-4939-2190-4_18

tant functions in all of these processes (Riikonen and Vanhala 1999; Nelson et al. 2001, 2006; Miyazaki et al. 2004; Connolly et al. 2006; Hashimoto et al. 2006). Moreover, disruption of synapse formation, stabilization and function, observed in autism (Zoghbi 2003), may be brought about by abnormal neurotrophin expression (Zagrebelsky and Korte 2014). Arguments favor the hypothesis that altered expression of neurotrophins early during the development contributes to the autistic pathology (Nelson et al. 2001). Furthermore, altered neurotrophin expression may be brought about by the environmental triggers as discussed below.

Animal data suggest a brain region-specific developmental expression of individual neurotrophins (Das et al. 2001). In rat cerebellum NT-3 is most abundantly expressed during embryonic development with a peak at postnatal day 1 and a subsequent decrease (Das et al. 2001). In monkey cerebellum the level of NT-3 was also higher during embryonic stages and decreased in adulthood, while the level of NT-4/5 increased during both embryonic and postnatal development, gradually declining with age (Takumi et al. 2005). If a similar developmental profile occurs in the human cerebellum, then the persistent elevation in NT-3 could upset the balance of neurotrophic factors and affect brain growth and development. Specifically, over expression of NT-3 could contribute to the cerebellar overgrowth observed in some autistic cases (Courchesne et al. 2001).

18.2 Environmental/Infection Effect of Neurotrophins

Evidence in support of the environmentally-derived disruption of neurotrophin expression comes from several lines of observations such as increased levels of hippocampal BDNF in response to lead (Chao et al. 2007), increased expression of NGF in C6 glioma cells following exposure to polychlorinated biphenyls (PCBs; Gurley et al. 2007), or increased production of NGF by fibroblasts in response to methyl mercury (Söderström and Ebendal 1995).

Recent studies have indicated that maternal infection during fetal brain development may be one of the risk factors for autism. Furthermore, it has been observed that maternal infection during pregnancy results in decreased amniotic fluid levels of NGF and BDNF (Marx et al. 1999). Study of the effect of the Borna disease virus (BDV) infection on the hippocampal neurons showed inhibition of BDNF-induced ERK 1/2 phosphorylation, BDNF-induced expression of synaptic vesicle proteins and severely impaired synaptogenesis and synaptic organization (Hans et al. 2004). Thus viral infection can interfere with neurotrophin-regulated neuroplasticity and neuronal connectivity. More recently, using lipopolysaccharide (LPS)-exposed rat model of infection we have shown elevation in cerebellar NT-3 levels in pups of LPS exposed dams (Xu et al. 2013a). Furthermore using the same model we have observed a significant elevation in BDNF gene expression (Xu et al. 2013b).

18.3 Possible Consequences of Altered Neurotrophins (Excess vs. Deficit)

Neurotrophins, and specifically, BDNF and NGF are important for the survival, maintenance and regeneration of specific neuronal populations in the adult brain and their depletion has been linked to neurodegenerative diseases such as Parkinson's, Alzheimer's disease (AD) and Huntington's disease. BDNF and NT-3, implicated in neuronal growth and synapse activity, decrease in different brain regions of patients with AD (Corbett et al. 2013).

Studies in transgenic mice indicated that NGF changes induced activation of amyloidogenesis and tau processing and provided link between neurotrophin signaling deficit and AD neurodegeneration (Cattaneo and Callisano 2012). Furthermore, experiments in neuronal culture revealed anti-amyloidogenic action of NGF/TrkA and suggested the importance of homeostatic balance between the neurotrophins and their receptors (Cattaneo and Callisano 2012). The expression of p75NTR receptor is increased in the AD hippocampus and Aβ-bound p75NTR triggers cell death (Armato et al. 2013).

It is important to realize that neurotrophin levels in normal controls are not constant but alter according to genetically determined trajectories. In humans, BDNF increases with age, while NT-3 and NT4/5 concentrations decrease (Nelson et al. 2006). The level of NT-3 decreases with age in the rodent brain (Das et al. 2001). Consequently, if a similar developmental down regulation occurs in the human cerebellum, then prolonged and persistent elevation of NT-3 in autism suggested by our data (Sajdel-Sulkowska et al. 2009, 2011) may have profound consequences on neuronal growth and synapse formation. Furthermore, while in healthy controls serum BDNF increases with age decreasing slightly in adulthood, it is abnormally low in children with autism suggesting a delay in normal age-dependent increase in BDNF (Katoh-Semba et al. 2007).

In addition to the trophic function, NT-3 may also under certain conditions exacerbate oxidative stress (Bates et al. 2002) as a part of a response to hypoxia (Pasarica et al. 2005) affecting survival of Purkinje cells in vitro (Morrison and Mason 1998). In that respect, NT-3 behaves like NO playing an important role in neuronal differentiation by nitrative modification of specific proteins (Cappelletti et al. 2003). However overproduction of nitric oxide leads to peroxynitrite formation as a source of neurotoxicity (Vargas et al. 2004). Increase in peroxynitrite results in nitration of free- and protein-bound tyrosine residues leading to 3-nitrotyrosine (3-NT) formation and protein modification as observed in a number of human pathologies.

It has been suggested that the negative action of NT-3 may be related to altered glutamate receptors (Behrens et al. 1999). Thus it is of interest that glutamatergic system abnormalities (Yip et al. 2007) have been implicated in selective Purkinje cell decrease in autism (Rout and Dhossche 2008).

Interestingly, BDNF has been implicated in mediating several of the effects of estrogen in hippocampus; in turn estrogen regulates BDNF as well as trkB and p75NTR of the mossy fiber pathway with altered hippocampal BDNF levels poten-

tially affecting hippocampal functions (Harte-Hargrove et al. 2013). Estrogen and the neurotrophin ligand-receptor complexes have been implicated in etiology of diverse types of neurological or psychiatric disorders (Harte-Hargrove et al. 2013). This estrogen-BDNF interaction may contribute to the sex-dependent differences in brain development.

18.4 Changes in Neurotrophins in Autism

Most of the data on neurotrophin levels in autism are derived from the analysis of blood levels (Kozlovskaia et al. 2000; Nelson et al. 2001, 2006; Miyazaki et al. 2004; Tsai 2005; Connolly et al. 2006; Nelson et al. 2006; Katoh-Semba et al. 2007; Croen et al. 2008; Tostes et al. 2012). In this context, it is important to realize that these do not necessarily reflect the brain neurotrophin levels because of the blood–brain-barrier. A few of the results derived from the direct measurement of the brain neurotrophin levels suggest an independent regulation of the two pools.

The hypothesis of early hyperactivity of BDNF in autism (Tsai 2005) is supported by increased levels of BDNF in the serum (Nelson et al. 2001) and may be associated with early brain outgrowth (Courchesne et al. 2001). BDNF levels were also elevated in newborn sera of children with autism (Connolly et al. 2006), but so were elevated autoantibodies suggestive of interaction between immune system and BDNF (Connolly et al. 2006). Others observed increased blood BDNF levels in young adults with autism (Miyazaki et al. 2004). Furthermore, elevated BDNF levels were also observed in basal forebrain of young adults with autism (Perry et al. 2001); NGF levels remained normal and NT-3 levels were not measured in this study. However, in adult male patients with autism the levels of BDNF were reduced as compared to normal controls (Hashimoto et al. 2006).

In healthy controls the serum BDNF concentrations increased over the first several years, then decreased after reaching the adult level. In autism, the serum levels of BDNF were lower in children 0–9 years compared to teenagers and adults indicating a delayed increase of this neurotrophin with development (Katoh-Semba et al. 2007). On the other hand population-based case control studies of archived maternal mid-pregnancy and neonatal blood did not show changes in BDNF levels in autism (Croen et al. 2008). BDNF expression in the peripheral blood lymphocytes of the drug naïve autism patients was found to be significantly higher than in the control group suggestive of a possible pathologic role of BDNF on the serotonergic system (Nishimura et al. 2007). Additionally, an increased level of BDNF in the basal forebrain in autism has also been reported (Nelson et al. 2006).

An association between BDNF gene polymorphism and autism was found in Chinese population (Cheng et al. 2009). More recently we have observed abnormal expression of brain derived neurotrophic factor (BDNF) gene in autism (Khan et al. 2014). Study of rs6265(BDNF) as a genetic marker of anxiety, ADHD and tics found BDNF genotype marginally significant for social phobia in children with ASD (Gadow et al. 2009)

Elevation of blood serum levels of NGF in early autism was reported (Kozlovskaia et al. 2000), but the analysis of CSF showed normal levels of NGF in children with autism (Riikonen and Vanhala 1999). The analysis of basal forebrain in autism showed no changes in NGF in teenage cases (Nelson et al. 2006).

It has been previously reported that blood NT-3 levels were significantly lower in autistic neonates (Nelson et al. 2006); others supported this observation and reported plasma levels of NT-3 that were significantly lower in children with autism (Tostes et al. 2012). NT-3 is specifically involved in neuronal differentiation (Ghosh and Greenberg 1995) neurite fasciculation (Segal et al. 1995) and axonal targeting. In the prenatal system, overexpression of NT-3 affects the formation of specific synapses. Our data indicating increased NT-3 expression in brain tissue derived from a subset of autistic cases including older donors suggest an abnormal synapse formation in autism (Sajdel-Sulkowska et al. 2009). Specifically, we reported an increase in NT-3 in cerebellar hemispheres, dorsolateral prefrontal cortex, Wernicke's area and cingulate gyrus suggesting brain region specific NT-3 changes (Sajdel-Sulkowska et al. 2011).

Neurotrophin 4/5 (NT-4/5) were also elevated in autism; furthermore that elevation was observed in peripheral blood in the first days of life (Nelson et al. 2001).

Thus several lines of evidence point out to altered neurotrophin levels in autism; complementary changes in related trophic factors have also been recently reviewed (Jockschat and Miche 2011).

18.5 Relationship Between Neurotrophin Changes and Autistic Pathology

Consistent neuropathological findings in autism include an early increase in brain size (Courchesne et al. 2001; Sparks et al. 2002), decrease in Purkinje cells, smaller neuronal size and decreased dendritic branching in the cerebellum (Bailey et al. 1998), hippocampus and amygdala (Kemper and Bauman 1998; 2002) and reduced neuronal and dendritic pruning (Ben Bashat et al. 2007; Palmen et al. 2005). Additionally, reduced connectivity in autistic brain regions is associated with impaired social cognition as documented by diffusion tensor imaging of white matter structure (Barnea-Goraly et al. 2004). Decrease in executive function in autism has been suggested by functional magnetic resonance imaging studies (Dawson et al. 2002). However, the link between neurotrophin abnormalities and autistic symptoms, is supported by a very small number of animal studies and human observations.

The hypothesis that early BDNF hyperactivity may play a role in autism etiology is supported by an association between the early increase in BDNF levels in both serum and brain tissue in autistic children and early brain outgrowth in autism (Tsai 2005).

We reported an increase in NT-3 in cerebellar hemispheres, dorsolateral prefrontal cortex, Wernicke's area and cingulate gyrus suggesting brain region specific NT-3 changes in autism (Sajdel-Sulkowska et al. 2011). Significantly, these particular brain regions are functionally altered in autism.

Importantly, variants of BDNF genes are linked to the decline in executive function with aging (Erickson et al. 2008; Raz et al. 2009) and animal data suggest that this effect may be mediated through a BDNF effect on synaptic plasticity in the prefrontal cortex (Sakata et al. 2009).

Further neurotrophin-behavioral link is provided by a transgenic mouse model deficient in the BDNF that exhibited diminished brain circuitry and selective deficit in social- and anxiety-related behaviors (Sadakata et al. 2012). Independently, a knockout mouse model of fragile X syndrome with a reduction in BDNF expression showed a deficit in cognitive skills (Uutela et al. 2012). However, at this point, the link between neurotrophin abnormalities and autistic pathology awaits further clinical and experimental confirmation.

18.6 Neurotrophin Receptor Abnormalities in Autism and Their Implications

Neurotrophins are synthesized as pro-neurotrophins and are then converted to the active neurotrophins. Both forms interact with the neurotrophin receptors, p75 and a member of the tyrosine kinase family (Trk), located on the target cells. The interaction of neurotrophins with the p75 receptor is of low affinity type and nonspecific. Binding to the Trk receptors is of high affinity type and specific for individual neurotrophins; BDNF binds to TrkA, B, and C, NT-3 binds to TrkC and NT-4/5 binds to TrkB receptors (Jockschat and Miche 2011).

It has been proposed that alterations in BDNF/tyrosine kinase B (TrkB) contribute to autistic pathology (Correia et al. 2010). Further, the activation of proapoptotic process may be related to lower BDNF levels and down regulation of Akt kinase and Bcl2 antiapoptotic signaling pathway in autistic brain (Sheikh et al. 2010).

Finally, a significant association with autism was determined for single nucleotide polymorphism in the TRK2 gene supporting its role as a susceptibility factor for the disorder (Correia et al. 2010).

18.7 Neurotrophins and Their Receptors as New Therapeutic Targets

Neurotrophin abnormalities are being targeted in new therapies of AD. Successful clinical NGF trials in AD are ongoing and preclinical BDNF studies are promising (Allen et. al. 2013). The results of these studies in AD offer new hope for novel therapies in autism. However, the blood–brain-barrier presents a problem and the neurotrophins must be infused either directly to the brain, produced internally by recombinant vectors or by the implanted protein-secreting cells or provided by active transport, or in a shorter, modified form.

Independently, another way of correcting neurotrophin deficit in AD brain involves stimulation of astrocytic BDNF and NT-3 expression by sodium phenylbutyrate (NaPB; drug for hyperammonemia); initial results indicate that NaPB-induced astroglial neurotrophins are functionally active and that oral administration of the drug increases neurotrophin levels in the CNS (Corbett et al. 2013). However, its potential therapeutic use in children with autism could be limited by cross-reactivity with other medications and availability of taste-masking formulation (Guffon et al. 2012).

Among the important issues to consider is the question whether autistic pathology is related to either neurotrophin or neurotrophin receptor deficits or disruption of neurotrophin-receptor homeostasis.

Encouraging are the observations that enriched environment upregulates Akt pathway as well as expression of BDNF, NGF and TrkB (Hu et al. 2013). This observation not only supports the key role in early intervention but offers new therapeutical avenues of correcting neurotrophin imbalance in autism noninvasively.

In summary, evidence albeit scarce from animal and human studies implicates both neurotrophins and their receptors in autistic pathology. Alterations in blood and brain tissue levels in neurotrophins indicates both an increase and decrease in their levels, and a deviation from normal developmental trajectory. An association with autism for polymorphism in the TRK2 gene supports the notion of neurotrophin receptor abnormalities in autism. A hypothesis of distorted neurotrophin-receptor abnormality in autism has been formulated. Therapies addressing abnormalities in neurotrophin in AD are cautiously promising and suggest hope for similar therapies in autism.

References

Allen SJ, Watson JJ, Shoemark DK, Barua NU, Patel NK (2013) GDNF, NGF and BDNF as therapeutic options for neurodegeneration. Pharmacol Ther 138:155–175

Armato U, Chakravarthy B, Pacchiana R, Whitfield JF (2013) Alzheimer's disease: an update of the roles of receptors, astrocytes and primary cilia. Int J Mol Med 31:3–10

Bailey A, Luthert P, Dean A, Harding B, Janota I, Montgomery M, Rutter M, Lantos P (1998) A clinicopathological study of autism. Brain 121:889–905

Barnea-Goraly N, Kwon H, Menon V, Eliez S, Lotspeich L, Reiss AL (2004) White matter structure in autism: preliminary evidence from diffusion tensor imaging. Biol Psychiatry 55:323–326

Bates B, Hirt L, Thoma SS, Akbarian S, Le D, Amin-Hanjani S, Whalen M, Jaenisch R, Moskowitz MA (2002) Neurotrophin-3 promotes cell death induced in cerebral ischemia, oxygen-glucose deprivation, and oxidative stress: possible involvement of oxygen free radicals. Neurobiol Dis 9:24–37

Behrens MM, Strasser U, Lobner D, Dugan LL (1999) Neurotrophin-mediated potentiation of neuronal injury. Microsc Res Tech 45:276–284

Ben Bashat D, Kronfeld-Duenias V, Zachor DA, Ekstein PM, Hendler T, Tarrasch R, Even A, Levy Y, Ben Sira L (2007) Accelerated maturation of white matter in young children with autism: a high b value DWI study. Neuroimage 37:40–47

Cappelletti G, Maggioni MG, Tedeshi G, Maci R (2003) Protein tyrosine nitration is triggered by nerve growth factor during neuronal differentiation of PC12 cells. Exp Cell Res 288:9–20

Cattaneo A, Callisano P (2012) Nerve growth factor and Alzheimer's disease: new facts for an old hypothesis. Mol Neurobiol 46:588–604

Chao SL, Moss JM, Harry GJ (2007) Lead-induced alterations of apoptosis and neurotrophic factor mRNA in the developing rat cortex, hippocampus, and cerebellum. J Biochem Mol Toxicol 21:265–272

Cheng L, Ge Q, Sun B, Yu P, Ke X, Lu Z (2009) Polyacrylamide gel-based microarray; a novel method applied to the association study between the polymorphisms of BDNF gene and autism. J Biomed Nanotechnol 5:542–550

Connolly AM, Chez M, Streif EM, Keeling RM, Golumbek PT, Kwon JM, Riviello JJ, Robinson RG, Neuman RJ, Deuel RM (2006) Brain-derived neurotrophic factor and autoantibodies to neural antigens in sera of children with autistic spectrum disorders, Landau-Kleffner syndrome, and epilepsy. Biol Psychiatry 59:354–363

Corbett GT, Roy A, Pahan K (2013) Sodium phenylbutyrate enhances astrocytic neurotrophin synthesis via protein kinase C (PKC)-mediated activation of cAMP-response element-binding protein (CREB); implications for Alzheimer disease therapy. J Biol Chem 288:8299–8312

Correia CT, Coutinho AM, Sequeira AF, Sousa IG, Lourenco Venda L, Almeida JP, Abreu RL, Lobo C, Miguel TS, Conroy J, Cochrane L, Gallagher L, Gill M, Ennis S, Oliveira GG, Vicente A (2010) Increased BDNF levels and NTRK2 gene association suggest a disruption of BDNF/TrkB signaling in autism. Genes Brain Behav 9:841–848

Courchesne E, Karns CM, Davis HR, Ziccardi R, Carper RA, Tigue ZD (2001) Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. Neurology 57:245–254

Croen LA, Goines P, Braunschweig D, Yolken R, Yoshida CK, Grether JK, Fireman B, Kharrazi M, Hansen RL, Van de Water J (2008) Brain-derived neurotrophic factor and autism: maternal and infant peripheral blood levels in the early markers for autism (EMA) study. Autism Res 1:130–137

Das KP, Chao SL, White LD, Haines WT, Harry GJ, Tilson HA, Barone S Jr (2001) Differential patterns of nerve growth factor, brain-derived neurotrophic factor and neurotrophi-3 mRNA and protein levels in developing regions of rat brain. Neuroscience 103:739–761

Dawson G, Webb S, Schellenberg GD, Dager S, Friedman S, Aylward E, Richards T (2002) Defining the broader phenotype of autism: genetic, brain, and behavioral perspectives. Dev Psychpatol 14:581–611

Erickson KI, Kim JS, Suever BL, Voss MW, Francis BM, Kramer AF (2008) Genetic contributions to age related decline in executive function: a 10-year longitudinal study of COMT and BDNF polymorphism. Front Hum Neurosci 2:11

Gadow KD, Roohi J, DeVincent CJ, Kirsch S, Hatchwell E (2009) Association of COMT (Val-158Met) and BDNF (Val66Met) gene polymorphisms with anxiety, ADHD and tics in children with autism spectrum disorder. J Autsm Dev Disord 39:1542–1551

Ghosh A, Greenberg ME (1995) Distinct roles for bFGF and NT-3 in the regulation of cortical neurogenesis. Neuron 15:89–103

Guffon N, Kibleur Y, Copalu W, Tissen C, Breitkreutz J (2012) Developing a new formulation of sodium phenylbutyrate. Arch Dis Child 97:1081–1085

Gurley GH, Jelaso AM, Ide CF, Spitsbergen JM (2007) Effects of polychlorinated biphenyls (PCBs) on expression of neurotrophic factors in C6 glial cells in culture. Neurotoxicology 28:1264–1271

Hans A, Bajramovic JJ, Syan S, Perret E, Dunia I, Brahic M, Gonzales-Dunia D (2004) Persistent, noncytolytic infection of neurons by borna disease virus interferes with ERK ½ signaling and abrogates BDNF-induced synaptogenesis. FASEB J 18:863–865

Harte-Hargrove LC, Maclusky NJ, Scharfman HE (2013) Brain-derived neurotrophic factor-estrogen interactions in the hippocampal mossy fiber pathway: implications for normal brain function and disease. Neurosci 239:46–66

Hashimoto K, Iwata Y, Nakamura K, Tsujii M, Tsuchiya KJ, Sekine Y, Suzuki K, Minabe Y, Takei N, Iyo M, Mori N (2006) Reduced serum levels of brain-derived neurotrophic factor in adult male patients with autism. Progr Neuropsychopharmacol Biol Psychiatry 30:1529–1531

Hu YS, Long N, Pigino G, Brady ST, Lazarov O (2013) Molecular mechanisms of environmental enrichment: impairments in Akt/GSK3β, neurotrophin-3 and CREB signaling. PLoS One 8:e64460

Jockschat TN, Miche TM (2011) The role of neurotrophic factors in autism. Mol Psychiatry 16:478–490

Katoh-Semba R, Wakako R, Komori T, Shigemi H, Miyazaki N, Ito H, Kumagai T, Tsuzuki M, Shigemi K, Yoshida F, Nakayama A (2007) Age-related changes in BDNF protein levels in human serum: differences between autism cases and normal controls. Int J Dev Neurosci 25:367–372

Kemper TL, Bauman M (1998) Neuropathology of infantile autism. J Neuropathol Exp Neurol 57:645–652

Kemper TL, Bauman M (2002) Neuropathology of infantile autism. Mol Psychiatry 7:7S12–13

Khan A, Harney JW, Zavacki AM, Sajdel-Sulkowska EM (2014) Disrupted brain thyroid hormone (TH) homeostasis and altered TH-dependent brain gene expression in autism spectrum disorders. J Physiol Pharmacol 65:257–272

Kozlovskaia GV, Kliushnik TP, Goriunova AV, Turkova IL, Kalinina MA, Sergienko NS (2000) Nerve growth factor auto-antibodies in children with various forms of mental dysontogenesis and in schizophrenia high risk group. Zh Nevrol Psikhiatr Im S S Korsakova 100:50–52

Marx CE, Vance BJ, Jarskog LF, Chescheir NC, Gilmore JH (1999) Nerve growth factor, brain derived neurotrophic factor and neurotrophin-3 levels in human amniotic fluid. Am J Obstet Gynecol 181:1225–1230

McAllister AK, Katz LC, Lo DC (1999) Neurotrophins and synaptic plasticity. Annu Rev Neurosci 22:295–318

Miyazaki K, Narita N, Sakuta R, Miyahara T, Naruse H, Okado N, Narita M (2004) Serum neurotrophin concentrations In autism and mental retardation: a pilot study. Brain Development 26:292–295

Morrison ME, Mason CA (1998) Granule neuron regulation of Purkinje cell development: striking a balance between neurotrophin and glutamate signaling. J Neurosci 18:3563–3573

Nelson KB, Grether JK, Croen LA, Dambrosia JM, Dickens BF, Jelliffe LL, Hansen RL, Philips TM (2001) Neuropeptides and neurotrophin in neonatal blond of children with autism or mental retardation. Ann Neurol 49:597–606

Nelson PG, Kuddo T, Song EY, Dambrosia JM, Kohler S, Satyanarayana G, Vandunk C, Grether JK, Nelson KB (2006) Selected neurotrophin, neuropeptides, and cytokines: developmental trajectory and concentrations in neonatal blood of children with autism or Down syndrome. Int J Dev Neurosci 24:73–80

Nishimura K, Nakamura K, Anitha A, Yamada K, Tsujii m, Iwayama Y, Hattori E, Toyota T, Takei N, Miyachi T, Iwata Y, Suzuki K, Matsuzaki H, Kawai M, Sekine Y, Tsuchiya k, Sughihara G, Suda S, Ouchi Y, Sugiyama T, Yoshikawa T, Mori N (2007) Genetic analyses of the brain-derived neurotrophic factor (BDNF) gene in autism. Biochem Biophys Res Comm 356:200–206

Palmen SJ, Hulshoff Pol HE, Kemner C, Schnack HG, Durston S, Lahuis BE, Kahn RS, Van Engeland H (2005) Increased grey matter volume in medication naïve high functioning children with autism spectrum disorder. Psychol Med 35:561–570

Pasarica D, Gheorghiu M, Toparceanu F, Bleotu C, Ichim L, Trandafir T (2005) Neurotrophin-3, TNF-alpha and IL-6 relations in serum and cerebrospinal fluid of ischemic stroke patients. Roum Arch Microbiol Immunol 64:27–33

Perry EK, Lee ML, Martin-Ruiz CM, Court JA, Volsen SG, Merrit J, Folly E, Iversen PE, Bauman ML, Perry RH, Wenk GL (2001) Cholinergic activity in autism: abnormalities in the cerebral cortex ad basal forebrain. Am J Psychiatry 158:1058–1066

Raz N, Rodrigue KM, Kennedy KM, Land S (2009) Genetic and vascular modifiers of age sensitive cognitive skills: effects of COMT, BDNF, ApoE, and hypertension. Neuropsychology 23:105–116

Riikonen R, Vanhala R (1999) Levels of cerebrospinal fluid nerve-growth factor differ in infantile autism and Rett syndrome. Dev Med Child Neurol 41:148–152

Rout UK, Dhossche DM (2008) A pathogenic model of autism involving Purkinje cell loss through anti GAD antibodies. Med Hypotheses 71:218–221

Sadakata T, Shinoda Y, Oka M, Sekine Y, Sato Y, Saruta C, Miwa H, Tanaka M, Itohara S, Furuichi T (2012) Reduced axonal localization of a Caps2 splice variant impairs axonal release of BDNF and causes autistic-like behavior in mice. Proc Nat Acad Sci USA 109:21104–21109

Sajdel-Sulkowska EM, Xu M, Koibuchi N (2009) Increase in cerebellar neurotrophin-3 and oxidative stress markers in autism. Cerebellum 8:366–372

Sajdel-Sulkowska EM, Xu M, McGinnis W, Koibuchi N (2011) Brain region-specific changes in oxidative stress and neurotrophin levels in autism spectrum disorders (ASD). Cerebellum 10:43–48

Sakata K, Woo NH, Martinowich K, Greene JS, Schloesser RJ, Shen L, Lu B (2009) Critical role of promoter IV-driven BDNF transcription in GABAergic transmission and synaptic plasticity in prefrontal cortex. Proc Nat Acad Sci USA 106:5942–5947

Segal RA, Pomeroy SL, Stiles CD (1995) Axonal growth and fasciculation linked to differential expression of BDNF and NT-3 receptors in developing granule cells. J Neurosci 15:4970–4981

Sheikh AM, Malik M, Wen G, Chauhan A, Chauhan V, Gong CX, Liu F, Brown WT, LiX (2010) BDNF-Akt-Bcl2 antiapoptotic signaling pathway is compromised in the brain of autistic subjects. J Neurosci Res 88:2641–2647

Söderström S, Ebendal T (1995) In vitro toxicity of methyl mercury: effects on nerve growth factor (NGF)-responsive neurons and on NGF synthesis in fibroblasts. Toxicol Lett 75:133–144

Sparks BF, Friedman SD, Shaw DW, Aylward EH, Echelard D, Artru AA, Maravilla KR, Giedd JN, Munson J, Dawson G, Dager SR (2002) Brain structural abnormalities in young children with autism spectrum disorder. Neurology 59:184–192

Takumi K, Mori T, Shimizu K, Hayashi M (2005) Developmental changes in concentrations and distributions of neurotrophins in the monkey cerebellar cortex. J Chem Neuroanat 30:212–220

Tostes MH, Teixeira HC, Gattaz WF, Brandao MA, Raposo NR (2012) Altered neurotrophin, neuropeptide, cytokines and nitric oxide levels in autism. Pharmacopsychiatry 45:241–243

Tsai SJ (2005) Is autism caused by early hyperactivity of brain-derived neurotrophic factor? Med Hypotheses 65:79–82

Uutela M, Lindholm J, Louhivuori V, Wei H, Louhivuori LM, Pertovaara A, Akerman K, Castren E, Castren ML (2012) Reduction of BDNF expressionin Fmr1 knockout mice worsens cognitive deficit but improves hyperactivity and sensorimotor deficits. Genes Brain Behav 11:513–523

Whitney ER, Kemper TL, Bauman ML, Rosene DL, Blatt GJ (2008) Cerebellar Purkinje cells are reduced in a subpopulation of autistic brains: a stereological experiment using calbindin-D28k. Cerebellum 7:406–416

Xu M, Sajdel-Sulkowska EM, Iwasaki T, Koibuchi N (2013a) Aberrant cerebellar neurotrophin-3 expression induced by lipopolysaccharide exposure during brain development. Cerebellum 12:316–318

Xu M, Sulkowski ZL, Parekh P, Khan A, Chen T, Midha S, Iwasaki T, Shimokawa N, Koibuch N, Zavacki AM, Sajdel-Sulkowska EM (2013b) Effects of perinatal Lipopolysaccharide (LPS) exposure on the developing rat brain; modeling the effect of maternal infection in the developing human CNS. Cerebellum 12:572–586

Yip J, Soghomonian JJ, Blatt GJ (2007) Decreased GAD67mRNA levels in cerebellar Purkinje cells in autism: pathophysiological implications. Acta Neuropathol 113:559–568

Zagrebelsky M, Korte M (2014) Form follows function: BDNF and its involvement in sculpting the function and structure of synapses. Neuropharmacology 76:628–638

Zoghbi HY (2003) Postnatal neurodevelopmental disorders: meeting at the synapse? Science 302:826–830

Chapter 19

Cognition, Motor Control and Other Aspects of Autism: A Pragmatic Review

James W. Howell and Detlef H. Heck

Abstract Evolutionists point out that ASD's continued presence in the human population may be because of the need for a few individuals to have extreme focus at the expense of human interaction. Regardless of the validity of this hypothesis, in a modern society autism places a considerable burden on the quality of life for patients and their caregivers. To date, the study of Autistic Spectrum Disorders (ASD) has yielded no specific common cause. This has made diagnosis and treatment difficult. This chapter reviews a variety of variables that have over the past years received clinical and/or animal experimental support for their relation to autism. They range from neuropathologies affecting individual cells or the size of entire brain areas, to considerations of the role of inflammation or environmental factors and autism. Important clues may also come from comparative studies of subjects with ASD and with schizophrenia, two disorders known to be associated with neuropathologies in the cerebellum and prefrontal cortex. Clearly, ASD research now faces the considerable challenge of integrating large amounts of diverse data. Qualitatively new insights will most likely arise from integrative approaches considering interactions between prominent variables.

Keywords Cerebral cortex · Cerebellum · Minicolumns · Evolution · Sensorimotor and cognitive dysfunction · Sports · Schizophrenia · Animal models · Intelligence · Environment · Stress · Brain size · Prefrontal cortex · Brain development · Self · Memory · Autoimmunity · Leaky gut · Dopamine · Pruning · Inflammation

Autism in humans is a complex array of loosely related conditions rightly called Autism Spectrum Disorders (ASD). A specific cause common to all forms of ASD has not been identified and, as this brief review will demonstrate, most likely never will be because of the substantial array of manifestations and degrees of severity of ASD. Several other facets of understanding ASD, including diagnostic methods,

D. H. Heck (✉)
Department of Anatomy and Neurobiology,
University of Tennessee Health Science Center,
855 Monroe Avenue, Suite 515, Memphis, TN 38163, USA
e-mail: dheck@uthsc.edu

J. W. Howell
The Urban Child Institute and the Department of Psychiatry,
University of Tennessee Health Science Center, Memphis, TN, USA

S. Hossein Fatemi (ed.), *The Molecular Basis of Autism,*
Contemporary Clinical Neuroscience, DOI 10.1007/978-1-4939-2190-4_19

objective measures, and similarities with other neurological conditions, have served to further complicate our knowledge of this spectrum of disorders.

If one compares an autistic brain with one from the general population, there is little in the way of gross abnormalities. Casanova (2005) discusses some of the few differences in his consideration of the minicolumns of cells in the cerebral cortex. He emphasizes the fact that few actual brains of autistic people have been studied. This continues to be a serious problem. Casanova found the cortical minicolumns of the autistic brain to be reduced in size, thus one dendrite will connect with more minicolums in the autistic person than in the normal.

The place of autism in the evolutionary history of mankind is interesting to consider and can give us an insight into the big picture of what autism is. Reser (2011) considers the possibility that autistic individuals during the time of early man fit into the scheme of daily life because food was scarce and the presence of "solitary foragers" could be a distinct advantage because large groups most likely had to disband periodically to survive. He says such an environment would favor the mildly autistic but that assortative mating would occasionally produce the more severe cases of autism. He suggests that autism is not necessarily a disease but a way of suppressing social information to focus on more specific tasks at hand.

Ploeger and Galis (2011) do consider autism a disorder that is detrimental to reproductive success, certainly a negative factor in evolution. They say that autism has not been eliminated by evolution because autism is a polygenic disorder whose genes interact. Probably, they say, these genes are also involved in positive traits, specifically increased intelligence which is related to reproductive success. Their hypothesis is that these genes normally contribute to normal or higher intelligence while unlucky individuals have to live with autism when the genes interact in such a way or spontaneously mutate to leave the individual in this low-fitness extreme.

Further, these authors describe the defensive responses of the autistic individuals as being more like that of the reptilian than the mammalian brain. A mammal tends to dampen the defensive system in response to stress by interaction in social situations while the reptile is in a chronic state of hypervigilance or shutdown. For the reptile and its environment, this strategy works, but for the mammal this is not an adaptive strategy. In other words, the autistic brain is not utilizing the full cognitive capacity of the mammalian brain in reaction to stressors.

In discussing the relationship between sensorimotor and cognitive dysfunction in autism and related disorders we take a general view of the link between brain function in general and motor control. Sessile organisms such as plants, have evolved successfully without the possession of a nervous system. The ability of an organism to move, however, whether in aquatic or terrestrial environments, seems to be inextricably linked with the possession of a nervous system, capable of sensing environmental and internal states and of generating movements appropriate for survival.

Based on evolutionary and neurophysiological considerations it seems possible to argue that cognitive and sensorimotor processes are closely related in terms of their underlying neuronal principles. A quote attributed to the late Walle Nauta (1916–1994) elegantly summarizes this idea: "What is a thought except a movement that is not connected to a motor neuron". This view implies that the challenges and

advantages associated with the ability to move around freely led to the evolution of neuronal mechanisms which can also support the development of complex cognitive processes such as self awareness, planning and decision making. In his 2002 book "I of the vortex", Rodolpho Llinás (2002) also argues in favor of a common evolutionary origin of the neuronal mechanisms underlying sensorimotor and cognitive processes, suggesting that thoughts are "internalized movements" (Llinas 2002).

The brain structure most implicated in cognitive function is the neocortex, particularly its prefrontal areas, which are not primarily involved in sensory or motor processes.

The question arises as to whether the characteristic neural circuitry of the neocortex can be used to generate both movements and mental processes. The most fundamental forms of movement, such as swimming, walking, breathing or chewing, are also the ones most relevant for survival and basic life support. These movements are typically highly rhythmic and controlled by semi-autonomous neuronal circuits called "central pattern generators" (CPGs), located in the spinal cord and brain stem (Grillner 2006; Harris-Warrick 2010). A characteristic feature of CPGs is that they can generate functional output independent of sensory inputs but can be modulated by sensory activity or top-down signals from other parts of the brain. In an opinion article that appeared in the journal Nature Review Neurocience, Yuste and colleagues argued that neocortical circuits could be considered analogous to CPGs as the neocortex is able to generate output in the absence of sensory inputs but can also be strongly modulated or engaged by sensory or other inputs (Yuste et al. 2005).

Together the above ideas suggest that sensorimotor and cognitive processes cannot be considered independent of each other as they are generated by circuits based on common neuronal principles and consequently common vulnerabilities to disease. As a consequence, pathological changes affecting the function of basic neocortical circuits will inevitably cause both sensorimotor and cognitive deficits and those would not be limited to a specific cognitive disorder but rather apply to a broad spectrum of those.

Too often, generalizations about the effect of autism on motor function have, to a great extent, ignore the fact that a significant number of people with autism have become celebrated athletes, even in sports requiring keen sensorimotor coordination such as archery, surfing, weightlifting, running, and karate. These accomplishments are beyond what is often seen in special Olympics and amateur sports. Often the athletes themselves have said ASD has in a way been an advantage because the condition allows intense focus and the ability to isolate themselves from what would otherwise be considered the hyper-excitement of the moment (See the extensive number of reports on the popular autism internet general information sites concerning this and including discussion of people successful in such a diverse array of sports. Examples: http://www.attentionlearningcenter.com/add-athletes-autism.htm, http://autismandathletics.wordpress.com/about/). Indeed, that this is possible demonstrates that autism is a spectrum of disorders.

Two prominent cognitive disorders, autism and schizophrenia, are also associated with neuropathology of the cerebellum, a brain structure long believed to be exclusively involved in the coordination of movements and motor learning. However,

recent years have seen a fast accumulation of evidence for a role of the cerebellum in cognition as well (Schmahman 1998; Strick et al. 2009). The neocortex and the cerebellum have coevolved and are heavily interconnected, suggesting a close functional interaction between the two structures, which is likely to be affected in ASD brains (Heck and Howell 2013). Here we approach the question of the relationship between motor and cognitive deficits in autism and other cognitive disorders by considering the neocortex and cerebellum as a functional entity and by reviewing known and proposed mechanisms of cerebro–cerebellar interaction.

Fatemi et al. published an exhaustive review of the pathological role of the cerebellum in autism (2012). The authors concluded their paper with the caution that "More definitive behavioral tests are required to confirm the validity of animal models for autism".

Autism in the human presents both a positive and a negative opportunity for understanding the disorder. Invasive experiments are limited to animal models, but behavioral and clinical observations and direct questioning of patients are equally important and useful, provided they are conducted with sufficient caution.

Many primary questions about autism are still much argued in the literature. A good example of this is a study described by Dawson et al. (2007) concerning the level of autistic intelligence compared to that of non-autistic control subjects. Their conclusion was that the intelligence of autistic people has been too often underestimated, but the true value of the paper was their careful and systematic development and presentation of their methodology. This was a refreshing, educated insight into the nature of autism.

Of course we know that genetic mutations have been linked to autism. It is thought these could number in the hundreds (Buxbaum et al. 2012). Epigenetics also plays a role (Shulha et al. 2012), just as it does in normal brain development.

Epigenetics would be expected to play a role in the development of autism in response to stress. In fact, stress has been implicated and studied extensively by many as being a possible cause of ASD.

Prenatal stressors were correlated with an increased incidence of autism if they occurred during 21–32 weeks of pregnancy, with a peak between 25 and 28 weeks (Beversdorf et al. 2005). This was seen to be consistent with particular pathological outcomes of neuroanatomical development, e.g. showing pathological changes in the cerebellum, but the authors called for more studies related to their findings.

Kern and Jones (2006) implicated neuronal cell death, and brain damage from insult after birth caused by the effects of toxicity and oxidative stress. This, they said, was related to the loss of Purkinje cells found in autism and the related increase in the volume of the brain.

Eric Courchesne and others have taken good advantage of the limited amount of brain tissue available from autistic people who died and donated their brains. From these studies he found a 67% increase in the number of brain cells of the prefrontal cortex when compared to corresponding tissue from people of the same age who were not autistic (Courchesne et al. 2011). Brain weight and overall size were also increased in autistic individuals (Courchesne et al. 2011). This was consistent with earlier reports of autism being related to overgrowth of the head and brain in the

frontal region. In fact, Stanfield et al. (2007) pointed out that in autistic subjects they studied with magnetic resonance imaging (MRI), although the corpus callosum was reduced, the total brain, the caudate nucleus, and the cerebral hemispheres exhibited an increased volume.

Several investigators over the years have discussed the overgrowth of nerve cells in the prefrontal cortex as characteristic of autism and Courchesne's continuation of this study has quantified some specific processes leading to this condition. The lack of selective pruning of collaterals during development in this region of the brain seems to be a primary factor. We know that pruning of nerve cells is a major component of brain development in the early years of life. This has been studied in several specific areas of brain development. One of the most striking investigations of this was of the role of nerve cell pruning in the development of stereo vision (Ding and Elberger 2000, 2001). Improper pruning would lead to impaired vision or even blindness. In the case presented by Courchesne et al. (2011) the massive prenatal build up of nerve cells resulting from lack of pruning in the prefrontal cortex might leave the individual with increased "neuronal noise" in this portion of the cognitive system.

Evidence suggests that the cerebral–cerebellar connection is greatly impaired in autism (Skoyles 2002) and this portion of the problem seems to be a central factor of the pathology of autism. That cerebral–cerebellar function of fine-tuning movement and muscle action is impaired, is easily seen in many people with autism because of their lack of smooth movement and various other motor deficits (Donnellan et al. 2013). On a simplistic level, this is understandable considering that it is generally accepted that the prefrontal cortex normally takes longer to mature than the cerebellum and, in autism, the communication between the cerebellum and prefrontal cortex is likely to be impaired due to the overpopulation of the prefontal cortex with nerve cells and the possibly resulting increase in "noise" (Courchesne et al. 2011).

Bernard Crespi (Crespi 2010; Crespi et al. 2010) hypothesized that autism spectrum disorder (ASD) and psychotic-affective spectrum disorders (schizophrenia) both involve problems with social interaction. He characterized ASD as exhibiting an over-development and schizophrenia as exhibiting an under-development of what he called the "human-specific social brain phenotypes." He and his colleagues supported their view with studies of how autism risk alleles affect manifestations of the disorder and through analysis of the literature. Developmental neurogenesis work and physiological and genetic studies of synaptic function in the mouse support Crespi's hypothesis (Kaphzan et al. 2012; Heck and Lu 2012; Hoerder-Suabedissen et al. 2013).

Another comparison between autism and schizophrenia can be made relative to the awareness of self or self-consciousness. Schizophrenia has been called a disorder of self which can take various forms and to varying degrees, but there is a general distortion of consciousness and lack of self presence (Sass and Parnas 2003). Autism can also be characterized by a lack of self-consciousness, but high-functioning autism or people with Asperger syndrome may have or acquire self-consciousness through learning (Frith and Happe 1999). Memory tests by Toichi et al. (2002) also revealed deficits in the consciousness of self by people with autism. It is noteworthy

that many of the genes implicated in autism and schizophrenia are active only during specific stages of the developing brain (Hoerder-Suabedissen et al. 2013), suggesting the existence of critical periods for the normal development of consciousness of self.

Self in people with autism is often compromised because memories are persistent, not affected by emotion or discussed with others (Jordan 2008). Access to memories is not efficient and the memories are often not coherent. The proper handling of memory needs to be taught because otherwise it is difficult to ascribe particular memories as part of self. Because the autistic person can have obsessive chains of negative thoughts making him or her vulnerable to anxiety with depression being many times the result (Jordan 2008).

Raison et al. (2006) pointed out that stress (anxiety would certainly be a stress) can lead to inflammation in the nervous system associated with the pathogenesis of depression. In this situation, it becomes a difficult "chicken or the egg" question. These authors cite many studies which explore the association of inflammation and depression continuing through the adult life of the individual. Using brain tissue from the autopsies of 11 autistic patients, neuroinflammatory markers were found in the cerebellum and other areas by Vargas et al. (2004). The authors also point out that neuroglial reactions are manifested by immune responses, focused in the resulting chronic inflammatory condition of the cerebellum. The detected presence of antiinflammatory cytokines and proinflammatory chemokines support their conclusion that this condition is a characteristic of some autistic brains. They believe addressing these neuroglial conditions might be a viable therapy in some people with ASD.

We know that adverse neonatal experiences can alter brain development (Anand and Scalzo 2000). Brain inflammation is often hypothesized as a possible cause of several neurological disorders. Alzheimers is just one that is pointed out as a disease possibly resulting from brain inflammation. See the paper by Yao et al. (2004) as just one study in support of this concept.

One review of the literature (Angelidou et al. 2012) revealed a consensus that several genes exist which can make an embryo susceptible to autism, but environment is a big factor leading to the actual development of autism. A partially developed gut–blood–brain barrier would not protect against neurotoxins and stressors can cause autoimmune problems for the mother, which can make the embryo susceptible to the development of autism. Thus, stress can lead to brain inflammation, resulting in autism for embryos genetically predisposed to this disorder.

This view supports the earlier work of Pardo et al. (2005) who strongly suggested environmental factors and stressors could combine with genetic susceptibility in increasing the possibility an embryo would develop autism.

These two latter papers also emphasized the importance of the presence of an autoimmune condition and the role of auto-antibodies and neuroglia in the brain's reaction to stress.

One argument has been presented in the literature, supported by several researchers, that schizophrenia and some other autoimmune diseases, such as autism, could result from what has been termed the "leaky gut" in which neuropeptides of

food proteins escape the gut and get past the blood-brain barrier resulting in CNS dysfunction (Dohan 1988; Eaton et al. 2006). Of course, inflammation is part of the mechanism of this insult on the brain and resulting inflammation can come from many sources, both internal and external.

There is a wealth of epidemiological evidence that the environment might affect the prevalence of ASD in certain geographical areas (Fombonne 2005, 2006). We have not addressed this here. However, the importance of the environment on the occurrence of autism is quite apparent from an extensive recent study by Suren and colleagues (Suren et al. 2013) of over 85,000 children. Those children whose mothers took folic acid around the time of conception had a distinctly lower risk of autistic disorder than those whose mothers did not. This and similar studies strongly suggest that more effort should be directed towards the study of environmental factors in ASD.

Experiments in animals, in particular in genetic mouse models of ASD, provide important clues as to possible neuronal pathways and mechanisms of interaction between the cerebellum and the cerebral prefrontal cortex and their involvement in autism. Tracing studies in primates have shown that the cerebellum projects (via the thalamus) to the prefrontal cortex (Middleton and Strick 2001). In turn, the prefrontal cortex projects back to the cerebellar areas from where prefrontal cortical projections originated, forming what might amount to parallel loops of cerebral–cerebellar connections (Kelly and Strick 2003). While these anatomical connections clearly suggest targeted neuronal interactions between the two structures, how cerebellar activity modulates prefrontal cortical activity and vice versa and how their interaction is related to behavior is poorly understood.

Recent experiments in mice have revealed a surprising new aspect of prefrontal cortical interaction with the cerebellum. When stimulating the cerebellar dentate nucleus in healthy mice, Mittleman and colleagues discovered an increase in dopamine release in the medial prefrontal cortex (Mittleman 2008). Dopamine is a neuromodulatory transmitter generated by cells in the substantia nigra and ventral tegmental area (VTA) and is most widely known for its importance in the failure and disinhibition of movement initiation in Parkinson's and Huntington's disease respectively (Graybiel et al. 1994). But dopamine is also strongly implicated in reward and pleasure seeking behavior, drug addiction and cognitive functions (Iversen et al. 2010). Abnormal function of the dopaminergic system has been implicated in a variety of cognitive disorders including autism spectrum disorders and schizophrenia (Dichter et al. 2012a, b; Sesack and Carr 2002). Cerebellar controlled dopamine release in the mouse medial prefrontal cortex was mediated by two independent and equally contributing pathways, one involving the ventral tegmental area (VTA) which contains dopaminergic cells projecting to the prefrontal cortex, the other involving the thalamus (Rogers et al. 2011). Increased dopamine release via cerebellar activated thalamic projections involved stimulation of mesocortical dopaminergic terminals via appositional excitatory glutamatergic synapses (Del Arco and Mora 2005; Pinto et al. 2003). A recent study in a mouse model of fragile X syndrome, an autism spectrum disorder, showed a reorganization of the pathways involved in cerebellar modulation of mPFC dopamine release resulting in a

weakening of the VTA and a strengthening of the thalamic pathway via the ventrolateral nucleus (Rogers et al. 2013). Together with the known cerebellar deficits associated with autism (Courchesne 1997; Skoyles 2002; Webb et al. 2009; Fatemi et al. 2012) these findings suggest that a dysfunction of cerebellar dependent reward circuits may play a role in at least some forms of autism. Consistent with this hypothesis are recent findings by Dichter and colleagues who used functional magnetic resonance imaging (fMRI) to compare reward circuit responses in autistic and control subjects and reported hypo-activation of reward circuit activity in autistic individuals (Dichter et al. 2012b). While more studies are needed to determine how strongly the cerebellum is involved in reward seeking tasks like those chosen by Dichter and colleagues, a role of the cerebellum in human cocaine-related behavior has already been demonstrated (Anderson et al. 2006). Together these studies suggest that the cerebellum modulates prefrontal cortical activity via dopamine, thus contributing to a broad spectrum of sensorimotor and cognitive functions, especially behaviors involving reward seeking and positive reinforcement (Iversen et al. 2010).

These dopamine mediated modulatory actions are unlikely to take place at the millisecond temporal resolution often associated with cerebellar coordination of movements (Braitenberg 1967; Welsh et al. 1995; Heck et al. 2007; Jacobson et al. 2008; Yarom and Cohen 2002). Mittleman et al. reported that the time course of PFC dopamine release in response to cerebellar stimulation could span tens of seconds (Mittleman et al. 2008). This suggests that the cerebellum can operate at much slower time scales, modulating PFC activity during slow complex processes, such as the analysis and interpretation of facial expressions, social contexts and theory of mind. Whether cerebellar modulation of PFC dopamine release does indeed serve any of these functions remains to be shown. But with the close association of cerebellar and PFC abnormalities with autism (Townsend et al. 2001; Carper and Courchesne 2000) it is at least an intriguing hypothesis yet to be tested, especially considering the accumulating evidence for a cerebellar role in cognition.

The above studies focused on the interaction between the cerebellum and the prefrontal cortex, which is an association cortical area not involved in motor control. But the most obvious clinical symptoms of cerebellar dysfunction are ataxic movements, balance and eye movement deficits. Motor deficits are common in ASD patients (Donnellan et al. 2013; Leary and Hill 1996) and at least some of those seem to be caused by cerebellar neuropathology (Takarae et al. 2004; Fatemi et al. 2012). Several studies in mouse models of single gene autism spectrum disorders such as Angelman, fragile X syndrome and Smith-Magenis syndrome have documented motor deficits in the mutant mice (e.g. Roy et al. 2011, 2012; Heck et al. 2008, 2012). It is thus likely that cerebellar deficits associated with ASD contribute to both, motor and cognitive deficits. Further complicating the issue from a translational perspective is the recent discovery suggesting that prefrontal cerebellar interactions in rodents are involved in motor learning (Kalmbach et al. 2009). Whether the same holds true for humans remains to be determined.

This review is an overview of a variety of lines of research on the causes and neuronal mechanism of autism. We began by focusing on a possible link between

motor control and cognitive function. However, faced with the sheer variety of ASDs it soon became clear that attempts at generalizations were futile. We thought it more useful to investigate the breadths of the spectrum of possible causes currently explored. We mostly covered neuronal aspects of ASD. The most extensive research efforts are currently directed towards genetic causes of autism, extensively reviewed elsewhere (Folstein and Rosen-Sheidley 2001; Veenstra-Vanderweele and Cook 2004; Miles 2011; Carter and Scherer 2013).

From what we have seen, inflammation plays a role in many of the theories, either in a primary or secondary manner. And, genetics provides the background for susceptibility. Given the overall knowledge thus far in the literature, we think that any conjecture as to the pathogenesis or description of ASD should consider both, inflammation and genetics.

Our review is far from comprehensive. Not covered here but adding to the puzzling variety of relevant clues are studies linking autism to, for example, autoimmune disorder (Brimberg et al. 2013), some forms of epilepsy (Sansa et al. 2011) or prenatal exposure to bisphenol A, a common compound found in plastic food and drink containers (Wolstenholme et al. 2013). It is clear that no single line of investigation will lead to a comprehensive understanding of the etiology of autism. Autism research now faces the considerable challenge of integrating large amounts of diverse data (Williams 2009). Qualitatively new insights will most likely arise from integrative approaches considering interactions between prominent variables.

Based on our limited review of the literature, there are several other opportunities in the study of autism that cry out for attention:

1. Much can be learned about schizophrenia and autism from constant comparisons in the studies of the two conditions. Following Bernard Crespi's lead, studies of everything from genomics to perception in people who have one of these conditions will enhance our understanding of both.
2. We know that much of the proper development of the brain depends on the mechanisms involved in selective pruning of nerve cells and collaterals. We need to know more about neurological pruning overall and specifically about its role in cognitive brain function.
3. The communication systems between the prefrontal cortex and the cerebellum have been studied to some extent, but we are still far from understanding how these systems operate in the normal brain, which is likely a prerequisite to understanding its dysfunction in autism.
4. As we continue to explore the human genome our appreciation of its complexity grows and this will provide new avenues to try to understand autism. For example, we know that the retrogene GLUD2 is derived from glutamate dehydrogenase. This agent is responsible for clearing the by-products of neuron activity from the system (Burki and Kaessmann 2004). The possible excessive buildup of by-products from the overpopulation of nerve cells of the prefrontal cortex of autism patients may be one of many factors contributing to the ill effects of autism. Or does some other gene compensate?
5. There is a special opportunity in the study of autism to learn much more about the basic nature of human intelligence.

Acknowledgement DHH is supported by grants from the National Institute of Health (NIH, NS060887, NS067201 and R01NS063009). The content of this publication is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

References

Anand KJS, Scalzo FM (2000) Can adverse neonatal experiences alter brain development and subsequent behavior? Biol Neonate 77:69–82

Anderson CM, Maas LC, Frederick B, Bendor JT, Spencer TJ, Livni E, Lukas SE, Fischman AJ, Madras BK, Renshaw PF, Kaufman MJ (2006) Cerebellar vermis involvement in cocaine-related behaviors. Neuropsychopharmacology 31:1318–1326

Angelidou A, Asadi S, Alysandratos K-D, Karagkouni A, Kourembanas S, Theoharides TC (2012) Perinatal stress, brain inflammation and risk of autism-Review and proposal. BMC Pediatrics 12:89

Beversdorf DQ, Manning SE, Hillier A, Anderson SL, Nordgren RE, Walters SE, Nagaraja HN, Cooley WC, Gaelic SE, Bauman ML (2005) Timing of prenatal stressors and autism. J Autism Dev Disord 35:471–478

Braitenberg V (1967) Is the cerebellar cortex a biological clock in the millisecond range? In: Fox CA, Snider RS (eds) Progress in brain research, The Cerebellum, vol 25. Elsevier, Amsterdam, pp 334–346

Brimberg L, Sadiq A, Gregersen PK, Diamond B (2013) Brain-reactive IgG correlates with autoimmunity in mothers of a child with an autism spectrum disorder. Mol Psychiatry 18:1171–1177

Burki F, Kaessmann H (2004) Birth and adaptive evolution of a hominoid gene that supports high neurotransmitter flux. Nat Genet 36:1061–1063

Buxbaum JD, Daly MJ, Devlin B, Lehner T, Roeder K, State MW, The Autism Sequencing Consortium (2012) The autism sequencing consortium: large-scale, high-throughput sequencing in autism spectrum disorders. Neuron 76:1052–1056

Carper RA, Courchesne E (2000) Inverse correlation between frontal lobe and cerebellum sizes in children with autism. Brain 123:836–844

Carter MT, Scherer SW (2013) Autism spectrum disorder in the genetics clinic: a review. Clin Genet 83:399–407

Casanova MF (2005) Minicolumnar pathology in autism. In Casanova MF (ed.) Recent developments in Autism research. Nova Biomedical Books, New York, pp 133–143

Courchesne E (1997) Brainstem, cerebellar and limbic neuroanatomical abnormalities in autism. Curr Opin Neurobiol 7: 269–278

Courchesne E, Mouton PR, Calhoun ME, Semendeferi K, Ahrens-Barbeau C, Hallet MJ, Barnes CC, Pierce K (2011) Neuron number and size in prefrontal cortex of children with autism. JAMA 306:2001–2010

Crespi BJ (2010) Revisiting Bleuler: relationship between autism and schizophrenia. Br J Psychiatry 196:495; (author reply 495–496)

Crespi B, Stead P, Elliot M (2010) Evolution in health and medicine Sackler colloquium: comparative genomics of autism and schizophrenia. Proc Natl Acad Sci U S A 107:1736–1741.

Dawson M, Soulieres I, Gernsbacher MA, Mottron L (2007) The level and nature of autistic intelligence. Psychol Sci 18:657–662

Del Arco A Mora F (2005) Glutamate-dopamine in vivo interaction in the prefrontal cortex modulates the release of dopamine and acetylcholine in the nucleus accumbens of the awake rat. J Neural Transm 112:97–109

Dichter GS, Damiano CA, Allen JA (2012a) Reward circuitry dysfunction in psychiatric and neurodevelopmental disorders and genetic syndromes: animal models and clinical findings. J Neurodev Disord 4:19

Dichter GS, Felder JN, Green SR, Rittenberg AM, Sasson NJ, Bodfish JW (2012b) Reward circuitry function in autism spectrum disorders. Soc Cogn Affect Neurosci 7:160–172

Ding SL, Elberger AJ (2000) Neuropeptide Y- and somatostatin-immunoreactive axons in the corpus callosum during postnatal development of the rat. Brain Res Dev Brain Res 124:59–65

Ding SL, Elberger AJ (2001) Postnatal development of biotinylated dextran amine-labeled corpus callosum axons projecting from the visual and auditory cortices to the visual cortex of the rat. Exp Brain Res 136:179–193

Dohan FC (1988) Genetic hypothesis of idiopathic schizophrenia: Its exorphin connection. Schizophia Bulletin 11:489–494

Donnellan AM, Hill DA, Leary MR (2013) Rethinking autism: implications of sensory and movement differences for understanding and support. Front Integr Neurosci 6:124

Eaton WW, Byrne M, Ewald H, Mors O, Chen C-Y, Agerbo E, Mortensen PB (2006) Association of schizophrenia and autoimmune diseases: linkage of Danish national registers. Am J Psychiatry 163:521–528

Fatemi SH, Aldinger KA, Ashwood P, Bauman ML, Blaha CD, Blatt GJ, Chauhan A, Chauhan V, Dager SR, Dickson PE, Estes AM, Goldowitz D, Heck DH, Kemper TL, King BH, Martin LA, Millen KJ, Mittleman G, Mosconi MW, Persico AM, Sweeney JA, Webb SJ, Welsh JP (2012) Consensus paper: pathological role of the cerebellum in autism. Cerebellum 11:777–807

Folstein SE, Rosen-Sheidley B (2001) Genetics of autism: complex aetiology for a heterogeneous disorder. Nat Rev Genet 2:943–955

Fombonne E (2005) The epidemiology of pervasive developmental disorders. In: Casanova MF (ed) Recent developments in autism research. Nova Biomedical Books, New York, pp 1–25

Fombonne E (2006) Past and future perspectives on autism epidemiology. In: Moldin SO, Rubenstein JL (eds) Understanding autism: from basic neuroscience to treatment. CRC Press, Boca Raton, pp 25–48

Frith U, Happe F (1999) Theory of mind and self-consciousness: what is it like to be autistic? Mind and Language 14:1–22

Graybiel AM, Aosaki T, Flaherty AW, Kimura M (1994) The basal ganglia and adaptive motor control. Science 265:1826–1831

Grillner S (2006) Biological pattern generation: the cellular and computational logic of networks in motion. Neuron 52:751–766

Harris-Warrick RM (2010) General principles of rhythmogenesis in central pattern generator networks. Prog Brain Res 187:213–222

Heck DH, Lu L (2012) The social life of neurons: synaptic communication deficits as a common denominator of autism, schizophrenia, and other cognitive disorders. Biol Psychiatry 72:173–174

Heck DH, Howell JW (2013) Prefrontal cortical—cerebellar interaction deficits in autism spectrum disorders. Autism Open Access S 4:001

Heck DH, Thach WT, Keating JG (2007) On-beam synchrony in the cerebellum as the mechanism for the timing and coordination of movement. Proc Natl Acad Sci U S A 104:7658–7663

Heck DH, Zhau Y, Roy S, LeDoux MS, Reiter LT (2008) Analysis of cerebellar function in ube3a deficient mice reveals novel genotype specific behaviors. Hum Mol Gen 17:2181–2189

Heck DH, Gu W, Cao Y, Qi S, Lacaria M, Lupski JR (2012) Opposing phenotypes in mice with Smith-Magenis deletion and Potocki-Lupski duplication syndromes suggest gene dosage effects on fluid consumption behavior. Am J Med Genet A 158A:2807–2814

Hoerder-Suabedissen A, Oeschger FM, Krishnan ML, Belgard TG, Wang WZ, Lee S, Webber C, Petretto E, Edwards AD, Molnár Z (2013) Expression profiling of mouse subplate reveals a dynamic gene network and disease association with autism and schizophrenia. Proc Natl Acad Sci U S A 110:3555–3560

Iversen L, Iversen S, Dunnett S, Bjorklund A (2010) Dopamine handbook. Oxford University Press, Oxford

Jacobson GA, Rokni D, Yarom Y (2008) A model of the olivo-cerebellar system as a temporal pattern generator. Trends Neurosci 31:617–625

Jordan RR (2008) Practical implications of memory characteristics in autistic spectrum disorders. In: Boucher J, Bowler D (eds), Memory in autism: theory and evidence. Cambridge University Press, Cambridge, pp 293–3010

Kalmbach BE, Ohyama T, Kreider JC, Riusech F, Mauk MD (2009) Interactions between prefrontal cortex and cerebellum revealed by trace eyelid conditioning. Learn Mem 16:86–95.

Kaphzan H, Hernandez P, Jung JI, Cowansage KK, Deinhardt K, Chao MV, Abel T, Klann E (2012) Reversal of impaired hippocampal long-term potentiation and contextual fear memory deficits in angelman syndrome model mice by erbB inhibitors. Biol Psychiatry 72:182–190

Kelly RM, Strick PL (2003) Cerebellar loops with motor cortex and prefrontal cortex of a nonhuman primate. J Neurosci 23:8432–8444

Kern JK, Jones AM (2006) Evidence of toxicity, oxidative stress, and neuronal insult in autism. J Toxicol Environ Health B Crit Rev 9:485–499

Leary MR, Hill DA (1996) Moving on: autism and movement disturbance. Ment Retard 34:39–53

Llinás RR (2002) I of the vortex. From neurons to self. MIT Press, Cambridge

Middleton FA, Strick PL (2001) Cerebellar projections to the prefrontal cortex of the primate. J Neurosci 21:700–712

Miles JH (2011) Autism spectrum disorders—a genetics review. Genet Med 13:278–294

Mittleman G, Goldowitz D, Heck DH, Blaha CD (2008) Cerebellar modulation of frontal cortex dopamine efflux in mice: relevance to autism and schizophrenia. Synapse 62:544–550

Pardo CA, Vargas DL, Zimmerman AW (2005) Immunity, neuroglia and neuroinflammation in autism. Int Rev Psychiatry 17:485–495

Pinto A, Jankowski M, Sesack SR (2003) Projections from the paraventricular nucleus of the thalamus to the rat prefrontal cortex and nucleus accumbens shell: ultrastructural characteristics and spatial relationships with dopamine afferents. J Comp Neurol 459:142–155

Ploeger A, Galis F (2011) Evolutionary approaches to autism—an overview and integration. MJM 13:38–43

Raison CL, Capuron L, Miller AH (2006) Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol 27:24–31

Reser KE (2011) Conceptualizing the autism spectrum in terms of natural selection and behavioral ecology: the solitary forager hypothesis. Evol Psychol 9:207–238

Rogers TD, Dickson PE, Heck DH, Goldowitz D, Mittleman G, Blaha CD (2011) Connecting the dots of the cerebro–cerebellar role in cognitive function: neuronal pathways for cerebellar modulation of dopamine release in the prefrontal cortex. Synapse 65:1204–1212

Rogers TD, Dickson PE, McKimm E, Heck DH, Goldowitz D, Blaha CD, Mittleman G (2013) Reorganization of circuits underlying cerebellar modulation of prefrontal cortical dopamine in mouse models of autism spectrum disorder. Cerebellum 12:547–556

Roy S, Zhao Y, Allensworth M, Farook MF, Reiter LT, Heck DH (2011) Comprehensive motor testing in Fmr1-KO mice exposes temporal defects in oromotor coordination. Behav Neurosci 125:962–969

Roy S, Watkins N, Heck DH (2012) Comprehensive analysis of ultrasonic vocalizations in a mouse model of Fragile X syndrome reveals limited, call type specific deficits. PLoS One 7:e44816

Sansa G, Carlson C, Doyle W, Weiner HL, Bluvstein J, Barr W, Devinsky O (2011) Medically refractory epilepsy in autism. Epilepsia 52:1071–1075

Sass LA, Parnas J (2003) Schizophrenia, consciousness, and the self. Schizophr Bull 29:427–444

Schmahmann JD (1998) The cerebellum and cognition. San Diego: Academic Press

Sesack SR, Carr DB (2002) Selective prefrontal cortex inputs to dopamine cells: implications for schizophrenia. Physiol Behav 77:513–517

Shulha HP, Cheung I, Whittle C, Wang J, Virgil D, Lin CL, Guo Y, Lessard A, Akbarian S, Weng Z (2012) Epigenetic signatures of autism: trimethylated H3K4 landscapes in prefrontal neurons. Arch Gen Psychiatry 69:314–324

Skoyles JR (2002) Is autism due to cerebral–cerebellum disconnection? Med Hypotheses 58:332–336

Stanfield AC, McIntosh AM, Spencer MD, Philip R, Gaur S, Lawrie SM (2007) Towards a neuroanatomy of autism: a systematic review and meta-analysis of structural magnetic resonance imaging studies. Eur Psychiatry 23:289–299

Suren P, Roth C, Bresnahan M, Haugen M, Hornig M, Hirtz D, Lie KK, Lipkin WI, Magnus P, Reichborn-Kjennerud T, Schjølberg S, Smith GD, Øyen A-S, Susser E, Stoltenberg C, (2013) Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children. JAMA 309:570–577

Takarae Y, Minshew NJ, Luna B, Sweeney JA (2004) Oculomotor abnormalities parallel cerebellar histopathology in autism. J Neurol Neurosurg Psychiatry 75:1359–1361

Toichi M, Kamio Y, Okada T, Sakihama M, Youngstrom EA, Findling RL, Yamamoto K (2002) A lack of self-consciousness in autism. Am J Psychiatry 159:1422–1424

Townsend J, Westerfield M, Leaver E, Makeig S, Jung T, Pierce K, Courchesne E (2001) Event-related brain response abnormalities in autism: evidence for impaired cerebello-frontal spatial attention networks. Brain ResCogn Brain Res 11:127–145

Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. (2004) Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann Neurol 57:67–81

Veenstra-Vanderweele J, Cook EH Jr (2004) Molecular genetics of autism spectrum disorder. Mol Psychiatry 9:819–832

Webb SJ, Sparks BF, Friedman SD, Shaw DW, Giedd J, Dawson G, Dager SR (2009) Cerebellar vermal volumes and behavioral correlates in children with autism spectrum disorder. Psychiatry Res 172:61–67

Welsh JP, Lang EJ, Suglhara I, Llinas R (1995) Dynamic organization of motor control within the olivocerebellar system. Nature 374:453–457

Williams RW (2009) Herding cats: the sociology of data integration. Front Neurosci 3:154–156

Wolstenholme JT, Goldsby JA, Rissman EF (2013) Transgenerational effects of prenatal bisphenol A on social recognition. Horm Behav 64:833–839

Yao Y, Chinnici C, Tang H, Trojanowski JQ, Lee VMY, Praticò D (2004) Brain inflammation and oxidative stress in a transgenic mouse model of Alzheimer-like brain amyloidosis. J Neuroinflammation 1:21

Yarom Y, Cohen D (2002) The olivocerebellar system as a generator of temporal patterns. Ann NY Acad Sci 978:122–134

Yuste R, MacLean JN, Smith J (2005) The cortex as a central pattern generator. Nat Rev Neurosci 6:477–483

Chapter 20

Increased Vulnerability to Oxidative Stress and Mitochondrial Dysfunction in Autism

Abha Chauhan and Ved Chauhan

Abstract The exact cause of autism is not known but the role of genetic and environmental factors as well as oxidative stress have been suggested in autism. Accumulating evidence also suggests defects in mitochondrial electron transport chain complexes in the individuals with autism, which may also lead to abnormal energy metabolism and increased oxidative stress. Several studies have reported increased oxidative damage as evident by increased lipid peroxidation, protein and DNA oxidation as well as decreased antioxidant status (enzymatic and non-enzymatic) indicated by glutathione—redox imbalance and decreased activities of antioxidant enzymes in the blood, urine and brain tissues of subjects with autism. Here, we review the evidence of oxidative stress, mitochondrial dysfunction and the genetic variations of enzymes that can increase vulnerability to oxidative stress in autism.

Keywords Antioxidants · Autism · Glutathione · Mitochondrial dysfunction · Oxidative stress

Autism spectrum disorders (ASDs) are behaviorally defined neurodevelopmental disorders that are characterized by impairment in social interactions, verbal and nonverbal skills, and restricted, repetitive and stereotyped patterns of behavior (Lord et al. 2000). ASDs include autism, Asperger syndrome and Pervasive developmental disorder-not otherwise specified. The symptoms of ASDs are typically present before the age of 3 years which can be regressive or non-regressive. In non-regressive autism, children do not adopt the normal social and language development. On the other hand, children with regressive autism first show normal social and language development, but lose these developmental skills at 15–24 months (Ozonoff et al. 2005). Last few decades have experienced a rapid increase in the number of ASD cases. Recently, the Centers of Disease Control and Prevention reported the prevalence of ASD to be 1 in 68 children in United States (Wingate et al. 2014). This increase cannot be completely attributed to an increased awareness or to changes in diagnosis criteria of autism. While the cause of autism

V. Chauhan (✉) · A. Chauhan
New York State Institute for Basic Research in Developmental Disabilities,
1050 Forest Hill Road, Staten Island, NY 10314, USA
e-mail: ved.chauhan@opwdd.ny.gov

S. Hossein Fatemi (ed.), *The Molecular Basis of Autism,*
Contemporary Clinical Neuroscience, DOI 10.1007/978-1-4939-2190-4_20

remains elusive, it is considered a multifactorial disorder that is influenced by environmental and genetic factors. Multiple lines of evidence suggest high prevalence of oxidative stress and mitochondrial dysfunction in the subjects with autism (Chauhan and Chauhan 2006; Kern and Jones 2006; Deth et al. 2008; Chauhan et al. 2009a, 2012b; Rossignol and Frye 2012a, b).

20.1 Oxidative Stress

There is oxidative stress when the generation of free radicals i.e., reactive oxygen species (ROS) overpowers the antioxidant defense capacity of the cell. The brain is highly vulnerable to oxidative stress due to its limited antioxidant capacity, high energy requirement and high amounts of polyunsaturated lipids and iron (Juurlink and Paterson 1998). Oxidative stress can occur due to increased production of free radicals originating from endogenous or exogenous sources, decreased antioxidant defense or both. In most cases, endogenous source of increased ROS is damaged or unhealthy mitochondria (Fig. 20.1). The mitochondrial respiratory chain, also known as the electron transport chain (ETC), consisting of five enzymes, i.e., complexes I–V, is responsible for the generation of energy in the form of ATP (Szewczyk and Wojtczak 2002; Bertram et al. 2006). ETC complexes I–IV generate a proton gradient, i.e., mitochondrial membrane potential (MMP), which is needed by complex V (ATP synthase) for ATP production. The mitochondrial dysfunction results in increased generation of free radicals, i.e., ROS and reactive nitrogen species (RNS), and also to oxidative stress and apoptosis (Cadenas and Davies 2000; Lenaz 2001; Brookes et al. 2002; Polster and Fiskum 2004). While oxidative phosphorylation in the mitochondria generates superoxide anions, enzymatic oxidation of biogenic amines by monoamine oxidase (MAO) in the outer mitochondrial membrane produces hydrogen peroxide (H_2O_2).

Mitochondrial ETC complexes I and III are the main sites of production of superoxide radical by mitochondria (Barja 1999; Muller et al. 2004). The leaked electron reduces molecular oxygen to superoxide anion, which is an initial step for subsequent production of other ROS. Superoxide anion can be converted to H_2O_2 by superoxide dismutase (SOD). Alternatively, H_2O_2 can also be produced by the actions of xanthine oxidase (XO), MAO or nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase (Granger 1988; Dupuy et al. 1991). It can get further converted to highly reactive hydroxyl radicals, which requires a two step reaction (Fig. 20.1). In the first step (I), superoxide gives rise to molecular oxygen by Haber-Weiss reaction where Fe^{3+} is converted to Fe^{2+}. In the second step (II), Fe^{2+} converts H_2O_2 to hydroxyl radical by Fenton reaction. In the reaction catalyzed by myeloperoxidase (MPO), H_2O_2 can also react with chlorides to form hydrochlorous acid (HOCl), which can either get converted to singlet oxygen, or to hydroxyl radicals by

reacting with Fe^{2+} or superoxide. In addition, superoxide can also react with nitric oxide (NO) resulting in the formation of peroxynitrite ($ONOO^{\cdot}$).

There is substantial evidence suggesting that environmental factors can increase vulnerability to oxidative stress, and may lead to clinical phenotype in autism. Our previous reviews have discussed the role of endogenous and exogenous environmental pro-oxidants such as NO, XO, homocysteine, heavy metals (mercury, lead), maternal prescription drugs (thalidomide and valproic acid), air pollutants, chemicals and toxins, pathologenic bacteria and viral infections that can lead to oxidative stress in autism (Chauhan and Chauhan 2006; Chauhan et al. 2009a).

It is widely accepted that an excess of ROS is toxic and damages essential cell components such as nucleic acids, proteins and lipids, leading to cell death. ROS can lead to oxidation of amino acid side chains, formation of protein-protein cross-linkages, and oxidation of the protein backbone resulting in protein fragmentation. Although all DNA bases are susceptible to damage, guanine is most prone to ROS-mediated oxidation of DNA and is therefore a primary target of oxidative modification (Cadet et al. 2002). Brain is a rich source of polyunsaturated fatty acids, which are easy targets of oxidation. The most common oxidative markers of protein oxidation are protein carbonyls and protein nitrates. The common markers of lipid peroxidation by free radicals are malonyldialdehyde (MDA), 4-hydroxynonenal (HNE), and F2-isoprostanes. The nucleic acid oxidation products that are frequently used for measurement of oxidative stress are: 8-hydroxy deoxyguanosine for DNA, and 8-hydroxyguanosine for RNA.

20.2 Antioxidant Defense

Antioxidants can be enzymatic or non-enzymatic in nature. As shown in Fig. 20.1, major enzymes that participate in anti-oxidant activity include SOD, catalase and glutathione peroxidase (GPx).Non-enzymatic antioxidants include redox proteins such as thioredoxins, peroxiredoxins, and glutaredoxins (Birben et al. 2012), and low molecular weight compounds such as glutathione (GSH), vitamin C, vitamin E and beta-carotene. Among these antioxidants, GSH is a major endogenous antioxidant that protects cells from endogenous and exogenous toxins, particularly in the central nervous system.

20.3 Oxidative Stress in Autism

Oxidative stress in autism can be caused by increased ROS generation, decreased anti-oxidant defense (enzymatic or non-enzymatic), and mitochondrial dysfunction.

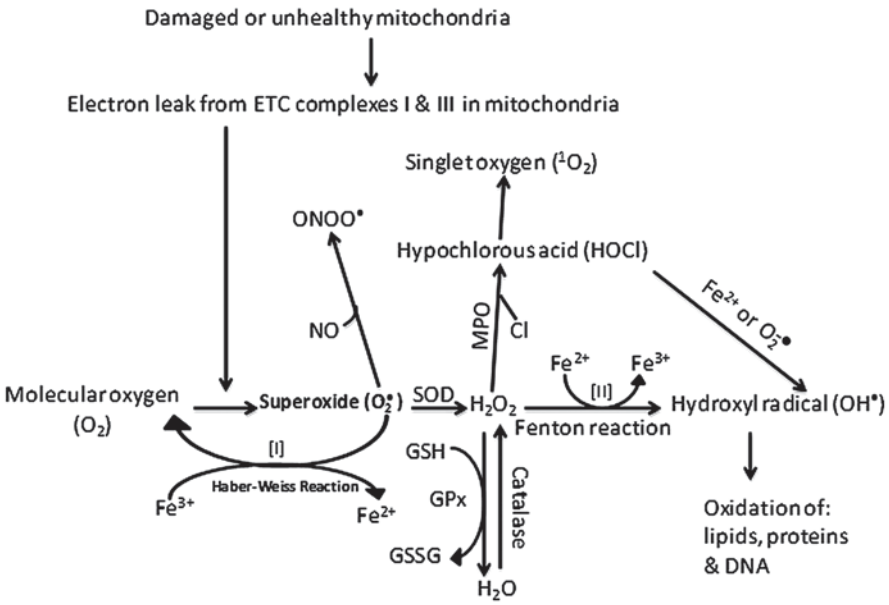


Fig. 20.1 Mechanism of generation of reactive oxygen species and oxidative stress. Leaked electrons from mitochondrial ETC complexes I and III react with molecular oxygen to produce superoxide (first free radical). The superoxide is then converted to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD). Superoxide can also react with nitric oxide (NO) to form peroxynitrite ($ONOO^{\bullet}$), and it can also contribute to the formation of hydroxyl ($OH^{\bullet}$) radical through Haber-Weiss and Fenton reactions. On the other hand, antioxidant enzymes (catalase and glutathione peroxidase, GPx) can convert H_2O_2 to H_2O. Glutathione (GSH) is a major antioxidant, which is converted to its oxidized form (GSSG) by GPx. A highly potent free radical, $OH^{\bullet}$ oxidizes lipids, proteins and nucleic acids. Myeloperoxidase (MPO) can also convert H_2O_2 in the presence of chloride to hypochlorous acid (HOCl), which can react with Fe^{2+} and superoxide to form $OH^{\bullet}$. HOCl can also get converted to singlet oxygen.

20.3.1 Increased ROS-Mediated Oxidative Damage in Autism

It has been suggested that increased vulnerability to oxidative stress by endogenous or environmental pro-oxidants in conjunction with genetic susceptibility factors may contribute to the development and clinical manifestations of autism (Chauhan and Chauhan 2006; Kern and Jones 2006; Deth et al. 2008; Chauhan et al. 2009a). In support of this hypothesis, there are numerous reports indicating increase in markers of lipid peroxidation, protein oxidation, and DNA oxidation, and reduced antioxidant status in blood and urine samples and in the post-mortem brain tissue samples from subjects with autism compared with age-matched control subjects.

We reported increased levels of MDA, a marker of lipid peroxidation, in the plasma of children with autism compared to their typically developing siblings

(Chauhan et al. 2004), and also increased lipid peroxidation (Muthaiyah et al. 2009; Chauhan et al. 2011b), DNA oxidation (Chauhan et al. 2011a), and protein oxidation (Chauhan et al. 2010) in the cerebellum and frontal and temporal regions of the brain in the subjects with autism as compared to age-matched control subjects. Oxidative stress in autism is brain-region specific because increased lipid peroxidation, DNA and protein oxidation were not observed in parietal and occipital cortices of autistic subjects compared with age-matched control subjects (Muthaiyah et al. 2009; Chauhan et al. 2011b; Chauhan et al. 2011a; Chauhan et al. 2010). Other studies have also indicated increased levels of lipid peroxidation and protein oxidation markers in autism, i.e., increased thiobarbituric acid (TBA)–reactive substances in the erythrocytes of autism subjects (Zoroglu et al. 2004); increased excretion of F2-isoprostane, and 8-OH deoxyguanosine in the urine of children with autism (Ming et al. 2005); increased levels of lipid-derived oxidative protein modification, i.e., carboxyethyl pyrrole and iso 4-levuglandin E2-protein adducts, in the brain, primarily in the white matter of autistic subjects (Evans et al. 2008); increased levels of 3-nitrotyrosine (a specific marker for oxidative damage of protein) and 8-OH deoxyguanosine in the cerebellum and temporal cortex of autistic subjects (Sajdel-Sulkowska et al. 2009; Rose et al. 2012a). The density of lipofuscin, a matrix of oxidized lipid and cross-linked protein, was also observed to be greater in the cortical brain areas involved in social behavior and communication in autism (Lopez-Hurtado and Prieto 2008). Recently, Pecorelli et al. (2013) reported increased levels of HNE in the erythrocytes and plasma of children with autism as compared to controls.

20.3.2 Glutathione Redox Imbalance in Autism

Glutathione is the most important endogenous antioxidant for detoxification and elimination of environmental toxins and free radicals. Several studies have reported lower levels of reduced glutathione (GSH), higher levels of oxidized glutathione (GSSG), and a lower redox ratio of GSH/GSSG in the plasma of individuals with autism (James et al. 2004, 2006; Al-Gadani et al. 2009; Geier et al. 2009; Bertoglio et al. 2010; Adams et al. 2011a, b). Al-Yafee et al. (2011) also reported reduced levels of GSH, GSH/GSSG, and increased levels of thioredoxins and thioredoxine reductase in the plasma of children with autism as compared to controls. In a systemic review and metanalysis of oxidative stress-related markers in autism, Frustaci et al. (2012) reported that ASD subjects had reduced levels of GSH, GPx, methionine and cysteine by 27, 18, 13 and 14% respectively, and increased concentration of GSSG by 45% in the blood. Pecorelli et al. (2013) observed decreased levels of GSH in the erythrocytes in the subjects with autism as compared to control subjects. Other studies have also demonstrated decreased ratio of GSH/GSSG in the lymphoblastoid and primary immune cells from autistic subjects (James et al. 2009; Rose et al. 2012b).

We have reported brain-region specific glutathione redox imbalance in autism (Chauhan et al. 2012a). Reduced levels of GSH, increased levels of GSSG, and a decrease in the ratio of GSH/GSSG were observed in the cerebellum and temporal cortex of autistic subjects compared with age-matched control subjects (Chauhan et al. 2012a). GSH levels were significantly decreased by 34.2 and 44.6 %, with a concomitant increase in the levels of GSSG by 38.2 and 45.5 %, respectively in these brain tissues in autism, as compared to the control group. There was also a significant decrease in the levels of total GSH (tGSH) by 32.9 % in the cerebellum, and by 43.1 % in the temporal cortex of subjects with autism. The redox ratio of GSH to GSSG was significantly decreased by 52.8 % in the cerebellum and by 60.8 % in the temporal cortex of subjects with autism, suggesting glutathione redox imbalance in the brain of individuals with autism (Chauhan et al. 2012a). Such alterations in glutathione levels were not observed in the parietal and occipital cortices of subjects with autism as compared to age-matched control subjects. Our findings on decreased GSH levels in the cerebellum of subjects with autism as compared to age-matched controls have been confirmed by Rose et al. (2012a).

Recently, we also reported impaired activities of glutathione-related enzymes involved in the antioxidant defense, detoxification, GSH regeneration and synthesis of glutathione in the cerebellum of subjects with autism as compared to age-matched controls (Gu et al. 2013a). There was significant reduction in the activities of glutamate cysteine ligase (GCL)—a rate limiting enzyme for GSH synthesis, glutathione S-transferase (GST)—an antioxidant detoxification enzyme, as well as GPx activity in the cerebellum of the subjects with autism as compared to age-matched controls (Gu et al. 2013a).

20.3.3 Relationship Between Homocysteine, Methionine and Oxidative Stress in Autism

Methionine is a main amino acid in the metabolism of glutathione. Several reports have suggested aberrant metabolism of the methionine cycle in autism. Ali et al. (2011) reported hyperhomocysteinemia, an indicator of impaired folate-dependent methionine metabolism, and increased oxidative stress in a case-control study of Omani children with autism. Hyperhomocysteinemia can cause oxidative stress via a number of mechanisms such as auto-oxidation of homocysteine to form ROS (Heinecke et al. 1987), increased lipid peroxidation (Jones et al. 1994), and reduced production of GPx (Upchurch et al. 1997). Pasca et al. (2006) reported higher levels of total homocysteine in the plasma of autistic children compared to control subjects. In the autistic group, a strong negative correlation was observed between homocysteine levels and GPx activity, suggesting an association between high levels of homocysteine and oxidative stress in autism. A clinical study has reported lower concentrations of methionine, cystathionine and cysteine as well as a decreased ratio of S-adenosylmethionine (SAM)/*S*-adenosinehomocysteine (SAH), an indicator of decreased methylation capacity in the plasma of children with autism (James

et al. 2004). According to the "redox/methylation hypothesis of autism" proposed by Deth et al. (2008), oxidative stress initiated by environmental factors in genetically vulnerable individuals can lead to impaired methylation and neurological deficits. Interestingly, the parents of autistic children shared similar metabolic deficits in methylation capacity and GSH-dependent antioxidant/ detoxification capacity, as observed in autistic children (James et al. 2008).

20.3.4 Decreased Activities of Antioxidant Enzymes in Autism

Not only the generation of ROS is increased in autism, the activities of antioxidant enzymes are also decreased, thus resulting in oxidative stress in autism. Decreased activity of GPx in erythrocytes and plasma (Yorbik et al. 2002; Pasca et al. 2006), and decreased activities of catalase (Zoroglu et al. 2004) and SOD (Yorbik et al. 2002) in erythrocytes have been reported in autism. We also reported increased oxidative damage and free radical generation, coupled with reduced activities of antioxidant enzymes, in lymphoblastoid cells from autistic subjects compared with age-matched control subjects (Essa et al. 2009). In the cerebellum, we recently reported significantly reduced activity of GPx in autism as compared to age-matched control group (Gu et al. 2013a). In a study of Egyptian children, oxidative stress was found in 88.6 % of autistic children, as revealed by elevated plasma F2-isoprostane and/or reduced GPx levels (Mostafa et al. 2010). In Saudi children with autism, decreased GSH levels and SOD activity were observed in erythrocytes (Al-Gadani et al. 2009). Meguid et al. (2011) reported lower levels of SOD and GPx, and increased lipid peroxidation in the blood samples from autistic children as compared with control subjects. Parellada et al. (2012) reported reduced levels of antioxidant status including non-enzymatic antioxidants (GSH and homocysteine) and antioxidant enzymes (catalase, SOD, GPX) in the blood of subjects with Asperger syndrome. Recently, meta-analysis of antioxidant enzymes in autism were completed by two groups (Frustaci et al. 2012; Main et al. 2012), which showed that GPx activity in erythrocytes was significantly lower in subjects with ASD than that in controls, while the activity of SOD in blood failed to demonstrate any significant association with autism.

20.3.5 Metals and Autism

Trace metals (such as copper and iron) and heavy metals (such as lead, cadmium and mercury) are known to induce oxidative stress. Adams et al. (2013) reported higher levels of lead in erythrocytes, and higher urinary levels of lead, thallium, tin and tungsten in the children with autism, which strongly correlated with severity of autism. In the hair samples of subjects with autism, Al-Farsi et al. (2013) reported higher levels of heavy metals ranging from 150 to 365 % of controls.

Copper and iron play important roles in the redox metabolism, and act as pro-oxidants. Their levels are regulated by two transporting proteins: ceruloplasmin (a copper-transporting protein) and transferrin (an iron-transporting protein). Both of these proteins are antioxidant proteins that are synthesized in several tissues, including brain (Arnaud et al. 1988; Loeffler et al. 1995). Ceruloplasmin inhibits the peroxidation of membrane lipids catalyzed by metal ions, such as iron and copper (Gutteridge 1983). We reported reduced levels of ceruloplasmin and transferrin in the blood of children with autism as compared to their developmentally normal siblings (Chauhan et al. 2004). Most importantly, the levels of ceruloplasmin and transferrin were significantly reduced in children with regressive autism who had lost previously acquired language skills (Chauhan et al. 2004).

Faber et al (2009) reported that plasma Zn/Cu ratio of 0.608 in children with autism was below the 0.7 % cut-off of the lowest 2.5 % of healthy children. Zinc deficiency, copper toxicity and low Zn/Cu in children with ASDs may indicate altered functioning of metallothionein system. Other preliminary studies have also suggested altered serum Cu/Zn ratios in autism (McGinnis 2004). In other study, Russo and Devito (2011) reported higher levels of Cu and lower levels of Zn in the plasma of subjects with autism as compared with controls, and that zinc therapy could decrease the plasma levels of copper indicating an association between plasma copper levels and autism. Similarly, Lakshmi and Geetha (2011) reported increased levels of copper in the hair and nails of children with autism. In a recent study, Percorelli et al. (2013) observed increased levels of non-protein bound iron in the erythrocytes and plasma of children with autism as compared with controls.

20.3.6 Xanthine Oxidase (XO) and NO (pro-oxidants) in Autism

XO and NO have been implicated in the etiology of autism. XO is an endogenous pro-oxidant enzyme that is involved in the production of superoxide radicals during conversion of xanthine to uric acid (Kellogg and Fridovich 1975). Increased XO activity has been reported in the erythrocytes of autistic subjects (Zoroglu et al. 2004).

NO is synthesized from L-arginine by nitric oxide synthase (NOS). NO is another toxic free radical that can react with superoxide anion and generate cytotoxic peroxynitrite anions ($ONOO^{\cdot}$) (Fig. 20.1). NO is known to affect the development and function of the central nervous system. NO has been implicated in neurotransmitter release (Lonart et al. 1992), neurite growth (Hindley et al. 1997), synaptogenesis (Truman et al. 1996), memory and learning (Holscher and Rose 1992), and macrophage-mediated cytotoxicity (Hibbs et al. 1988). Sogut et al. (2003) reported increased NO levels in the RBCs of autistic subjects, and suggested that NOS may be activated in autism. Elevated levels of NO have also been reported in the plasma of autistic subjects (Zoroglu et al. 2003; Sweeten et al. 2004; Tostes et al. 2012).

20.4 Genetic Variations of Enzymes Involved in Oxidative Stress in Autism

Recent evidence suggests association of gene mutations/deletions, copy number variations, and other genetic abnormalities with autism (Sutcliffe 2008). Some of the genetic variations can also increase vulnerability to oxidative stress in autism, and are discussed in the following sections:

20.4.1 Nitric Oxide Synthase

Fatemi et al (2000) reported that prenatal viral infection affects brain development via pathological involvement of neuronal NOS (nNOS) expression. Genotyping of single-nucleotide polymorphisms (SNPs) in the NOS-I gene and NOS-IIA gene, and haplotype analysis in 151 Korean ASD trios showed significant evidence for an association between NOS-IIA and ASD (Kim et al. 2009).

20.4.2 Glyoxalase 1

Glyoxalase 1 (Glo 1) has a critical role in the detoxification of dicarboxylic compounds, thereby reducing the formation of advanced glycation end products.Glo 1 uses GSH as a cofactor to detoxify cytotoxic 2-oxoaldehydes, such as methylglyoxal, which are produced by lipid peroxidation, glycation, and degradation of glycolytic intermediates (Thornalley 2003). While two studies reported a SNP in Glo 1 in autism (Junaid et al. 2004, Sacco et al. 2007), other studies did not find an association between Glo 1 gene and autism (Rehnstrom et al. 2008; Wu et al. 2008). However, in a recent review, the role of Glo 1 and methylglyoxal in behavior has been described (Distler and Palmer 2012).

20.4.3 Monoamine Oxidase A (MAOA)

This enzyme catalyzes the oxidation of endogenous amine-containing neurotransmitters such as serotonin and norepinephrine (Fitzpatrick 2010). The role of MAOA in autism is of particular interest because this enzyme affects the levels of serotonin, which are known to be abnormal in some individuals with autism (Hranilovic et al. 2007). An association of the 3-repeat MAOA and a 30-base pair (bp) repeat polymorphism (uVNTR) allele (low activity) with increased severity of autism has been reported (Cohen et al. 2003; Roohi et al. 2009; Cohen et al. 2011). Yoo et al. (2009) also reported preferential transmission of the 3-repeat allele of a MAOA-uVNTR marker in ASDs in a Korean population. Davis et al. (2008) reported an association between MAOA-uVNTR polymorphism and brain growth in autism. Their magnetic

resonance imaging (MRI) studies showed an increase in the volume of white matter in the brains of children with autism who had the low-activity, 3-repeat allele compared to those with a high-activity, 4-repeat allele. In a case-control study, Tassone et al. (2011) reported that male children carrying 4 tandem repeats in the promoter region of MAOA gene had a two-fold higher risk of autism. In a recent study, MAO A and MAO A/B knockout mice showed behavioral abnormalities such as social and communication impairments, perseverative and stereotypical responses, and behavioral inflexibility similar to those observed in ASDs (Bortolato et al. 2013).

20.4.4 Cyclooxygenase (COX)

This enzyme is responsible for the formation of important biological mediators called prostanoids, including prostaglandins, protacyclin, and thromboxane. During inflammation, COX-2 is rapidly induced by growth factors, cytokines, and inflammatory molecules. Recently, Asadabadi et al. (2013) reported that combination of risperidone (antipsychotic drug) and celecoxib (COX-2 inhibitor) was better than only risperidone in treating irritability, social withdrawal and stereotyped behavior of children with autism. Yoo et al. (2008) examined the relationship between ASDs and polymorphism of PTGS2 (the gene encoding COX-2) in 151 Korean family trios including children with autism. They reported a significant association of one intronic SNP (OS2745557) and the GAAA haplotypes with ASDs.

20.4.5 Genes Involved in Glutathione Homeostasis

Polymorphism of genes related to glutathione metabolism has been reported in autism (James et al. 2006; Williams et al. 2007; Ming et al. 2010; Bowers et al. 2011). Wiliams et al. (2007) reported increased risk of autistic disorder in the children of mothers having glutathione S-transferase P1 haplotype. An association between polyalanine repeat polymorphism in GPx gene (GPx-1) and autism has been observed (Ming et al. 2010). Bowers et al. (2011) examined genetic variations in 42 genes related to GSH metabolism in autism samples obtained from Autism Genetic Resources Exchange (AGRE), and reported 308 SNPs in 318 families. Their analysis showed significant association between cystathione gamma lyase, glutaredoxin, glutaredoxin 3 and autism (Bowers et al. 2011).

20.4.6 Genes Involved in Folate Metabolism

Folate plays an important role in the metabolism of neurotransmitters, and the synthesis of methionine and thymine. The role of the folate gene polymorphism has also been suggested in autism. Adams et al. (2007) reported that the 19-bp deletion

polymorphism of dihydrofolate reductase is a significant risk factor in autism. James et al. (2006) reported differences in allele frequency and/or significant gene-gene interactions for genes encoding the reduced folate carrier and methylene tetrahydrofolate reductase (MTHFR). In other studies, MTHFR C677T was suggested as a risk factor for autism (Mohammad et al. 2009; Guo et al. 2012). Recently, Schmidt et al. (2012) reported association of maternal periconceptional folic acid intake with reduced ASD risk, which was strongest for mothers and children with MTHFR C677T variant genotype. Folate deficiency can increase oxidative stress by increasing the levels of homocysteine. Ali et al. (2011) observed decreased levels of folate and increased levels of homocysteine in the serum of Omani children with autism. Kaluzna-Czaplinska et al. (2011) reported increased urinary excretion of homocysteine in children with autism, thus suggesting a deficiency of folate in blood.

20.5 Role of Mitochondria in Free Radical Generation, and Mitochondrial Dysfunction (MD) in Autism

Free radicals are generated endogenously during oxidative metabolism and energy production by mitochondria in the cell (Cadenas and Davies 2000; Lenaz 2001). In a recent meta-analysis of data in the literature, Rossignol and Frye (2012b) reported the strongest evidence for immune dysregulation/inflammation and oxidative stress, followed by toxicant exposures and mitochondrial dysfunction in autism. Several studies with blood, muscle biopsy, and postmortem brain tissue samples have suggested mitochondrial dysfunction in a subset of individuals with autism (Gargus 2008; Haas 2010; Palmieri and Persico 2010; Chauhan et al. 2011b, 2012b; Gu et al. 2013b; Rossignol and Frye 2012a, b). The review of previous reports and meta-analysis conducted by Rossignol and Frye (2012a) suggested deficiencies of mitochondrial ETC complexes I, III, V, IV, and II in 53, 30, 23, 20 and 9% of children with ASD and concomitant MD, respectively. Multiple complex deficiencies were reported in 36% of the children with ASD/MD. In the lymphoblastoid cells from autistic subjects, we reported reduced mitochondrial membrane potential (MMP) and increased free radical generation (Chauhan et al. 2009b). Recently, we also reported brain region–specific changes in the levels of mitochondrial ETC complexes in the subjects with autism (Chauhan et al. 2011b). In autistic children (4–10 years of age), we observed significantly lower levels of complexes III and V in the cerebellum, of complex I in the frontal cortex, and of complexes II, III, and V in the temporal cortex as compared to age-matched control subjects. In the cerebellum and temporal cortex, no overlap was observed in the levels of these complexes between autism and control subjects. In the frontal cortex, decreased levels of ETC complexes were observed in 60% of autism cases for complexes I, II and V, and in 40% autism cases for complexes II and IV (Chauhan et al. 2011b).

We also recently reported alterations in the activities of mitochondrial ETC complexes and mitochondrial DNA copy number in the frontal cortex of subjects with

autism (Gu et al. 2013b). The activities of complexes I, V and pyruvate dehydrogenase (PDH) in the mitochondria were most affected in autism being significantly reduced by 31, 36 and 35%, respectively. When 99% confidence interval (CI) of control group was taken as a reference range, impaired activities of complexes I, III and V were observed in 43, 29 and 43% of autistic subjects, respectively. All of five ETC complexes showed reduced activities in 14% of autistic cases, and the activities of multiple complexes were decreased in 29% of autistic subjects. These results suggest that defects in complexes I and III (sites of mitochondrial free radical generation) and complex V (ATP synthase) are more prevalent in autism. PDH activity was also reduced in 57% of autistic subjects. The ratios of mtDNA of three mitochondrial genes ND1, ND4 and CytB (that encode for subunits of complexes I and III) to nuclear DNA were significantly increased in autism, suggesting a higher mtDNA copy number in autism. Furthermore, ND4 and Cyt B deletions were observed in 44 and 33% of autistic subjects, respectively. Our studies suggest that autism is associated with mitochondrial dysfunction in the brain (Chauhan et al. 2011b; Gu et al. 2013b). Recently, Anitha et al. (2013) reported reduced expression of several ETC genes in the brain of subjects with autism compared to controls. Brain region-specific expression of eleven genes of complex I, five genes each from complex III and complex IV, and seven genes of complex V were reduced in autism. Tang et al. (2013) also reported that mitochondrial function and intracellular redox status are compromised in pyramidal neurons in brains of ASD subjects, and that mitochondrial dysfunction occurs during early childhood when ASD symptoms appear.

20.6 Summary

There has been a rapid increase in the number of autism spectrum cases, which could be attributed, in part, to the combined effect of environmental factors with an inter-play with genetic factors. Multiple lines of evidence suggest that oxidative stress may be a common link between interactions of environmental factors and genetic factors. It is not clear whether oxidative stress is the cause or an effect of autism but several reports indicate oxidative stress in individuals with ASD as evident from increased levels of markers of oxidative stress and decreased antioxidant capacity in blood, urine and postmortem brain samples of subjects with autism. In addition, mitochondrial abnormalities may also contribute to increased oxidative stress in autism. Potential mechanism on the involvement of oxidative stress in autism is shown in Fig. 20.2.

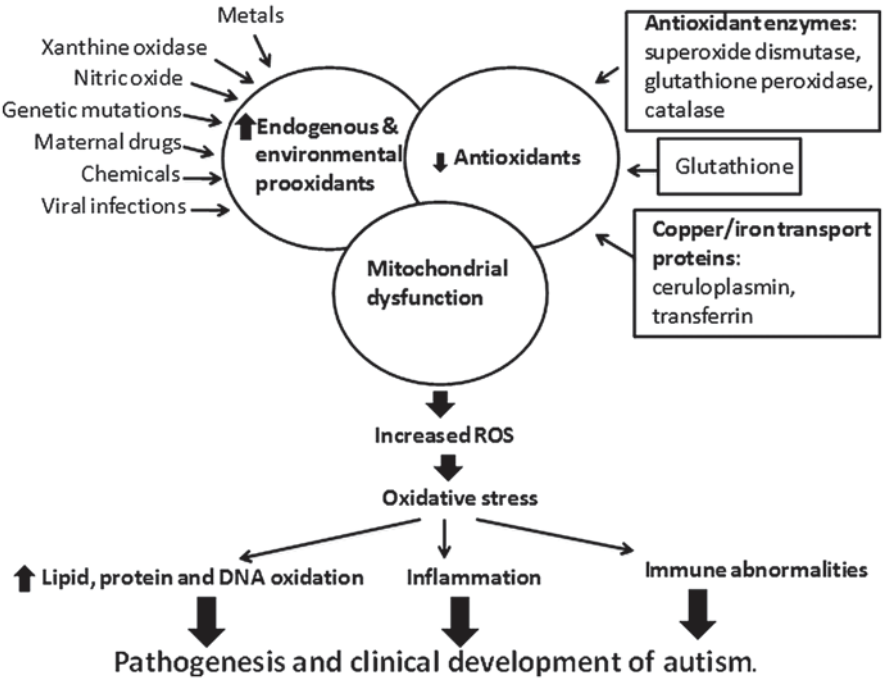


Fig. 20.2 Potential model depicting the role of oxidative stress and mitochondrial dysfunction in autism. It is not clear whether oxidative stress is the cause or an effect of autism. Nevertheless, increase in pro-oxidants, decrease in antioxidant defense, and mitochondria dysfunction will increase the production of ROS, which in turn will react with proteins, DNA and lipids resulting in oxidative damage, which are common abnormalities observed in individuals with autism. Alternatively, autism may lead to damaged mitochondria which may trigger ROS generation and oxidative stress, leading to clinical features seen in autism.

References

Adams M, Lucock M, Stuart J, Fardell S, Baker K, Ng X (2007) Preliminary evidence for involvement of the folate gene polymorphism 19 bp deletion-DHFR in occurrence of autism. Neurosci Lett 422:24–29

Adams JB, Audhya T, McDonough-Means S, Rubin RA, Quig D, Geis E, Gehn E, Loresto M, Mitchell J, Atwood S, Barnhouse S, Lee W (2011a) Nutritional and metabolic status of children with autism vs. neurotypical children, and the association with autism severity. Nutr Metab (Lond) 8:34–66

Adams JB, Audhya T, McDonough-Means S, Rubin RA, Quig D, Geis E, Gehn E, Loresto M, Mitchell J, Atwood S, Barnhouse S, Lee W (2011b) Effect of a vitamin/mineral supplement on children and adults with autism. BMC Pediatrics 11:111–131

Adams JB, Audhya T, McDonough-Means S, Rubin RA, Quig D, Geis E, Gehn E, Loresto M, Mitchell J, Atwood S, Barnhouse S, Lee W (2013) Toxicological status of children with autism vs. neurotypical children and the association with autism severity. Biol Trace Elem Res 151: 171–180

Al-Farsi YM, Waly MI, Al-Sharbati MM, Al-Shafaee MA, Al-Farsi OA, Al-Khaduri MM, Gupta I, Ouhtit A, Al-Adawi S, Al-Said MF, Deth RC (2013) Levels of heavy metals and essential

minerals in hair samples of children with autism in Oman: a case-control study. Biol Trace Elem Res 151:181–186
Al-Gadani Y, El-Ansary A, Attas O, Al-Ayadhi L (2009) Metabolic biomarkers related to oxidative stress and antioxidant status in Saudi autistic children. Clin Biochem 42:1032–1040
Al-Yafee YA, Al-Ayadhi LY, Haq SH, El-Ansary AK (2011) Novel metabolic biomarkers related to sulfur-dependent detoxification pathways in autistic patients of Saudi Arabia. BMC Neurol 11:139
Ali A, Waly MI, Al-Farsi YM, Essa MM, Al-Sharbati MM, Deth RC (2011) Hyperhomocysteinemia among Omani autistic children: a case-control study. Acta Biochimica Polonica 58:547–551
Anitha A, Nakamura K, Thanseem I, Matsuzaki H, Miyachi T, Tsujii M, Iwata Y, Suzuki K, Sugiyama T, Mori N (2013) Downregulation of the expression of mitochondrial electron transport complex genes in autism brains. Brain Pathol 23:294–302
Arnaud P, Gianazza E, Miribel L (1988) Ceruloplasmin. Methods Enzymol 163:441–452
Asadabadi M, Mohammadi MR, Ghanizadeh A, Modabbernia A, Ashrafi M, Hassanzadeh E, Forghani S, Akhondzadeh S (2013) Celecoxib as adjunctive treatment to risperidone in children with autistic disorder: a randomized, double-blind, placebo-controlled trial. Psychopharmacology 225:51–59
Barja G (1999) Mitochondrial oxygen radical generation and leak: sites of production in states 4 and 3, organ specificity, and relation to aging and longevity. J Bioenerg Biomembr 31:347–366
Bertoglio K, James SJ, Deprey L, Brule N, Hendren RL (2010) Pilot study of the effect of methyl B12 treatment on behavioral and biomarker measures in children with autism. J Altern Complement Med. 16:555–560
Bertram R, Gram Pedersen M, Luciani DS, Sherman A (2006) A simplified model for mitochondrial ATP production. J Theor Biol. 243:575–586
Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O (2012) Oxidative stress and antioxidant defense. World Allergy Organ J 5:9–19
Bortolato M, Godar SC, Alzghoul L, Zhang J, Darling RD, Simpson KL, Bini V, Chen K, Wellman CL, Lin RC, Shih JC (2013) Monoamine oxidase A and A/B knockout mice display autistic-like features. Int J Neuropsychopharmacol 16:869–888
Bowers K, Li Q, Bressler J, Avramopoulos D, Newschaffer C, Fallin MD (2011) Glutathione pathway gene variation and risk of autism spectrum disorders. J Neurodev Disord 3:132–143
Brookes PS, Levonen AL, Shiva S, Sarti P, Darley-Usmar VM (2002) Mitochondria: regulators of signal transduction by reactive oxygen and nitrogen species. Free Radic Biol Med 33:755–764
Cadenas E, Davies KJ (2000) Mitochondrial free radical generation, oxidative stress, and aging. Free Radic Biol Med 29:222–230
Cadet J, Bellon S, Berger M, Bourdat AG, Douki T, Duarte V, Frelon S, Gasparutto D, Muller E, Ravanat JL, Sauvaigo S (2002) Recent aspects of oxidative DNA damage: guanine lesions, measurement and substrate specificity of DNA repair glycosylases. Biol Chem 383:933–943
Chauhan A, Chauhan V (2006) Oxidative stress in autism. Pathophysiology 13:171–181
Chauhan A, Chauhan V, Brown WT, Cohen I (2004) Oxidative stress in autism: increased lipid peroxidation and reduced serum levels of ceruloplasmin and transferrin—the antioxidant proteins. Life Sci 75:2539–2549
Chauhan A, Chauhan V, Brown WT (eds) (2009a) Autism:oxidative stress, inflammation and immune abnormailities. CRC Press, Taylor and Francis Group, Florida, pp 1–427
Chauhan A, Essa MM, Muthaiyah B, Brown WT, Chauhan V (2009b) Mitochondrial abnormalities in lymphoblasts from autism. J Neurochem 109 (Suppl. 1):273
Chauhan A, Essa MM, Muthaiyah B, Brown WT, Wegiel J, Chauhan V (2010) Increased protein oxidation in cerebellum, frontal and temporal cortex in autism. International Meeting for Autism Research (Abstract)
Chauhan A, Audhya T, Chauhan V (2011a) Increased DNA oxidation in the cerebellum, frontal and temporal cortex of brain in autism. Trans Am Soc 42:81

Chauhan A, Gu F, Essa MM, Wegiel J, Kaur K, Brown WT, Chauhan V (2011b) Brain region-specific deficit in mitochondrial electron transport chain complexes in children with autism. J Neurochem 117:209–220

Chauhan A, Audhya T, Chauhan V (2012a) Brain region-specific glutathione redox imbalance in autism. Neurochem Res 37:1681–1689

Chauhan A, Gu F, Chauhan V (2012b) Mitochondrial respiratory chain defects in autism and other neurodevelopmental disorders. J Pediatr Biochem 2:213–223

Cohen IL, Liu X, Schutz C, White BN, Jenkins EC, Brown WT, Holden JJ (2003) Association of autism severity with a monoamine oxidase A functional polymorphism. Clin Genet 64:190–197

Cohen IL, Liu X, Lewis ME, Chudley A, Forster-Gibson C, Gonzalez M, Jenkins EC, Brown WT, Holden JJ (2011) Autism severity is associated with child and maternal MAOA genotypes. Clin Genet 79:355–362

Davis LK, Hazlett HC, Librant AL, Nopoulos P, Sheffield VC, Piven J, Wassink TH (2008) Cortical enlargement in autism is associated with a functional VNTR in the monoamine oxidase A gene. Am J Med Genet B Neuropsychiatr Genet 147B:1145–1151

Deth R, Muratore C, Benzecry J, Power-Charnitsky VA, Waly M (2008) How environmental and genetic factors combine to cause autism: a redox/methylation hypothesis. Neurotoxicology 29:190–201

Distler MG, Palmer AA (2012) Role of Glyoxalase 1 (Glo1) and methylglyoxal (MG) in behavior: recent advances and mechanistic insights. Front Genet 3: 250–260

Dupuy C, Virion A, Ohayon R, Kaniewski J, Deme D, Pommier J (1991) Mechanism of hydrogen peroxide formation catalyzed by NADPH oxidase in thyroid plasma membrane. J Biol Chem 266:3739–3743

Essa MM, Muthaiyah B, Chauhan V, Brown WT, Chauhan A (2009) Increased oxidative damage in lymphoblasts form autism: enhanced free radical generation coupled with reduced antioxidant status. J Neurochem 108 (Suppl 1):73

Evans TA, Siedlak S, Lu L, Fu X, Wang Z, McGinnis W R, Fakhoury E, Castellani RJ, Hazen SL, Walsh WJ, Lewis AT, Salomon RG, Smith MA, Perry G, Zhu X (2008) The autistic phenotype exhibits a remarkably localized modification of brain protein by products of free radical-induced lipid oxidation. Am J Biochem Biotech 4:61–72

Faber S, Zinn GM, Kern JC, 2nd, Kingston HM (2009) The plasma zinc/serum copper ratio as a biomarker in children with autism spectrum disorders. Biomarkers 14:171–180

Fatemi SH, Cuadra AE, El-Fakahany EE, Sidwell RW, Thuras P (2000) Prenatal viral infection causes alterations in nNOS expression in developing mouse brains. Neuroreport 11:1493–1496

Fitzpatrick PF (2010) Oxidation of amines by flavoproteins. Arch Biochem Biophys 493:13–25

Frustaci A, Neri M, Cesario A, Adams JB, Domenici E, Dalla Bernardina B, Bonassi S (2012) Oxidative stress-related biomarkers in autism: systematic review and meta-analyses. Free Radic Biol Med 52:2128–2141

Gargus JJ, Imtiaz F. (2008) Mitochondrial energy-deficient endophenotype in autism. Am J Biochem Biotech 4:198–207

Geier DA, Kern JK, Garver CR, Adams JB, Audhya T, Geier MR (2009) A prospective study of transsulfuration biomarkers in autistic disorders. Neurochem Res 34:386–393

Granger DN (1988) Role of xanthine oxidase and granulocytes in ischemia-reperfusion injury. Am J Physiol 255:H1269–1275

Gu F, Chauhan V, Chauhan A (2013a) Impaired synthesis and antioxidant defense of glutathione in the cerebellum of autistic subjects: alterations in the activities and protein expression of glutathione-related enzymes. Free Radic Biol Med 65:488–496

Gu F, Chauhan V, Kaur K, Brown WT, Lafauci G, Wegiel J, Chauhan A (2013b) Alterations in mitochondrial DNA copy number and the activities of electron transport chain complexes and pyruvate dehydrogenase in the frontal cortex from subjects with autism. Transl Psychiatry 3:e299

Guo T, Chen H, Liu B, Ji W, Yang C (2012) Methylenetetrahydrofolate reductase polymorphisms C677T and risk of autism in the Chinese Han population. Genet Test Mol Biomarkers 16:968–973

Gutteridge JM (1983) Antioxidant properties of caeruloplasmin towards iron- and copper-dependent oxygen radical formation. FEBS Lett 157:37–40

Haas RH (2010) Autism and mitochondrial disease. Dev Disabil Res Rev 16:144–153
Heinecke JW, Rosen H, Suzuki LA, Chait A (1987) The role of sulfur-containing amino acids in superoxide production and modification of low density lipoprotein by arterial smooth muscle cells. J Biol Chem 262:10098–10103
Hibbs JB, Jr., Taintor RR, Vavrin Z, Rachlin EM (1988) Nitric oxide: a cytotoxic activated macrophage effector molecule. Biochem Biophys Res Commun 157:87–94
Hindley S, Juurlink BH, Gysbers JW, Middlemiss PJ, Herman MA, Rathbone MP (1997) Nitric oxide donors enhance neurotrophin-induced neurite outgrowth through a cGMP-dependent mechanism. J Neurosci Res 47:427–439
Holscher C, Rose SP (1992) An inhibitor of nitric oxide synthesis prevents memory formation in the chick. Neurosci Lett 145:165–167
Hranilovic D, Bujas-Petkovic Z, Vragovic R, Vuk T, Hock K, Jernej B (2007) Hyperserotonemia in adults with autistic disorder. J Autism Dev Disord 37:1934–1940
James SJ, Cutler P, Melnyk S, Jernigan S, Janak L, Gaylor DW, Neubrander JA (2004) Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. Am J Clin Nutr 80:1611–1617
James SJ, Melnyk S, Jernigan S, Cleves MA, Halsted CH, Wong DH, Cutler P, Bock K, Boris M, Bradstreet JJ, Baker SM, Gaylor DW (2006) Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. Am J Med Genet B Neuropsychiatr Genet. 141B:947–956
James SJ, Melnyk S, Jernigan S, Hubanks A, Rose S, Gaylor DW (2008) Abnormal transmethylation/transsulfuration metabolism and DNA hypomethylation among parents of children with autism. J Autism Dev Disord 38:1966–1975
James SJ, Rose S, Melnyk S, Jernigan S, Blossom S, Pavliv O, Gaylor DW (2009) Cellular and mitochondrial glutathione redox imbalance in lymphoblastoid cells derived from children with autism. FASEB J 23:2374–2383.
Jones BG, Rose FA, Tudball N (1994) Lipid peroxidation and homocysteine induced toxicity. Atherosclerosis 105:165–170
Junaid MA, Kowal D, Barua M, Pullarkat PS, Sklower Brooks S, Pullarkat RK (2004) Proteomic studies identified a single nucleotide polymorphism in glyoxalase I as autism susceptibility factor. Am J Med Genet A. 131:11–17
Juurlink BH, Paterson PG (1998) Review of oxidative stress in brain and spinal cord injury: suggestions for pharmacological and nutritional management strategies. J Spinal Cord Med 21:309–334
Kaluzna-Czaplinska J, Michalska M, Rynkowski J (2011) Homocysteine level in urine of autistic and healthy children. Acta Biochim Pol 58:31–34
Kellogg EW, 3rd, Fridovich I (1975) Superoxide, hydrogen peroxide, and singlet oxygen in lipid peroxidation by a xanthine oxidase system. J Biol Chem 250:8812–8817
Kern JK, Jones AM (2006) Evidence of toxicity, oxidative stress, and neuronal insult in autism. J Toxicol Environ Health B Crit Rev 9:485–499
Kim HW, Cho SC, Kim JW, Cho IH, Kim SA, Park M, Cho EJ, Yoo HJ (2009) Family-based association study between NOS-I and -IIA polymorphisms and autism spectrum disorders in Korean trios. Am J Med Genet B Neuropsychiatr Genet 150B:300–306
Lakshmi Priya MD Geetha A (2011) Level of trace elements (copper, zinc, magnesium and selenium) and toxic elements (lead and mercury) in the hair and nail of children with autism. Biol Trace Elem Res 142:148–158
Lenaz G (2001) The mitochondrial production of reactive oxygen species: mechanisms and implications in human pathology. IUBMB Life 52:159–164
Loeffler DA, Connor JR, Juneau PL, Snyder BS, Kanaley L, DeMaggio AJ, Nguyen H, Brickman CM, LeWitt PA (1995) Transferrin and iron in normal, Alzheimer's disease, and Parkinson's disease brain regions. J Neurochem 65:710–724
Lonart G, Wang J, Johnson KM (1992) Nitric oxide induces neurotransmitter release from hippocampal slices. Eur J Pharmacol 220:271–272

López-Hurtado E. Prieto JJ (2008) A microscopic study of language-related cortex in autism. Am J Biochem Biotech 4: 130–145
Lord C, Cook EH, Leventhal BL, Amaral DG (2000) Autism spectrum disorders. Neuron 28:355–363
Main PA, Angley MT, O'Doherty CE, Thomas P, Fenech M (2012) The potential role of the antioxidant and detoxification properties of glutathione in autism spectrum disorders: a systematic review and meta-analysis. Nutr Metab 9:35
McGinnis WR (2004) Oxidative stress in autism. Altern Ther Health Med 10:22–36
Meguid NA, Dardir AA, Abdel-Raouf ER, Hashish A (2011) Evaluation of oxidative stress in autism: defective antioxidant enzymes and increased lipid peroxidation. Biol Trace Elem Res 143:58–65
Ming X, Stein TP, Brimacombe M, Johnson WG, Lambert GH, Wagner GC (2005) Increased excretion of a lipid peroxidation biomarker in autism. Prostaglandins Leukot Essent Fatty Acids 73:379–384
Ming X, Johnson WG, Stenroos ES, Mars A, Lambert GH, Buyske S (2010) Genetic variant of glutathione peroxidase 1 in autism. Brain Dev 32:105–109
Mohammad NS, Jain JM, Chintakindi KP, Singh RP, Naik U, Akella RR (2009) Aberrations in folate metabolic pathway and altered susceptibility to autism. Psychiatr Genet 19:171–176
Mostafa GA, El-Hadidi ES, Hewedi DH, Abdou MM (2010) Oxidative stress in Egyptian children with autism: relation to autoimmunity. J Neuroimmunol 219:114–118
Muller FL, Liu Y, Van Remmen H (2004) Complex III releases superoxide to both sides of the inner mitochondrial membrane. J Biol Chem 279:49064–49073
Muthaiyah B, Essa MM, Chauhan V, Brown WT, Wegiel J, Chauhan A (2009) Increased lipid peroxidation in cerebellum and temporal cortex of brain in autism. J Neurochem 108 (Suppl 1): 73
Ozonoff S, Williams BJ, Landa R (2005) Parental report of the early development of children with regressive autism: the delays-plus-regression phenotype. Autism 9:461–486
Palmieri L, Persico AM (2010) Mitochondrial dysfunction in autism spectrum disorders: cause or effect? Biochim Biophys Acta 1797:1130–1137
Parellada M, Moreno C, Mac-Dowell K, Leza JC, Giraldez M, Bailon C, Castro C, Miranda-Azpiazu P, Fraguas D, Arango C (2012) Plasma antioxidant capacity is reduced in Asperger syndrome. J Psychiatr Res 46:394–401
Pasca SP, Nemes B, Vlase L, Gagyi CE, Dronca E, Miu AC, Dronca M (2006) High levels of homocysteine and low serum paraoxonase 1 arylesterase activity in children with autism. Life Sci 78:2244–2248
Pecorelli A, Leoncini S, De Felice C, Signorini C, Cerrone C, Valacchi G, Ciccoli L, Hayek J (2013) Non-protein-bound iron and 4-hydroxynonenal protein adducts in classic autism. Brain Dev 35:146–154
Polster BM, Fiskum G (2004) Mitochondrial mechanisms of neural cell apoptosis. J Neurochem 90:1281–1289
Rehnstrom K, Ylisaukko-Oja T, Vanhala R, von Wendt L, Peltonen L, Hovatta I (2008) No association between common variants in glyoxalase 1 and autism spectrum disorders. Am J Med Genet B Neuropsychiatr Genet 147B:124–127
Roohi J, DeVincent CJ, Hatchwell E, Gadow KD (2009) Association of a monoamine oxidase-A gene promoter polymorphism with ADHD and anxiety in boys with autism spectrum disorder. J Autism Dev Disord 39:67–74
Rose S, Melnyk S, Pavliv O, Bai S, Nick TG, Frye RE, James SJ (2012a) Evidence of oxidative damage and inflammation associated with low glutathione redox status in the autism brain. Transl Psychiatry 2:e134
Rose S, Melnyk S, Trusty TA, Pavliv O, Seidel L, Li J, Nick T, James SJ (2012b) Intracellular and extracellular redox status and free radical generation in primary immune cells from children with autism. Autism Res Treat 2012:986519
Rossignol DA, Frye RE (2012a) Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis. Mol Psychiatry 17:290–314

Rossignol DA, Frye RE (2012b) A review of research trends in physiological abnormalities in autism spectrum disorders: immune dysregulation, inflammation, oxidative stress, mitochondrial dysfunction and environmental toxicant exposures. Mol Psychiatry 17:389–401

Russo AJ, Devito R (2011) Analysis of copper and zinc plasma concentration and the efficacy of zinc therapy in individuals with Asperger's Syndrome, Pervasive developmental disorder not otherwise specified (PDD-NOS) and autism. Biomark Insights 6:127–133

Sacco R, Papaleo V, Hager J, Rousseau F, Moessner R, Militerni R, Bravaccio C, Trillo S, Schneider C, Melmed R, Elia M, Curatolo P, Manzi B, Pascucci T, Puglisi-Allegra S, Reichelt KL, Persico AM (2007) Case-control and family-based association studies of candidate genes in autistic disorder and its endophenotypes: TPH2 and GLO1. BMC Med Genet 8:11–20

Sajdel-Sulkowska EM, Xu M, Koibuchi N (2009) Increase in cerebellar neurotrophin-3 and oxidative stress markers in autism. Cerebellum 8:366–372

Schmidt RJ, Tancredi DJ, Ozonoff S, Hansen RL, Hartiala J, Allayee H, Schmidt LC, Tassone F, Hertz-Picciotto I (2012) Maternal periconceptional folic acid intake and risk of autism spectrum disorders and developmental delay in the CHARGE (CHildhood Autism Risks from Genetics and Environment) case-control study. Am J Clin Nutr 96:80–89

Sogut S, Zoroglu SS, Ozyurt H, Yilmaz HR, Ozugurlu F, Sivasli E, Yetkin O, Yanik M, Tutkun H, Savas HA, Tarakcioglu M, Akyol O (2003) Changes in nitric oxide levels and antioxidant enzyme activities may have a role in the pathophysiological mechanisms involved in autism. Clin Chim Acta 331:111–117

Sutcliffe JS (2008) Genetics. Insights into the pathogenesis of autism. Science 321:208–209

Sweeten TL, Posey DJ, Shankar S, McDougle CJ (2004) High nitric oxide production in autistic disorder: a possible role for interferon-gamma. Biol Psychiatry 55:434–437

Szewczyk A, Wojtczak L (2002) Mitochondria as a pharmacological target. Pharmacol Rev 54:101–127

Tang G, Gutierrez Rios P, Kuo SH, Akman O, Rosoklija G, Tanji K, Dwork A, Schon EA, Dimauro S, Goldman J, Sulzer D (2013) Mitochondrial abnormalities in temporal lobe of autistic brain. Neurobiol Dis 54:349–361

Tassone F, Qi L, Zhang W, Hansen RL, Pessah IN, Hertz-Picciotto I (2011) MAOA, DBH, and SLC6A4 variants in CHARGE: a case-control study of autism spectrum disorders. Autism Res 4:250–261

Thornalley PJ (2003) Glyoxalase I–structure, function and a critical role in the enzymatic defence against glycation. Biochem Soc Trans 31:1343–1348

Tostes MH, Teixeira HC, Gattaz WF, Brandao MA, Raposo NR (2012) Altered neurotrophin, neuropeptide, cytokines and nitric oxide levels in autism. Pharmacopsychiatry 45:241–243

Truman JW, De Vente J, Ball EE (1996) Nitric oxide-sensitive guanylate cyclase activity is associated with the maturational phase of neuronal development in insects. Development 122:3949–3958

Upchurch GR, Jr., Welch GN, Fabian AJ, Freedman JE, Johnson JL, Keaney JF, Jr., Loscalzo J (1997) Homocysteine decreases bioavailable nitric oxide by a mechanism involving glutathione peroxidase. J Biol Chem 272:17012–17017

Williams TA, Mars AE, Buyske SG, Stenroos ES, Wang R, Factura-Santiago MF, Lambert GH, Johnson WG (2007) Risk of autistic disorder in affected offspring of mothers with a glutathione S-transferase P1 haplotype. Arch Pediatr Adolesc Med 161:356–361

Wingate M, Kirby RS, Pettygrove S, Cunniff C, Schulz E, Ghosh T, Robinson C, Lee LC, Landa R, Constantino J, Fitzgerald R, Zahorodny W, Daniels J, Nicholas J, Charles J, McMahon W, Bilder D, Durkin M, Baio J, Christensen D, Braun KV, Clayton H, Goodman A, Doernberg N, Yeargin-Allsopp M, Lott E, Mancilla KC, Hudson A, Kast K, Jolly K, Chang A, Harrington R, Fitzgerald R, Shenouda J, Bell P, Kingsbury C, Bakian R, Henderson A, Arenson C, Washington A, Frenkel G, Wright V (2014) Prevalence of autism spectrum disorder among children aged 8 years autism and developmental disabilities monitoring network, 11 sites, United States, 20 I 0. MMWR Surveill Summ 63:1–21

Wu YY, Chien WH, Huang YS, Gau SS, Chen CH (2008) Lack of evidence to support the glyoxalase 1 gene (GLO1) as a risk gene of autism in Han Chinese patients from Taiwan. Prog Neuropsychopharmacol Biol Psychiatry 32:1740–1744

Yoo HJ, Cho IH, Park M, Cho E, Cho SC, Kim BN, Kim JW, Kim SA (2008) Association between PTGS2 polymorphism and autism spectrum disorders in Korean trios. Neurosci Res 62:66–69

Yoo HJ, Lee SK, Park M, Cho IH, Hyun SH, Lee JC, Yang SY, Kim SA (2009) Family- and population-based association studies of monoamine oxidase A and autism spectrum disorders in Korean. Neurosci Res 63:172–176

Yorbik O, Sayal A, Akay C, Akbiyik DI, Sohmen T (2002) Investigation of antioxidant enzymes in children with autistic disorder. Prostaglandins Leukot Essent Fatty Acids 67:341–343

Zoroglu SS, Yurekli M, Meram I, Sogut S, Tutkun H, Yetkin O, Sivasli E, Savas HA, Yanik M, Herken H, Akyol O (2003) Pathophysiological role of nitric oxide and adrenomedullin in autism. Cell Biochem Funct 21:55–60

Zoroglu SS, Armutcu F, Ozen S, Gurel A, Sivasli E, Yetkin O, Meram I (2004) Increased oxidative stress and altered activities of erythrocyte free radical scavenging enzymes in autism. Eur Arch Psychiatry Clin Neurosci 254:143–147

Index

S. Hossein Fatemi (ed.), *The Molecular Basis of Autism,*
Contemporary Clinical Neuroscience, DOI 10.1007/978-1-4939-2190-4