

# 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

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

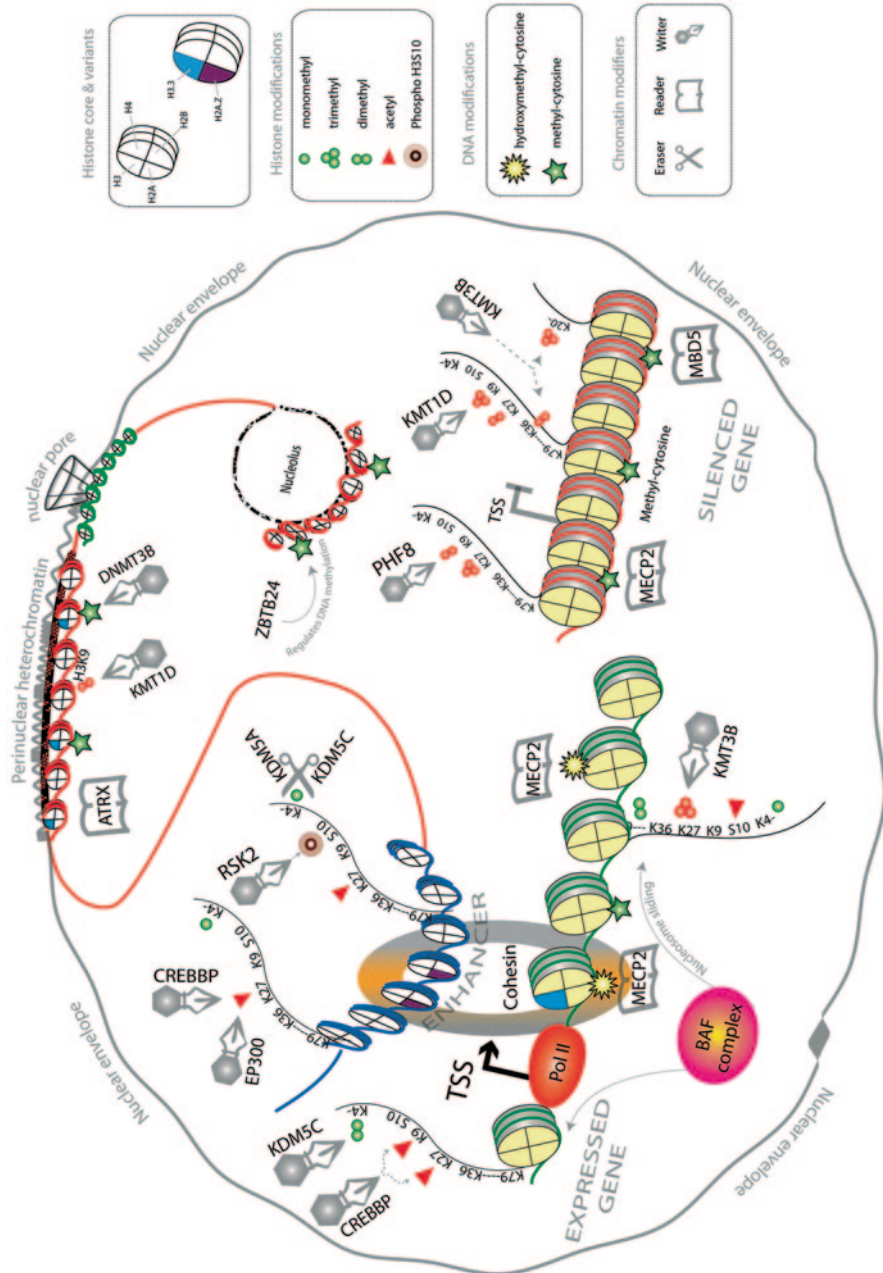
- i. a general introduction on the basic principles of epigenetic regulation, we will discuss
- ii. epigenetic heritability,
- iii. monogenic forms of ASD due to mutations in genes encoding chromatin regulatory proteins,
- iv. epigenetic regulation in the human brain during normal aging and development,
- 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 ‘supra-nucleosomal’ 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 mitochondrial 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 perturbations) (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, serotonergic, 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 (Siegmond 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 (Siegmond 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; Siegmond 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 perturbations, 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<sub>A</sub> and GABA<sub>B</sub> 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 severely 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 (Sapranaukas 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<sub>12</sub>-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, Stegheuis 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, Sznajder Y, Wijmenga C, van Eggermond MC, van Oostaijen-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, Gillissen-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 JL, 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, Biggins 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, Matevosian A, Jiang Y, Akbarian S (2006) Chromatin immunoprecipitation in post-mortem 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, Sagar 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, Konciewicz 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, Lalonde 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 Rееuwijk 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) Dnm3a regulates emotional behavior and spine plasticity in the nucleus accumbens. *Nat Neurosci* 13:1137–1143
- Le Meur E, Watrin F, Landers M, Sturny R, Lalonde 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, Lalonde 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, Tarakhovskiy 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, Esmaeili-Nieh S, Wiczorek 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
- Sapranasuskas 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, Rogojev 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, Biniszkiwicz 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 JL, 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, Catanch 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, Sevrerson 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* 49:589–593
- 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* 49:589–593