Autism: Comparative Genomics and Interactomics

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Introduction

Autism spectrum disorder (ASD) is a common neurodevelopmental disorder, diagnosable in early childhood, and causing lifelong morbidity in around 1 % of the population. ASD is diagnosed by significant deficits in communication and social skills and by the presence of narrow and/or repetitive behaviors and/or interests. The lifetime costs of ASD are high and include personal and family impacts on health and well-being, as well as significant financial costs. For example, the financial impact of ASD to society is estimated to be between $35 and $90 billion annually in the USA (IACC report 2011), and in the UK, the costs of supporting children or adults with ASDs are estimated to be £2.7 billion and £25 billion per year, respectively (Knapp et al. 2009).

One of the complex features of ASD, which is frequently referred to as "autism," is its clinical heterogeneity. Not only can the core symptoms of ASD each vary in severity, autism is frequently associated with features that are not part of the core diagnostic phenotype. For example, when criteria such as those described in the Diagnostic and Statistical Manual of Mental Disorders are used for diagnosis, individuals with ASD often fulfil the criteria for several diagnoses. For example, individuals with ASD may also fulfil the diagnostic criteria for mental retardation (MR), attention-deficit/hyperactivity disorder (ADHD), obsessive-compulsive disorder (OCD), as well as conduct, sleep, anxiety, eating, gender identity, depressive, schizophrenia, and tic disorders (Lichtenstein et al. 2010; Kohane et al. 2012). Medical disorders are also frequently reported in subsets of ASD patients, such as

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seizures/epilepsy, gastrointestinal disorders, biochemical abnormalities, gait and motor disturbances, physical anomalies, such as macro/microcephaly or dysmorphic facial features, and certain types of cancers (McDougle et al. 2005; Kao et al. 2010; Kohane et al. 2012). Immune system anomalies are also common in those with ASD (Singh 1996; Croonenberghs et al. 2002) and, like many of the autism comorbidities, may be either an interesting epiphenomenon, may be causative of some cases of autism, or may exacerbate the autism phenotype in a synergistic manner.

The behavioral diagnosis of ASD can also be applied to patients with specific genetic disorders, including Mendelian genetic disorders and those with specific chromosome abnormalities, including whole chromosome, microscopic, and sub-microscopic "copy number" changes (i.e., deletions or duplications). For example, ASD is frequently diagnosed in patients with fragile X syndrome, tuberous sclerosis (TS), neurofibromatosis, Smith–Magenis syndrome, Smith–Lemli–Opitz syndrome, Turner syndrome, 15q11–13 disorder, and 16p11.2 syndrome (Benvenuto et al. 2009; Caglayan 2010; El-Fishawy and State 2010; Ronald and Hoekstra 2011). Conversely, it has been estimated that 10–20 % of ASD cases have defined single-gene mutations or defined chromosomal abnormalities as the identifiable cause (Geschwind 2011), but identified causes may be as high as 40 % (Schaefer and Lutz 2006).

The association of autism with defined genetic disorders provided key evidence for redefining ASD from what was considered an environmentally induced disorder (the "refrigerator-mother" hypothesis), to its current definition as a typical complex genetic disorder. Indeed, multiple studies have demonstrated that ASD is highly heritable, with heritability estimates of 80–90 % being calculated (Ronald and Hoekstra 2011). However, not all ASD is inherited, and spontaneous genetic mutations (de novo mutations) are implicated in between 10 % and 20 % of cases of ASD (Hochstenbach et al. 2011). Factors such as increased parental age, multiple births, and fetal infection (particularly rubella) are known to increase the risk of de novo mutations and resulting genetic disorders and to increase the risk of ASD. The severity and presentation of ASD can also vary between monozygotic twins, and these differences suggest "post-twinning" de novo mutations and/or epigenetic modifications also impact on the final ASD phenotype (Zwijnenburg et al. 2010).

As described in detail elsewhere, ASD can be caused by both single-gene mutations (Table 1), or defined chromosomal mutations, but in the majority of cases is thought be complex in nature, with multiple alleles, with smaller effect size but higher population frequency, contributing to the development of an ASD phenotype (El-Fishawy and State 2010; Betancur 2011; Bishop et al. 2014). This finding is typical of complex genetic disorders, and genetic studies of MR, epilepsy, and ADHD are finding similar results. Furthermore, just as many behavioral studies have found phenotypic overlaps between ASD individuals and other DSM-defined disorders, genetic studies have likewise revealed that many of the genes implicated in ASD can increase the risk of a variety of neurological conditions (Lichtenstein et al. 2010; Talkowski et al. 2012). Therefore, it is expected that subsets of ASD genes will also affect biological processes contributing to the etiology of multiple, related, behaviorally-defined disorders.

Table 1 Syndromic autism genes

Official gene symbol[a]	Chromosomal location	Inheritance pattern[b]	Disorder	OMIM accession	Autism evidence level[c]
CACNA1C	12p13.3	AD	Timothy syndrome	601005	4
CNTNAP2	7q35	AR	Cortical dysplasia-focal epilepsy syndrome	610042	4
DHCR7	11q13.4	AR	Smith–Lemli–Opitz syndrome	270400	4
FMR1	Xq27.3	XL	Fragile X syndrome	300624	4
MECP2	Xq28	XL	Rett syndrome	312750	4
TSC1	9q34	AD	Tuberous sclerosis 1	191100	4
TSC2	16p13.3	AD	Tuberous sclerosis 2	191100	4
UBE3A	15q11.2	AD	Angelman syndrome	105830	4
ACSL4	Xq22.3-q23	XL	Non-syndromic X-linked mental retardation (MR)	300387	3
ADSL	22q13.1	AR	Adenylosuccinate lyase deficiency	103050	3
AFF2	Xq28	XL	Fragile X mental retardation 2 (FRAXE type)	309548	3
AGTR2	Xq22-q23	XL	Non-syndromic X-linked MR	300852	3
AHI1	6q23.3	AR	Leber congenital amaurosis 2	204100	3
ALDH5A1	6p22	AR	Succinic semialdehyde dehydrogenase deficiency	271980	3
ALDH7A1	5q31	AR	Pyridoxine-dependent epilepsy	266100	3
ARHGEF6	Xq26.3	XL	X-linked form of mental retardation (type 46)	300436	3
ARX	Xp21	XL	X-linked MR	300419	3
ATRX	Xq21.1	XL	ATRX (alpha-thalassemia/mental retardation) syndrome	301040	3
BRAF	7q34	AD	Cardio-facio-cutaneous syndrome	115150	3
CACNA1F	Xp11.23	XL	X-linked incomplete congenital stationary night blindness, type 2A (CSNB2)	300071	3
CDKL5	Xp22	XL	Epileptic encephalopathy, early infantile, 2	300672	3
CEP290	12q21.32	AR	Joubert syndrome 5	610188	3
CHD7	8q12.2	AD	CHARGE syndrome	214800	3
CREBBP	16p13.3	AD	Rubinstein–Taybi syndrome	180849	3
DCX	Xq22.3-q23	XL	Type 1 lissencephaly, X-linked	300067	3
DMD	Xp21.2	XL	Duchenne muscular dystrophy	310200	3
DMPK	19q13.3	AD	Myotonic dystrophy 1 (Steinert disease)	160900	3
EHMT1	9q34.3	AD	9q subtelomeric deletion syndrome (Kleefstra syndrome)	610253	3

(continued)

Table 1 (continued)

Official gene symbol[a]	Chromosomal location	Inheritance pattern[b]	Disorder	OMIM accession	Autism evidence level[c]
IGF2	11p15.5	AD	Beckwith–Wiedemann syndrome	130650	3
FGD1	Xp11.21	XL	Aarskog–Scott syndrome	305400	3
FOXG1	14q13	AD	Rett syndrome, variant	613454	3
FOXP1	3p14.1	AD	MR with language impairment and autistic features	613670	3
FTSJ1	Xp11.23	XL	Non-syndromic X-linked MR 9	309549	3
GAMT	19p13.3	AR	GAMT deficiency	612736	3
GRIA3	Xq25	XL	Non-syndromic X-linked MR 94	300699	3
HOXA1	7p15.3	AR	Bosley–Salih–Alorainy syndrome	601536	3
IL1RAPL1	Xp22.1-p21.3	XL	X-linked mental retardation	300143	3
IQSEC2	Xp11.22	XL	Non-syndromic X-linked MR 1	309530	3
KRAS	12p12.1	AD	Cardio-facio-cutaneous syndrome	115150	3
L1CAM	Xq28	XL	MASA syndrome	303350	3
MAP2K1	15q22.1-q22.33	AD	Cardio-facio-cutaneous syndrome	115150	3
MBD5	2q23.1	AD	2q23.1 microdeletion syndrome (MR)	156200	3
MED12	Xq13	XL	Lujan–Fryns syndrome	309520	3
MEF2C	5q14	AD	5q14.3 microdeletion syndrome	613443	3
MID1	Xp22	XL	Opitz syndrome	300000	3
MKKS	20p12	AR	Bardet–Biedl syndrome 6	209900	3
NDP	Xp11.4	XL	Norrie disease	310600	3
NF1	17q11.2	AD	Neurofibromatosis1	162200	3
NFIX	19p13.3	AD	Sotos-like overgrowth syndrome	117550	3
NHS	Xp22.13	XL	Nance–Horan syndrome	302350	3
NIPBL	5p13.2	AD	Cornelia de Lange Syndrome 1	122470	3
NLGN4X	Xp22.33	XL	X-linked mental retardation	300495	3
NRXN1	2p16.3	AD?/AR	Pitt–Hopkins-like mental retardation	600565	3
NSD1	5q35	AD	Sotos syndrome	117550	3
OCRL	Xq25	XL	Lowe syndrome	309000	3
OPHN1	Xq12	XL	XLMR with cerebellar hypoplasia and distinctive facial appearance	300486	3

Table 1 (continued)

Official gene symbol[a]	Chromosomal location	Inheritance pattern[b]	Disorder	OMIM accession	Autism evidence level[c]
PAH	12q22-q24.2	AR	Phenylketonuria	261600	3
PAFAH1B1	17p13.3	AD	Lissencephaly 1	607432	3
PCDH19	Xq13.3	XL	Sporadic early infantile epileptic encephalopathy	300088	3
POMGNT1	1p34.1	AR	Muscle–eye–brain disease	253280	3
PTEN	10q23.3	AD	PTEN hamartoma-tumor syndrome	601728	3
PTPN11	12q24	AD	Noonan syndrome 1	163950	3
PQBP1	Xp11.23	XL	Renpenning syndrome	309500	3
RAB39B	Xq28	XL	X-linked mental retardation 72	300271	3
RAI1	17p11.2	AD	Smith–Magenis syndrome	182290	3
RNF135	17q11.2	AD	Overgrowth MR syndrome	611358	3
RPE65	1p31	AR	Leber congenital amaurosis	204100	3
RPGRIP1L	16q12.2	AR	COACH syndrome	216360	3
SATB2	2q33	AD	2q33.1 microdeletion (cleft palate, MR) syndrome	119540	3
SCN1A	2q24.3	AD	Dravet syndrome	607208	3
SGSH	17q25.3	AR	Sanfilippo syndrome A (mucopolysaccharidisis type IIIA)	252900	3
SHANK3	22q13.3	AD	22q13 deletion syndrome	606232	3
SLC6A8	Xq28	XL	Creatine transporter deficiency syndrome	300352	3
SLC9A6	Xq26.3	XL	Mental retardation, microcephaly, epilepsy, and ataxia (Christianson type)	300243	3
TBX1	22q11.21	AD	Velocardiofacial syndrome	192430	3
UPF3B	Xq25-q26	XL	X-linked MR	300676	3
VPS13B	8q22.2	AR	Cohen syndrome	216550	3
YWHAE	17p13.3	AD	Miller–Dieker lissencephaly syndrome	247200	3

[a]Data obtained from Betancur (2011) and evidence scores from the AutismKB database (Xu et al. 2012)

[b]Abbreviations: *AR* autosomal recessive, *AD* autosomal dominant, *XL* X-linked

[c]Evidence level 1 and 2 genes are omitted, while evidence level 3 and 4 genes are presented, where level 1 indicates the gene has only been reported in single cases with ASD and/or autistic features and additional evidence is needed; level 2 indicates the gene has been reported in a single family with 2–3 males with ASD and/or autistic features and further evidence is required to confirm a role in ASD etiology; level 3 indicates the gene has been reported in more than one family with ASD and/or autistic features, but the disorder hasn't been a generally acknowledged ASD-related disorder; and level 4 indicates the disorder is widely acknowledged as an syndromic form of ASD

Studies on syndromic single gene causes of ASD (Table 1), such as TS, demonstrate that mutation of a single gene frequently leads to a phenotype of which ASD is just one part (Benvenuto et al. 2009; Caglayan 2010; Barth et al. 2014; Bishop et al. 2014). Another complication of many genetic disorders, including TS and other syndromic forms of ASD, is that different mutations in the same gene can lead to different phenotypes (Antonarakis and Beckmann 2006; Bishop et al. 2014). This latter finding is expected, as many gene products have multiple cellular roles, typically due to proteins having multiple binding partners. The field of interactomics is focused on identifying the binding (interacting) partners of gene products and is greatly assisting biologists to understand these phenomena. Therefore, despite the apparent complexities emerging in the phenotypic and genetics studies of ASD, it is interactomic and pathway analyses that have revealed the remarkable convergence of many of these genes on common cellular pathways, such as those affecting synapse development, morphology, and function (reviewed by Peça and Feng 2012). In addition to mutations directly affecting synaptic genes, defects in a variety of other cellular pathways also lead to synapse anomalies, such as those causing defects in calcium signaling, secretory pathway function, or mitochondrial activities (Krey and Dolmetsch 2007; Bourgeron 2009; Gargus 2009; Palmieri and Persico 2010; Aziz et al. 2011a, 2014; Barth et al. 2014; Peça and Feng 2012).

In this chapter, the key role that interactomics and pathway analyses play in understanding, explaining, and reducing the complexities of ASD genetics and pathobiology will be discussed. Use of these tools, supported by comparative genomic and interactomics, will lead to better understanding of the etiology of ASD and the development of beneficial therapeutics. Research efforts are presently aimed at reducing the complexities of "complex" genetic disorders, via interactomic and pathway analyses, while concurrently expanding the complexities of "simple" genetic disorders. An appreciation and understanding of the convergent nature of complex disorders, as well as the complexities of simple disorders, are of enormous importance for improving our understanding of all ASDs.

Biological Networks in Autism: Common Pathways and the Goldilocks Effect

As mentioned in the Introduction, the genetic basis of ASD only emerged in the 1980s, and the first clues as to the biological processes affected came from the co-occurrence of ASD with defined genetic disorders, such as fragile X syndrome (reviewed by Abrahams and Geschwind 2008). Genetic association studies for ASD only began around 20 years ago, followed by whole-genome linkage studies, and then CNV assessments (Abrahams and Geschwind 2008). Many databases are available collating the so-called autism genes and provide links to data supporting their involvement in the disorder, and some attempt to quantify the current strength of evidence that a particular gene is involved in ASD etiology (Table 2). To date, these studies and databases indicate there are hundreds of important ASD loci and

Table 2 Autism spectrum disorder genetic databases providing evidence evaluation

Database	Database descriptor	Website (and key reference)	Type of evaluation provided
AutDB	Autism database	www.mindspec.org/autdb.html (Basu et al. 2009)	Number of publications corresponding to each gene provided
SFARI	Simons Foundation Autism Research Initiative gene database	https://sfari.org/resources/sfari-gene (Banerjee-Basu and Packer 2010)	Score for each gene provided by an expert panel of researchers.
			Further information available from: https://s1gene.sfari.org/autdb/GS_Home.do and https://s1gene.sfari.org/autdb/GS_Statistics.do
AutismKB	Autism Knowledge Base	http://autismkb.cbi.pku.edu.cn (Xu et al. 2012)	Confidence level for each gene provided
			Level 1 – one reported case with autistic symptoms, level 2 – two to three cases in a single family, level 3 – cases in more than one family, level 4 – reported in multiple papers
AGD	Autism Genetic Database	http://wren.bcf.ku.edu/ (Matuszek and Talebizadeh 2009)	A three-level (1, 2, 3) classification system is used based on the authors' review of publications, where category 3 represents the strongest autism candidate genes, and category 1 the weakest

led to the definition of ASD as a prototypical complex genetic disorder. The major task now, in order to understanding the etiology of ASD, is to integrate the existing and emerging genetic candidates into the known synaptic, biological, and phenotypic anomalies found in ASD patients. The resulting data will be of vital importance for the development of ASD-specific therapeutics and diagnostics.

In April 2009, analysis of the strongest ASD candidate genes led to the proposal that disruption of genes affecting one of two independent pathways could lead to the synaptic abnormalities associated with autism (Bourgeron 2009). The first was the TSC/TOR pathway (see Fig. 1), based on syndromic forms of ASD where the genes encoding NF1, TSC1, TSC2, and PTEN are disrupted which, as described elsewhere in this book (Bishop et al. 2014; Barth et al. 2014), usually act as negative effectors of the rapamycin-sensitive TORC1 complex (Fig. 1). The second pathway suggested was one affected by mutations in synaptic genes encoding neurexins, neuroligins, and their binding partners (e.g., Homer3 and Shank3), which was proposed to dysregulate synapse homeostasis by impairing inhibitory–excitatory balance (Bourgeron 2009).
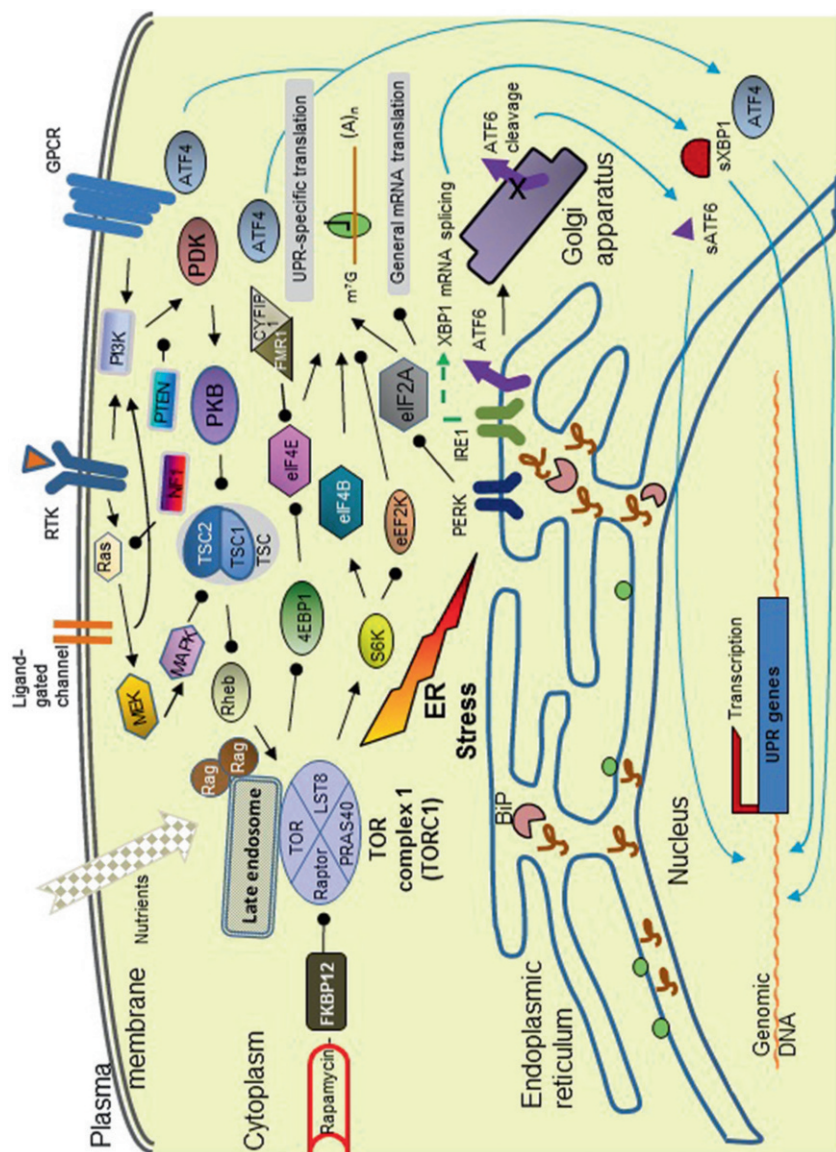
Fig. 1 (continued)

Later, in 2009, Ingenuity pathway analysis was used to evaluate the interaction between 33 different autism genes (Geschwind 2008). Ingenuity is a commercially available software package (see Table 3) and is designed to identify interactions between genes based on published evidence of binding partners, as well as common transcriptional and translational regulators. Of 33 candidate genes (see Table 1), direct or indirect interactions involving two or less degrees of separation were detected, thereby identifying a network of interactions between ASD genes (Geschwind 2008). This study established functional links between the TSC–TOR pathway and those gene products affecting synaptic function. Indeed, one well-known downstream effector of TOR, eIF4E (see Fig. 2), has also been implicated in ASD (Ehninger and Silva 2011). Therefore, interactomic data, as well as the functional data discussed elsewhere in this book (Barth et al. 2014), indicates many ASD-implicated genes are expected to interact with the TSC–TOR

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Fig. 1 Signaling upstream and downstream of the TORC1 complex. Various conditions that perturb endoplasmic reticulum (ER) homeostasis lead to accumulation of mis-folded proteins in the ER. This can be triggered by the inactivation of the TSC1/TSC2 complex (TSC), which leads to overactivity of the target of rapamycin (TOR) complex-1 (TORC1), as this leads to a constitutive increase in protein synthesis. The unfolded protein response (UPR) is triggered, which involves phosphorylation of PERK (pancreatic ER kinase), which leads to the upregulation of UPR genes via the activation of the transcription factor ATF4 (activating transcription factor 4) and activation of ATF6 to form sATF6, which is dependent on traffic to the Golgi apparatus and downregulation of general protein synthesis. Inositol-requiring enzyme-1 (IRE1) is also activated, which leads to the production of the splice variant of X-box-binding protein-1 (XBP1), sXBP1, which also assists in upregulating UPR gene transcription to produce factors such as ER chaperones, such as BiP (binding immunoglobulin protein; also known as glucose-regulated protein of 78 kDa, Grp78). Many cell-surface (plasma membrane-localized) receptors and channels act upstream of the TORC1 complex, and several gene products implicated in this signaling cascade are encoded by known "autism genes." These include PTEN, a phosphatidyl (3,4,5)-3 phosphatase, that antagonizes phosphoinositide 3-kinase (PI3K) signaling and neurofibromatosis 1 (NF1). There are many downstream signaling events from TORC1, and this diagram highlights those implicated in translational control and the UPR. Additional autism gene products also regulate cellular translation, such as FMRP1 (fragile X mental retardation 1), and its indirect binding partner, eIF4E (eukaryotic-translation initiation factor 4E), which is also implicated in autism (Neves-Pereira et al. 2009). The FMRP–CYFIP1 complex prohibits eIF4E-dependent initiation, thereby acting to repress translation. Rapamycin, and related compounds, binds to FKBP12 to inhibit TORC1 signaling. The intricacies of spatial regulation of TOR signaling are still emerging, and this complexity is largely absent from the diagram. However, on amino acid stimulation of cells, Rag protein heterodimers recruit the TOR complex to Rab7-positive late endosomes, and TOR is then activated on recruitment of Rheb to the same organelle. TSC2 is a transmembrane (TM) protein, localizing predominantly to the Golgi apparatus, while TSC1 is a soluble protein localizing to vesicles in the cytoplasm, detail not present in the schematic but discussed in the accompanying text. Other abbreviations: *CYFIP1* cytoplasmic FMR1-interacting protein 1, *eEF2* eukaryotic elongation factor-2 kinase, *eIF2A* eukaryotic-translation initiation factor 2A, *FKBP12* FK506-binding protein of 12 kDa, *GPCR* G protein-coupled receptor, *LST8* lethal with sec13 protein 8, *PDK* phosphoinositide-dependent protein kinase, *PKB* protein kinase B (alias AKT), *PRAS40* proline-rich AKT/PKB substrate of 40 kDa, *Rag* Ras-related GTP-binding protein, *Rheb* Ras homolog enriched in brain, *Rictor* rapamycin-insensitive companion of TOR, *RTK* receptor tyrosine kinase

Table 3 Selected tools for pathway analysis

Acronym or name	Descriptor	Website
IPA	Ingenuity pathway analysis software (commercial)	www.ingenuity.com
YFG	Your favorite gene (limited freely available version of Ingenuity from Sigma Life Science)	www.sigmaaldrich.com/life-science/your-favorite-gene-search.html
GO	Gene ontology analysis via Biobase	http://bklbiobase.de
STRINGS	Functional protein association networks	string.embl.de
IntNetDB	Integrated network database	http://hanlab.genetics.ac.cn/IntNetDB.htm
FunCoup	Networks of functional coupling	http://FunCoup.sbc.su.se
GeneMANIA	Network-based prediction of gene function	http://www.genemania.org
STARNET 2	Microarray-based network generation, with a protein–protein interaction module	http://vanburenlab.medicine.tamhsc.edu/starnet2.html
BioGRID	The biological general repository for interaction datasets	www.thebiogrid.org
GenMAPP 2	Gene map annotator and pathway profiler – version 2 (free software download)	www.genmapp.org
PIN	Protein interaction network at SFARI (autism specific)	https://sfari.org/autdb/PINHome.do
Autworks	Autism-network analysis tool	http://autworks.hms.harvard.edu
Predictive Networks	Integration and analysis of human gene networks (free software download)	https://sourceforge.net/projects/predictivenets
WGCNA	Software for weighted correlation network analysis of co-expression data	www.r-project.org
MetaCore	Pathway analysis of experimental data and gene lists (commercial)	http://www.genego.com/metacore.php
GEM-TREND	Gene expression data mining –toward relevant network discovery (using own data)	http://cgs.pharm.kyoto-u.ac.jp/services/network
Ariadne Pathway Studio	Commercial pathway analysis tool	www.ariadnegenomics.com/products/pathway-studio

pathway, and this in turn suggests that therapeutics based on modulation of this pathway will be widely applicable.

The determination that disruption of one or more common pathways is causative of many cases of ASD indicates that a single therapeutic may be able to effectively treat defined cohorts of ASD patients. However, no single treatment is expected to be valid for all individuals affected. The data that best demonstrate why a single therapeutic will not apply to all cases of ASD are based on the studies of patients with fragile X syndrome (FXS). Fragile X syndrome is caused by mutations in the X-linked *FMR1* gene, and the FMR1 protein is known to interact with *TSC2* transcripts, as well as transcripts of other autism genes. However, while TORC1 signaling is overactive in FXS, FMR1 may have additional effects on the

Fig. 2 Autoimmune disease genetics. The number of genes common to other autoimmune diseases (*black*), compared to genes that are specific (*gray*) to a particular disease. Abbreviations: *CS* celiac sprue disease, *CD* Crohn's disease, *MS* multiple sclerosis, *NUC* nonspecific ulcerative colitis, *P* psoriasis, *RA* rheumatoid arthritis, *SLE* systemic lupus erythematosus, *T1D* type 1 diabetes (Data for graph obtained from Baranov 2009)
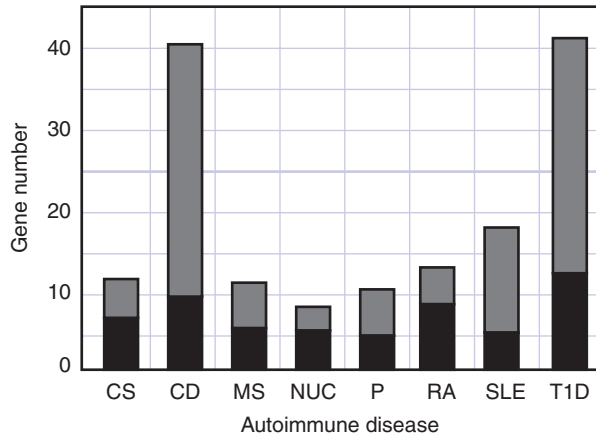
TSC–TOR pathway, as mGluR-LTD was altered in opposite directions in mouse disease models of FXS, compared to TSCD (Auerbach et al. 2011; Ehninger and Silva 2011; Troca-Marín et al. 2012). These data indicate the FXS synaptic phenotype could be corrected by the *inhibition* of mGluR5, while the effects of the TSC mutation were ablated by the *augmentation* of mGluR5. This research indicates that even when a common phenotype is caused by anomalies in a common cellular pathway, the same therapeutic approach will not always be applicable.

The principle that up- *or* downregulation of a key process can lead to a common defect is well known in biology. An extension of this principle is the so-called Goldilocks effect, where deletion or duplication of a genetic locus leads to a similar phenotype and gene copy number (dosage) needs to be "just right." Different mutations in the ASD gene, *SHANK2* (encoding ProSAP1), represent another "Goldilocks analogy" as, depending on which mutation in *Shank2* is recreated in mice, opposite effects on neuron signaling are triggered, although both demonstrate an ASD phenotype (Schmeisser et al. 2012; Won et al. 2012). Mice with a *Shank2* mutation in exon 7 show enhanced signaling from NMDA (*N*-methyl-d-aspartate) receptors (Schmeisser et al. 2012), while mice with *Shank3* lacking exons 6 and 7 have reduced NMDA receptor signaling (Won et al. 2012). Treatment of the latter mice with a positive allosteric modulator of mGluR5 led to enhanced NMDAR function and a marked increase in social behaviors. While again, emphasizing that treatment of ASD is effective, these findings not only emphasize that individual synaptic phenotypes of ASD patients differ and therapy must match the underlying synaptic phenotype but that dosage of any ASD therapeutic may need to be carefully adjusted.

The Goldilocks principle also applies to *UBE3A*, another ASD-implicated gene (see Table 1). Loss of *UBE3A* function causes Angelman syndrome, a syndromic form of autism, characterized by developmental delay, seizures, and impaired speech (reviewed by Jedele 2007). By contrast, patients with a gain of copy of *UBE3A* have a phenotype of cognitive impairment, gross motor delays, seizures,

and ASD (Battaglia 2008). Of note, these features are also reproducible in mouse models of *Ube3a* copy number (Smith et al. 2011; Baudry et al. 2012), and as discussed further toward the end of this chapter, model organisms have a vital part to play in furthering our understanding of ASD etiology and the evaluation of effective therapeutics.

Together these data indicate that an understanding of the aberrant synaptopathology and/or etiological mechanisms in each individual with ASD will be crucial for the provision of effective and appropriate therapies. At present, there is no clinical or biological marker to determine which ASD cases might be suitable for a particular type of therapy. Interactomics and pathway analysis, validation in model systems, and the use of biomarkers will together play an important part in teasing out different subtypes of ASD, diagnosing these, and matching individuals to appropriate therapeutic interventions.

The Power and Value of Network-Based Approaches for Understanding ASD

In addition to commercial packages for interaction network and pathway analysis, many similar tools are publically available for analysis of protein–protein interactions (Table 3) and yield comparable results (see Müller et al. 2011). There are caveats to the accuracy and completeness of such pathway and interaction analyses, as they depend highly on information from high-throughput studies and available published data, so the maps generated are still limited in coverage and accuracy. In high-throughput studies, weak or transient interactions may not be detected, while artifactual interactions may be detected, and the latter may not be biologically significant due to temporal or spatial differences in gene expression. To overcome these weaknesses, a variety of algorithms have been developed for pathway and network analyses, and these integrate data from increasing numbers of resources, including physical and genetic data from model organisms, functional annotation, GWAS, microarray, RNAomic, and proteomic studies, as well as algorithm-based computational predictions of protein and nucleic acid interactions (see Table 3). Therefore, while high-throughput data are considered error-prone and incomplete, integration of data from multiple sources and multiple organisms can provide robust information and can also be used to guide future studies.

Indeed, large numbers of novel genes have been identified through GWAS of ASD, as has been the case for many other common complex genetic diseases. However, the stringent statistical criteria used to minimize false positive results also lead to valid genes also being ignored. As susceptibility to common complex genetic disorders in most cases depends on the effects of several variants in multiple genes, which affect a common functional pathway, pathway analysis is also emerging as a tool to integrate the available data (Zhong et al. 2010; Lee et al. 2011; McKinney and Pajewski 2011). In most cases, however, this approach is limited to the inclusion of genes which are already known to have roles in a common

biological pathway. Genes of unknown function, or which are yet to be placed in a curated biological pathway, will still be disregarded using this approach. Fortunately, the applicability of pathway-based approaches to identify new members of biological pathways, and to predict function of uncharacterized genes, has been validated in studies of multiple complex genetic diseases, and predictive results using integrated approaches are proving highly accurate (Wang and Marcotte 2010; Lee et al. 2011; McKinney and Pajewski 2011).

Interaction networks also form the basis for algorithms used to predict epistatic interactions, the effects of modifier genes, and ultimately to calculate phenotype from genotype. Some such algorithms and models also incorporate variation from other data types, such as environmental factors, epigenetic modifications, and other factors modulating gene expression, as well as the effect of therapeutics, and predictions can be validated in model organisms or cell culture systems (Wang and Marcotte 2010; McKinney and Pajewski 2011). For example, interactomic and genetic analyses indicate a significant number of protein–protein interactions between TOR pathway kinases and proteins involved in endocytosis and actin organization (Aronova et al. 2007), and in addition to the mouse models described above, a cell-based assay has recently been developed to test the effects of unclassified *TSC2* mutations so these can be compared to better-characterized mutants (Coevoets et al. 2009).

Understanding epistatic interactions and the role of modifier genes is important for predicting phenotype from genotype. These types of interactions can lead to ASD genes, identified from GWAS for example, being discarded if phenotypic validation is not found in a single mouse strain. Unfortunately, for many model organisms, it is too costly and time-consuming to validate phenotypes in a variety of genetic backgrounds. Therefore, the ability to predict the impact of modifier genes on a phenotype is of value for understanding the etiology of complex genetic disorders and the penetrance of genetic mutations and variants. Once again, furthering our understanding of human modifier genes is dependent on a combination of network-based analyses combined with experimental findings from model organisms, where the predicted genetic interactions can be specifically and systematically studied (Nadeau 2003). Of further importance, a greater understanding of the effect of modifier genes may also lead to the development of therapeutic strategies aimed at mimicking protective allele function to reduce the impact of pathogenic ASD mutations.

Modifier genes affecting the TS phenotype are discussed in this book (Bishop et al. 2014), but examples are also emerging for other syndromic, as well as idiopathic, forms of ASD. It has recently emerged that the FXS phenotype can be modified dependent on whether single-nucleotide polymorphisms (SNPs), or deletion polymorphisms, are present in the promoter region of the serotonin transporter gene (*SLC6A4*), as these affect *SLC6A4* gene transcription. Those FXS patients with a highly transcribed variant (longer promoter) of *SLC6A4* demonstrate significantly more aggressive and destructive behaviors than those that do not, while those with the short promoter (with the deletion polymorphism) demonstrate the least aggression (Hessl et al. 2008).

Mutations of *FMR1* also cause variable behavioral phenotypes depending on which mouse strain carries the mutation (Spencer et al. 2011), and as described above mice with double *FMR1* and *TSC2* mutations have a normal phenotype. Another modifier of the FXS phenotype is the *RGS4* gene (which encodes the regulator of G-protein signaling-4 protein), which acts as a GAP (GTPase-activating protein) for G-protein receptors in the central nervous system, such as mGluR5 and GABA(B) receptors (Pacey et al. 2011). Genetic abrogation of RGS4 activity rescues many of the FXS symptoms in mice, and modification of this and perhaps other GTPases, using therapeutics, is another potential therapeutic strategy for ASD (reviewed by Hampson et al. 2012).

Even more exciting are the findings that some mice with autism-associated gene defects do not have autism-like behaviors, indicating modifier genes can protect against an autism phenotype. Dual FXS and TSCD mutations were used as an example earlier on in this chapter, and one further example is in mouse strains with mutations in the well-characterized ASD gene, *SEMA5A*. Depending on the genetic background, *Sema5a* mutations have no apparent effect, are lethal *in utero*, or produce mice with neurological abnormalities (Fiore et al. 2005; Gunn et al. 2011). Another example are mice with a mutation in the neuroligin-3 gene (*NLGN3*) corresponding to the human R451C (arginine to cysteine) *NLGN3* ASD mutation. One mouse model of this mutation shows autistic-like features, while another mouse model does not (Tabuchi et al. 2007; Chadman et al. 2008). Again, this is thought to be the result of the different genetic backgrounds of the mice used. The presence of modifier genes is proposed to protect novel mutations from elimination and play an important role in evolution, leading to the concept that the whole genome, not just a genotype, should be considered as the selective unit in evolution (Nadeau 2003). This model outlines one factor that may contribute to the high prevalence of ASDs in human populations.

In one final example, the phenotype produced by mutations in the *PTEN* gene, implicated in TSC-TORC1 signaling (see Fig. 1), is also affected by polymorphisms in the *SLC6A4* gene, at least in mice (Page et al. 2009). Female mice, haploinsufficient for *Pten*, have impairments in social behavior, and this phenotype is exacerbated by haploinsufficiency for *Slc6a4*. Dual haploinsufficiency also affects physical characteristics, as the macrocephaly detected in *Pten* haploinsufficient mice is exacerbated in those also haploinsufficient for *Slc6a4* (Page et al. 2009). Therefore, in a complex pathway, such as those causing ASD, modification of one gene can affect other genes in the pathway. Characterizing modifier genes will lead to a more detailed knowledge about the complex molecular interactions that are central to ASD etiology, will lead to further advances in ASD gene discovery, may aid the development of novel therapeutic targets, and/or may help define treatment subgroups.

Interacting Pathways and Pleiotropic Genes

As mentioned in the section above, mutations in the *Slc6A4* gene can modify the ASD phenotype of mice with *Pten* or with *Fmr1* mutations. However, in addition to altering the ASD phenotype triggered by mutation of other genes, modifier genes

such as *SLC6A4* independently express a phenotype and can modify the phenotype of other neurological disorders (Allen et al. 2008; Albani et al. 2009; Quaranta et al. 2009; Liu et al. 2011). Mutations in *SLC6A4* are independently linked to anxiety and mood disorders, and again, the phenotype in mice is dependent on genetic background (Holmes et al. 2003; Homberg et al. 2010). Furthermore, mutations in the synaptic scaffolding protein gene *SHANK3* are strongly implicated in both idiopathic ASD and Phelan–McDermid 22q13 deletion syndrome, considered a syndromic form of ASD. The precise location of the mutations within the *Shank3* gene appears as key to the phenotypic outcome, as the phenotype of gene deletions differs from that in which only the ankyrin repeat domain is deleted (Yang et al. 2012).

The phenomenon described above highlights two key factors that must be considered in ASD pathway analyses, which are the pleiotropic nature of many genes and the participation of genes in complex overlapping interaction networks. These types of interactions can greatly affect the phenotype of both monogenic and complex genetic disorders, as different mutations in the same gene can cause distinct phenotypes, while mutation of a single gene can also lead to an increased (and/or decreased) risk of additional disorders (Goh et al. 2007; Baranov 2009). Understanding these types of interactions will impact on how, and why, some ASD candidate genes have also been identified as risk genes in other neurological disorders and/or how single ASD gene mutations can contribute to multiple phenotypes (as described for TS elsewhere in this book: Barth et al. 2014).

Indeed, it is a combination of genes that mediates biological processes, and it is variations in the function of these genes which together lead to the development of a specific pathology (reviewed by Emmert-Streib and Glazko 2011). In each of these genetic networks, models of interactions indicate there are both central (or "hub") genes and auxiliary genes, with the latter often being referred to as pathway modifier genes. Again, this relates to the pleiotropic nature of many genes, as they may play roles in multiple functional genetic modules. This means that there may be some alleles that are specific to a particular disease but that many loci will contribute to multiple, related diseases, due to common genetic modules being involved in the etiology and pathogenesis of several diseases (Goh et al. 2007; Baranov 2009).

This overlap is best documented for autoimmune diseases (Goh et al. 2007; Baranov 2009) and is illustrated in Fig. 2. It is now known that about a third of the identified loci for allergic diseases are associated with two or more other such diseases, and therefore, there are many common candidate genes for celiac disease, Crohn's disease, multiple sclerosis, psoriasis, rheumatoid arthritis, lupus, diabetes, and ulcerative colitis (Goh et al. 2007; Baranov 2009).

A network incorporating the genes common to over 1,000 complex disorders has been created and named the human disease network (HDN) (Goh et al. 2007; Baranov 2009). Conversely, a network of genes common to various diseases has also been created, the disease genetic network or DGN (Goh et al. 2007; Baranov 2009). Combining HDN- and DGN-maps creates a "diseasome map" reflecting the

molecular pathways of these complex disorders (Goh et al. 2007; Baranov 2009). This analysis found every complex disorder could be caused by mutations in multiple different genes. Furthermore, the genes causative of each disorder interact in a functional network with a characteristic topology, consisting of central and peripheral genes, where almost all the complex disease networks connect with a number of other disease networks (Goh et al. 2007; Baranov 2009). This "diseasome" landscape is altering rapidly, as new genetic studies reveal additional genes contributing to multiple complex disorders at an ever-increasing pace and the interactions between diseases are increasing accordingly. The presence of overlapping functional genetic modules between complex disorders supports the concept of syntropy, which is based on the concept of "families" of etiologically related complex disorders (Goh et al. 2007; Baranov 2009). In particular for diseases where multiple genes are implicated in causality, the functional role of many genes may make small, yet significant, contribution to the overall risk of several common diseases.

Therefore, the recent explosion of data in the post-genomic era regarding the etiology of ASDs and other complex disorders has led to the conclusion that biological systems consist of complex molecular and functional networks that interact to give rise to physiological function and, indeed, dysfunction. The past decade has seen the advent of increasing numbers of new technologies and computational methods for studying these systems on a genome-wide scale and, in many cases, incorporate comparative genomic data. These new technologies facilitate the analysis of thousands of genes and gene products to gain novel insights into biological function and drive the rational design of laboratory experiments and development of therapeutics.

Co-expression Analysis: Adding Power to Network Analysis and Development of Biomarkers

Many genes identified in studies looking for those contributing to ASD etiology, such as linkage or GWAS, are poorly annotated, are of unknown biological function, and are largely ignored. Integrative interaction networks are one tool to predict function and provide evidence of interaction of uncharacterized genes with known molecular pathways associated with ASD. While data from large-scale transcriptomic analyses, using microarrays for example, are useful when incorporated into interaction networks, they are also a useful tool in their own right and can yield information even in the absence of correlation with protein interaction or functional pathway analysis. For example, microarrays may be useful for understanding ASD cases caused by multiple, common, low-penetrance polymorphisms, as these tools can ultimately facilitate the detection of an overall effect of multiple genetic mutations on cellular homeostasis at the transcript level. Identification of these transcriptional networks and their co-regulated components, together with other interactomic tools, provide a powerful framework for identifying and characterizing molecular pathways dysfunctional in ASD.

One early study of the power of these techniques was by detecting differences in gene expression profiles in monozygotic twins with differential severity of language impairment (Hu et al. 2006). Several research groups have applied network-based approaches to analyze transcriptomic data gained from specimens from ASD patients, including postmortem brain samples, and have found subsets of genes with different expression levels in ASD, compared to control, specimens (Voineagu et al. 2011; Voineagu 2012). Of note, when such gene expression data is analyzed using software designed to convert levels of gene co-expression to networks of similarly affected genes, these networks were found enriched for previously validated ASD risk genes and with genes with cellular trafficking and synapse function roles (Voineagu et al. 2011; Voineagu 2012). Many gene expression changes in the brain have also been found in studies of peripheral blood-derived samples from ASD patients, providing a rationale for the development of diagnostic biomarkers based on abnormal gene expression profiles (Voineagu 2012). They also may be used to provide further insight into how etiologically heterogeneous ASD is and whether multiple pathways can be causative, and overall patterns of transcription may be one way of distinguishing an ASD phenotype at the molecular levels from that of "overlapping" conditions, discussed above, such as ADHD, anxiety, OCD, MR, epilepsy, and schizophrenia.

However, several limitations of transcriptomic analyses must be kept in mind. These include difficulties in differentiating primary adaptive changes from secondary effects, although attempts at teasing out these effects in ASD analyses have been made (Voineagu et al. 2011), and that differences in mRNA abundance do not correlate in a linear manner to changes in protein abundance (the functional end product of most genes) and changes in gene expression can even be opposite to that of protein expression (Fournier et al. 2010). Overall, the correlation between mRNA and protein is low for both expression and co-expression (Ostlund and Sonnhammer 2012). This indicates that integrative analyses will be necessary for a full understanding of the etiology of ASD.
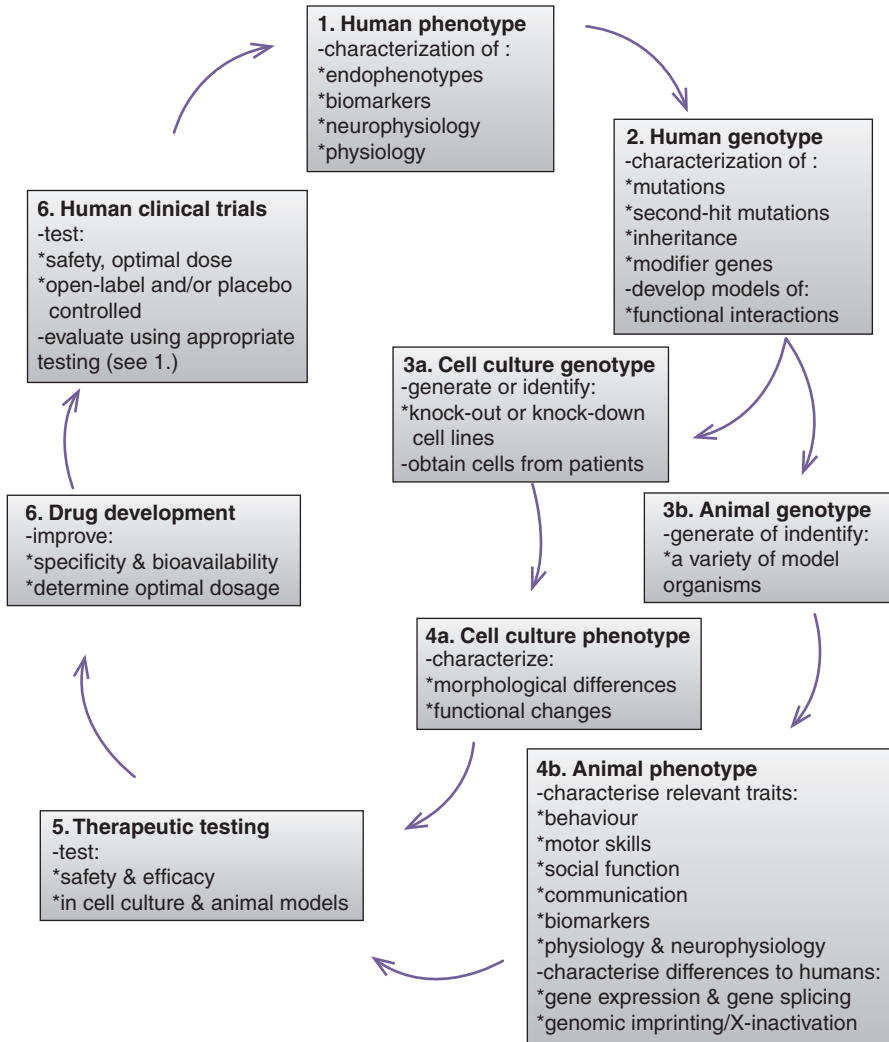
One elegant study recently underscored the power of integrated genetic, transcriptomic, and functional screening strategies, for the discovery of novel gene and network connections in human cancer and neurobiology and for the prediction of appropriate therapies (Scott et al. 2009). This work examined genes present in a region of chromosome 5 found duplicated in many different types of solid tumors. They used this integrated approach to identify a Golgi apparatus protein, Golph3, encoded at position 5p13, for further study. Gain- and loss-of-function studies in vitro and in vivo validated *GOLPH3* as a potent oncogene. Physically, Golph3 (Vp74p in yeast) was found to localize to the trans-Golgi network and interact with components of the retromer complex, such as Vps35, which had previously been linked to TSC–TOR signaling in yeast. Golph 3 was also found to regulate cell size, and enhance TORC1 signaling in human cancer cells, and alter the response to the TOR inhibitor, rapamycin, in vivo. Thus, integration of genomic, genetic, biological, functional, and biochemical data from both yeast and human systems established *GOLPH3* as a new human oncogene capable of modulating the response of treatment with the cancer drug, and potential ASD
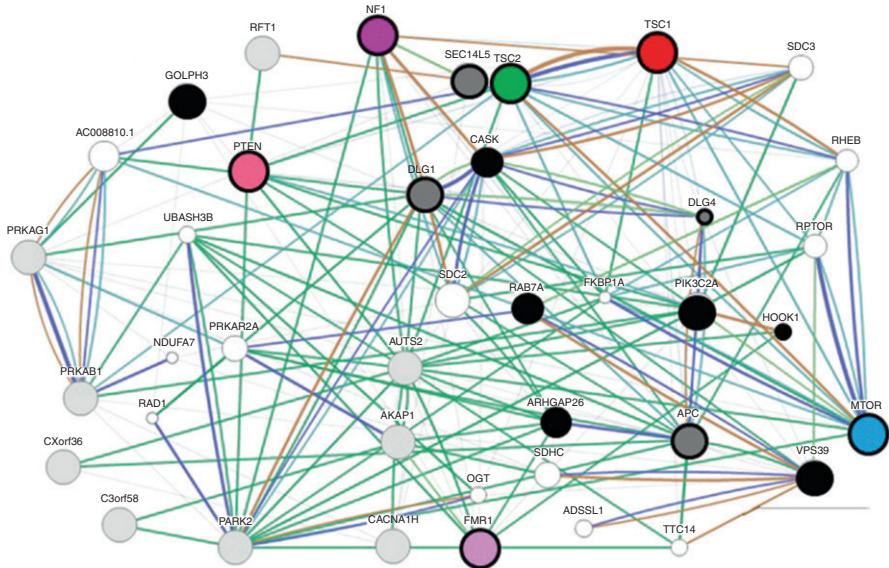
Fig. 3 Emerging functional links between TSC–TOR pathway gene products and cellular trafficking gene products. Physical (*blue*), predicted (*brown*), co-expression (*gray*), co-localization (*light blue*), genetic (*dark green*), and shared domain-based (*light green*) interaction network. Links to tools, gene and protein function can be found using resources in Tables 1 and 2. *Red circle* = TSC1; *green circle* = TSC2; *blue circle* = TOR; *pink circles* = key ASD gene products: NF1, PTEN, and FMR1. Proteins with well-characterized cellular trafficking roles are indicated by *black circles*. Those with emerging roles in secretion are indicated by *dark gray circles*. Golph3 is also known to bind Vps35

therapeutic, rapamycin (Scott et al. 2009). Tumors expressing high levels of Golph3 protein are more sensitive to rapamycin in vivo. Furthermore, as expected from the neurodevelopmental phenotype of TSCD patients, *GOLPH3* mutation is associated with behavioral phenotypes. *GOLPH3* is a candidate gene for speech delay (Murru et al. 2008) and schizotypal personality disorder (Bespalova et al. 2005), and more recently, *GOLPH3* was identified as a candidate gene in a family pedigree with learning and behavioral difficulties (Barber et al. 2011). This chromosomal region is adjacent to the cri du chat locus, and it has been suggested that there are two ASD genes in this region of chromosome 5, one at 5p13.3 and another at 5p13.2 (Harvard et al. 2005). A small-scale functional network analysis indicates Golph3 is part of a network of gene products linked to the TSC–TOR signaling pathway and other ASD-implicated genes (see Fig. 3).

Together, information from pathway analyses and data from studies in cell culture and animal models (see Fig. 4) are providing evidence that therapeutic interventions to manipulate the emerging common pathways are likely to benefit a wide range of ASD patients, despite diverse genetic causes. The recent funding

1. Human phenotype
-characterization of :
*endophenotypes
*biomarkers
*neurophysiology
*physiology

2. Human genotype
-characterization of :
*mutations
*second-hit mutations
*inheritance
*modifier genes
-develop models of:
*functional interactions

6. Human clinical trials
-test:
*safety, optimal dose
*open-label and/or placebo
 controlled
-evaluate using appropriate
testing (see 1.)

3a. Cell culture genotype
-generate or identify:
*knock-out or knock-down
 cell lines
-obtain cells from patients

3b. Animal genotype
-generate of indentify:
*a variety of model
organisms

6. Drug development
-improve:
*specificity & bioavailability
*determine optimal dosage

4a. Cell culture phenotype
-characterize:
*morphological differences
*functional changes

4b. Animal phenotype
-characterise relevant traits:
*behaviour
*motor skills
*social function
*communication
*biomarkers
*physiology & neurophysiology
-characterise differences to humans:
*gene expression & gene splicing
*genomic imprinting/X-inactivation

5. Therapeutic testing
-test:
*safety & efficacy
*in cell culture & animal models

Fig. 4 Key stages of research required for the development of autism therapeutics. Multidisciplinary research is required if safe and effective therapeutic agents are to be developed for ASD. Not all subtypes of ASD are expected to respond to the same therapeutic agents, due to factors discussed in the text. Kumar and colleagues (2011) provide a systematic approach to assessing the ASD phenotype in animal models

and establishment of European consortium on synaptic protein networks disrupted in neurological psychiatric diseases (EuroSPIN; see www.eurospin.mpg.de) highlights the growing momentum of this type of research, where outcomes will provide benefits applicable to a range of neurological and psychiatric diseases.

Model Systems: Essential for Understanding Key Pathways and Developing Therapeutics

The use of animal and cell culture models, as described above, is crucial for ASD research aimed at identifying, validating, and characterizing autism genes, understanding ASD etiology, and developing ASD diagnostics and therapeutics. Comparative genomics and interactomics have proved useful tools for predicting effects of genetic polymorphisms and gene mutations and are essential for drawing meaningful conclusions from model organism-derived data (see Aziz et al. 2011b, 2012) and are beneficial for generating more complete and accurate molecular interaction networks (see above). Even the use of the simplest of model organisms can provide vital information, but as for all other aspects of ASD biology, an integrated approach is the most beneficial. An example relevant to our focus on TSCD and the TSC/TOR pathway is provided in this penultimate chapter section.

The genes encoding the core components of the TSC–TOR signaling pathway are present in all major eukaryotic phyla, but this is not true of all components (see Table 4), indicating complexity has been added to the simple ancestral TSC–TOR signaling pathway (Serfontein et al. 2010, 2011). Indeed, researchers must be

Table 4 Homologs of human TSC–TORC1 pathway genes

Mammals[a]	Drosophila[b]	C. elegans[c]	S. cerevisiae[d]	S. pombe[e]	Dictyostelium[f]
TSC[g]					
TSC1	Tsc1	–	–	Tsc1	–
TSC2	Gig	–	–	Tsc2	Tsc2
TORC1[g]					
MTOR	Tor	let-363	TOR1	Tor1	Tor
			TOR2	Tor2	
RPTOR	raptor	daf-15	KOG1	Mip1	Raptor
MLST8	CG3004	C10H11.8	LST8	Pop3	Lst8
AKT1S1[h]	CG10109	–	–	–	–
TORC2[g]					
MTOR	Tor	let-363	TOR2	Tor1	Tor
				Tor2	
MAPKAP1	Sin1	sinh-1	AVO1	Sin1	RipA
Rictor	Rictor	Rict-1	AVO3	Ste16	PiaA
MLST8	CG3004	C10H11.8	LST8	pop3	Lst8

[a]Official Human Gene Nomenclature Committee (HGNC) name (www.genenames.org)
[b]*D. melanogaster* (fruit fly)
[c]*Caenorhabditis elegans* (worm)
[d]*Saccharomyces cerevisiae* (budding yeast)
[e]*Schizosaccharomyces pombe* (fission yeast)
[f]*Dictyostelium discoideum* (amoeba)
[g]Homologous genes whose products make up the tuberous sclerosis complex (TSC), TOR (target of rapamycin) complex 1 (TORC), and TOR complex 2 (TORC2) are listed. The TORC2 complex is a second cellular complex containing the TOR protein, which is not discussed further
[h]Alias *PRAS40*

acutely aware of which components of this pathway are conserved in their model organism of choice, to inform the interpretation of experiments (Aziz et al. 2012). For example, as with the *DIA1* and *DIA1R* "autism genes" (Aziz et al. 2011a), *TSC1* and *TSC2* homologs are not detectable in the *Caenorhabditis elegans* genome (see Table 4), and *TSC1* is only found in opisthokont species (animals and fungi). By contrast, while *TSC1* is absent in the model organism, *Dictyostelium discoideum*, an amoeboid species used to model many human diseases, *TSC2* and *TOR* are conserved (Rosel et al. 2012; Table 4). Signaling via TSC2 also differs between species, as indicated by sequence divergence and the lack of conservation of key phosphorylated residues of human TSC2, compared to that in other species disease (Serfontein et al. 2011). These differences highlight the need for a systematic characterization of evolutionary conservation between genes, and between functional pathways, in organisms used to model complex human diseases (Aziz et al. 2012).

Despite, or rather partly because of, these differences, model organisms often provide advantages for biological research and are being used to tease out the roles of different aspects of TSC–TOR pathway that would be difficult, or impossible, to achieve in the complex human system (see Serfontein et al. 2011). For example, one beneficial feature of the *Drosophila* (fruit fly) system is that it is easy to monitor the effects of "mosaic" mutations, and fly-based studies have also greatly enhanced the understanding of how insulin signaling pathways control cell mass versus cell growth and the poorly characterized effects on neural development and behavior, due to aberrant TSC–TOR pathway signaling (Neufeld 2004; Dimitroff et al. 2012). In the fission yeast *Schizosaccharomyces pombe*, deletion of *TSC1* or *TSC2* causes defects in the localization of amino acid permeases, which are retained in vesicles of the secretory pathway and are not delivered to the cell surface (Matsumoto et al. 2002). The power of genetic analysis and the ability to develop simple phenotypic assays in this fungal organism have tremendous potential and have already led to a greater understanding of epistatic events regulating the *TSC1*/*TSC2* mutant phenotype and the identification of TSC–TOR pathway modifier genes (Aspuria and Tamanoi 2008; Napolioni and Curatolo 2008).

It is good practice to use a wide variety of model systems to gain maximal knowledge and assess function, mutation effects, and therapeutics, on any key cellular process, and to be well informed about the similarities and differences existing between human and model systems, such as differences in gene expression, gene splicing, genomic imprinting, and X-inactivation (for X-linked human genes) (see Fig. 4). For example, the question why the brain, heart, and kidney are the organs most commonly affected in TSCD patients has been investigated using tissue expression profiling. While *TSC2* expression is detectable in all tissues, the highest levels are in the brain, heart, and kidney, and this correlates with those tissues most severely affected (Wienecke et al. 1996). By contrast, in rats, the highest levels of TSC2 are in the brain, liver, and testis. Therefore, despite the TSC/TOR pathway being highly conserved in rats and are good models for neurological TSCD phenotypes, heart and kidney disease effects of human TSCD may not be phenomimicked. However, it was in mouse models of TSCD that it was first

demonstrated that rapamycin not only reduced tumorigenesis but had benefit on the epileptic endophenotype, a finding subsequently confirmed in human clinical trials, leading to a new era of ASD research (reviewed by Napolioni and Curatolo 2008).

Conclusion

Network strategies, based on data from multiple sources, are powerful tools being effectively used to understand three of the greatest puzzles posed by ASDs. These are (i) the wide range of biochemical, cellular, and neurological comorbidities of ASD; (ii) the huge number and variety of genes implicated as contributing to an ASD, phenotype; and (iii) the cellular mechanisms underpinning ASD etiology. Mapping the biological pathways implicated in ASD is helping understand phenomena such as pleiotropy, differences in penetrance, synergistic genetic effects, and the mode of action of therapeutics and is informing present efforts to generate biomarker-based diagnostic tests. It is now clear that the multiple genes implicated in ASD act in common pathways that underpin the neurobiology of ASD and the core behavioral phenotype, and explain the phenotypic heterogeneity seen in ASD populations. Many autism genes affect TSC–TOR signaling and cellular secretion or act to cause dysfunction of synapses. Detailed analysis of the interaction networks of autism-implicated genes, and the biological pathways in which they function, is therefore shedding light on mechanisms behind ASD comorbidities, including epilepsy, cancer, gastrointestinal dysfunction, and immune anomalies.

An increase in knowledge about key pathways and their cellular effects (such as the TOR pathway on synapse function and the contribution of the secretory pathway to neuronal function) is leading to a greatly improved understanding of the etiology of ASD and illuminates novel targets for therapeutics that will address both the core- and noncore symptoms present in individuals with ASD. It is clear that significant progress has been made toward understanding the molecular pathways underlying several forms of syndromic ASD, such as tuberous sclerosis; however, many questions still remain, particularly about the links between TOR signaling and synapse function, memory, learning, and the role of this pathway in idiopathic ASD. Therefore, the importance of future studies on TOR signaling, calcium homeostasis, as well as ER, mitochondrial, and secretory pathway function are of vital importance for understanding autism and developing novel therapeutics.

Major progress has been made in recent years into targeted ASD therapies, and many of these drugs shown to be effective in animal models of ASD are currently being evaluated in human clinical trials. The studies to date with rapamycin, and related drugs, indicate the plausibility that a single therapeutic agent will simultaneously abrogate autistic, cognitive, tumorigenic, and epileptic phenotypes, as disruption of a common molecular pathway underpins the etiology of these disorders in at least a subset of ASD individuals. A caveat is that the single agent required may need to differ between different subgroups of individuals with ASD, dependent on where and how cellular pathways are disrupted and in which

"direction" from optimum this disruption is, due to phenomena such as the Goldilocks effect. Understanding these cellular processes, and the design and testing of novel therapeutic agents, will require the input and interaction between researchers from many disciplines, including systems biologists, cell biologists, neuroscientists, geneticists, psychologists, pediatricians, and psychiatrists. These same analyses also provide a framework for the rational design of biomarker-based assays which, in turn, could be used for predicting the most appropriate therapy for differing subgroups of ASD patients.

Key Terms

Epistasis. Where the effect(s) of a genetic mutation in a gene is modified variation or mutation in another gene(s), which can be referred to as modifier genes.

Goldilocks effect. A phenotype affected by too many, or too few, copies of a dosage-sensitive gene. Copy number of the gene needs to be "just right."

Haploinsufficiency. When loss or mutation of one, of the two, copy of a gene(s) or locus (in diploid somatic cells) causes a phenotype, often an undesirable phenotype.

Mendelian genetic disorder. A monogenic, or single-gene disorder, which is inherited following the classical inheritance patterns first described by Mendel: autosomal recessive, autosomal dominant, and sex-linked dominant and sex-linked recessive (usually X-linked and specific to females).

Modifier gene. A genetic variant or gene mutation which modifies the penetrance of a mutation present in another gene(s), either minimizing penetrance or enhancing penetrance.

Pleiotropic gene. A gene that affects multiple phenotypic traits if mutated, often due to the gene product having multiple binding partners.

Key Facts of OMICS

- The suffix -omics is widely used to refer to the collective study of certain aspects of biological research.
- The field of research corresponding to a given "-omic" ends in the suffic "-ome."
- For example, genomics refers to the study of genomes.
- Genomics can refer to the study of all genes and regulatory elements of a single genome or comparisons between genomes from different organisms.
- Proteomics refers to the large-scale experimental study of proteins and can involve comparisons of protein profiles from specific cells, tissues, or organisms.
- Transcriptomics refers to the analysis of mRNA profiles from cells, tissues, or organisms.
- RNAomics refers to the analysis of RNA molecules within cells or tissues but may extend to the study of interactions between RNA molecules and their regulation.

- Metabolomics is the large-scale study of metabolites or the products of metabolic reactions in cells, tissues, or organisms.
- Interactomics refers to the large-scale analysis of molecular interactions, including gene–gene, protein–protein, and protein–gene interactions.
- Other "omics" include secretomics, the large-scale study of secreted proteins; kinomics, the large-scale study of phosphorylated molecules; and lipidomics, the lipid profile of cells and tissues.
- A comprehensive list of "-ome" and "-omic" terminology and accepted definitions is maintained by the Gerstein Lab, Yale University, and can be viewed at http://bioinfo.mbb.yale.edu/what-is-it/omes/omes.html.

Summary Points

- Emerging evidence suggests the hundreds of genes implicated in autism act on common biological processes due to interactions between these gene products in complex molecular networks.
- Common effects of these genes on synapse plasticity are emerging, but opposing dysfunction in long-term synaptic depression are both associated with ASD.
- Disruption of a single gene in a molecular network can lead to a range of phenotypic effects.
- Different mutations in a single gene may affect different portions of a molecular network and therefore cause different phenotypes.
- Due to molecular interactions, variation in other genes or gene products in a network can affect the final phenotype caused by a specific genetic mutation.
- The phenomenon of pleiotropic effects, due to mutation in a single gene, demonstrates why a single therapeutic could abrogate both the core symptoms of autism and also the varying but etiologically related comorbid conditions.
- ASD appears to be a treatable and reversible disorder, even in adults.
- Interactomic and network analysis can enhance genetic and cellular studies, and vice versa.
- Integrating biological data from multiple sources, including model organisms, is providing valuable tools for the rational development of ASD diagnostics and therapeutics.

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