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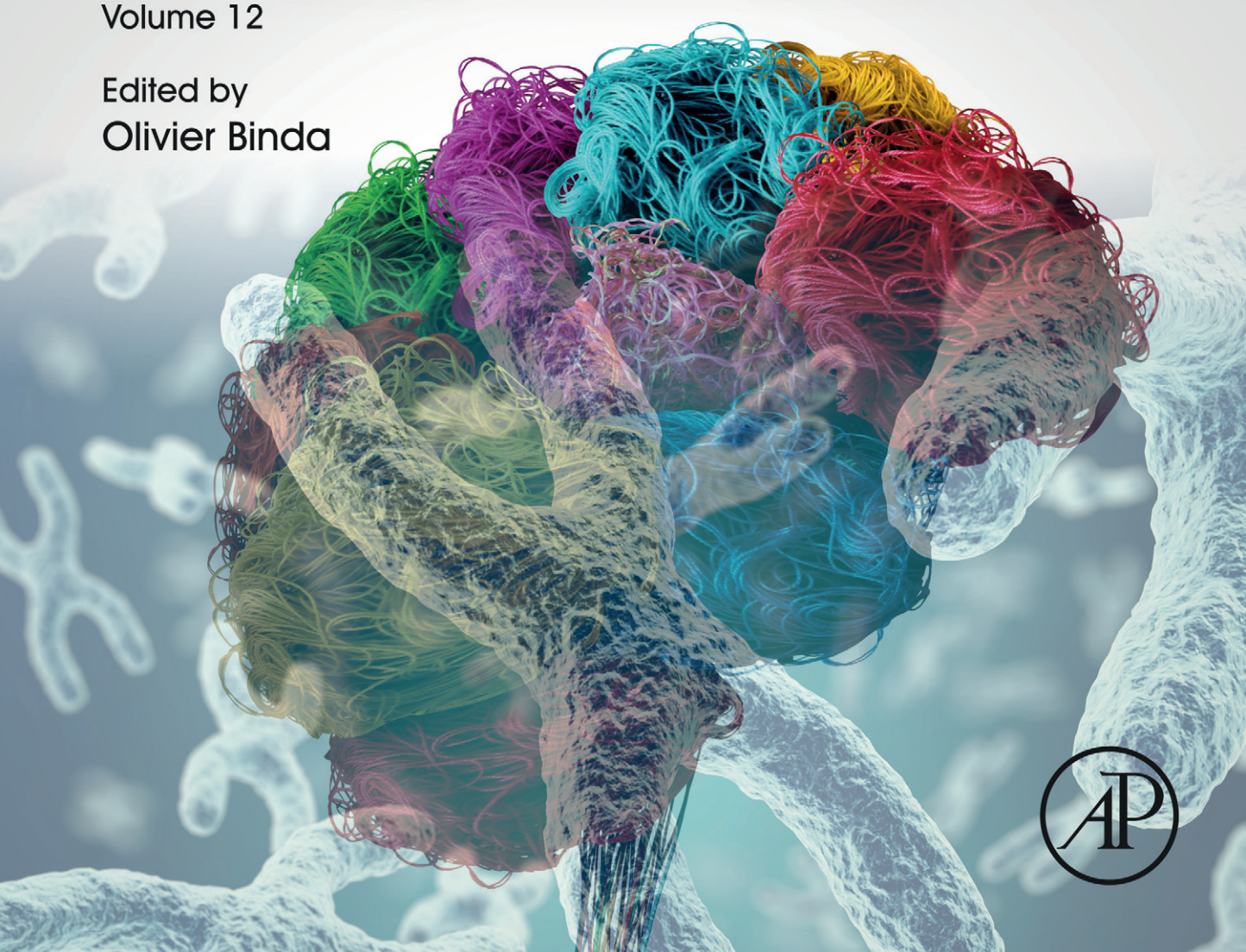

CHAPTER

The epigenetics of autism

13

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Chapter Outline

13.1 Autism

The term autism refers to a group of neurodevelopmental conditions characterized by difficulties in social interactions alongside stereotyped behavior and unusually narrow interests [1]. In addition, autistic individuals usually have other comorbid conditions including intellectual disability, epilepsy, sleep difficulties, and attention deficits (attention-deficit/hyperactivity disorder [ADHD]/attention deficit disorder) [1]. Males are diagnosed three times more with autism than females, reflecting biological sex differences in the condition or an underdiagnosis of females, or a combination of both. However, this sex difference in autism reduces with intellectual disability [2]. In the

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fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-V*), the Asperger syndrome and pervasive developmental disorder not otherwise specified (PDD-NOS) fall under the umbrella of autism spectrum disorders (ASDs) [3]. The use of the term autism spectrum conditions (ASCs) instead of ASDs is to avoid the pejorative connotation of the word "disorder," given that, although some characteristics of autism are disabling, others (such as excellent attention to and memory for detail) may even confer talent in some autistic individuals [1]. The prevalence of diagnosed autism has been steadily increasing since the term was coined (70 years ago), with a substantial rise in the past two decades. The worldwide prevalence of autism is currently 1–2% [4–7]. This increase in autism prevalence can be attributable to changes in the diagnostic criteria and greater public awareness, rather than an absolute increase in the condition [5]. Nonetheless, an increase in a risk factor could also be partially involved [1]. Epidemiologic studies have reported a link between some environmental factors (e.g., maternal health during pregnancy, prenatal exposure to chemicals, toxins) that put individuals at higher risk of developing ASCs. However, none of the studies prove a causal link with autism [8].

13.1.1 Heritability and genetics

It is clear that a considerable proportion of the risk for autism is genetic. In one of the earliest studies investigating the heritability of autism, Folstein and Rutter [9] studied a sample of 21 twin pairs and reported a monozygotic twin concordance of 36% and a dizygotic twin concordance of 0% for autism. Subsequently, several studies in much larger cohorts have established relatively high twin heritability for autism. A meta-analysis of twin studies [10] reported a heritability of 64–91%. While these studies focus on clinically defined autism, studies have also investigated the heritability of autistic traits (i.e., subthreshold autistic traits that are thought to be representative of the liability model of autism). Ronald and Hoekstra [11], in a meta-analysis, reported a heritability of between 60% and 90% for autistic traits. Heritability can also be investigated using familial recurrence. A study investigating insurance claims from over a third of the entire US population identified a heritability of approximately 92% for autism, which had the highest heritability of the 149 diseases and conditions investigated [12]. Similarly, familial recurrences in Sweden and Finland have identified a sibling recurrence risk of $\sim$10% [13,14] and heritability estimates of 83% [15].

Evidence of molecular genetics provides further support for the considerable heritability of autism. Several studies have investigated the additive single nucleotide polymorphism (SNP) heritability (i.e., the proportion of variance explained by all SNPs investigated) using multiple models. Cumulatively, SNPs investigated are thought to contribute to between 15% and 50% of the total risk for autism, with a higher contribution in multiplex autism families [16–18]. Although, *en masse*, these SNPs contribute to a substantial proportion of the heritability, the per-SNP effect is very small, suggesting a highly polygenic architecture of autism, similar to that of other complex conditions.

Several genome-wide association studies (GWASs) have sought to identify significant SNPs associated with autism at a *P*-value threshold $<5 \times 10^{-8}$. In a GWAS of autism, five independent loci were identified [19]. Candidate genes identified from this analysis includes *KMT2E*, which encodes the MLL5 tumor suppressor, and *MACROD2*, which encodes a macrodomain deacetylase that removes ADP-ribose from mono-ADP-ribosylated proteins [19]. Early studies used a case-pseudocontrol design in which they tested differences in allele frequencies between transmitted and

nontransmitted alleles in simplex trio families, which is statistically underpowered [20]. Newer studies have included a case-control design and have increased sample sizes by meta-analyzing genetic data from multiple different cohorts [19,21].

GWASs have also allowed for the dissection of directional pleiotropy between autism and other related traits by using genetic correlations. Multiple studies have identified that there is a modest positive genetic correlation between the measures of intelligence, including educational attainment and cognitive aptitude, and the risk for autism [22,23]. This is in contrast to studies on rare genetic variation, which identify an enrichment for *de novo* protein-truncating mutations in autistic individuals with below average IQ [24,25]. Genetic correlations have also identified a positive genetic correlation between autism and other psychiatric conditions such as depression, schizophrenia, and ADHD, suggesting shared underlying liabilities for autism and other psychiatric conditions [19]. Studies from our laboratory have shown that social traits related to autism, such as empathy and social relationship satisfaction, are also genetically correlated with autism: common alleles that are associated with the increased risk for autism are also associated with decreased social relationship satisfaction and empathetic abilities [26,27]. In contrast, alleles that are associated with the increased risk for autism are also associated with increased systemizing, in support of the hypersystemizing theory of autism [28].

In addition to common genetic variations, other classes of genetic variations contribute to the risk for autism. Notably, although rare, protein-truncating *de novo* mutations are enriched in the probands of simplex autism families, in autistic individuals with comorbid intellectual disability, and in female autistic probands [29]. Although the individual effect sizes are large, together, *de novo* variants are thought to account for a smaller proportion of the risk for autism. For example, rare genetic variants, both *de novo* and inherited, are thought to account for the risk in 10−30% of autistic individuals [30]. As of December 2017, as many as 78 genes have been identified as enriched for rare variation in autism (http://spark-sf.s3.amazonaws.com/SPARK_gene_list.pdf). Many of these genes encode chromatin modifiers (e.g., ASH1L, EHMT1, EP300, NSD1, SUV420H1) and transcription regulators (e.g., CHD8, MECP2, MED13L).

13.2 Epigenetics of autism

Epigenetic modifications function to maintain the architecture and the appropriate segregation of chromosomes during mitosis, to regulate repetitive elements and endogenous retroelements, and to regulate gene expression [34]. There are two main molecular epigenetic mechanisms involved in gene expression: covalent modification of DNA through methylation and chromatin structure regulation via histone posttranslational modification (PTM) [35]. These modifications arise from heritable modifications, environmental factors, and replication errors [31]. While the genetic replication error rate is 1:1000000, the usual error rate for replicating epigenetic elements is closer to 1:1000 [32]. There are also spontaneous changes that are thought to occur to the epigenome, known as epigenetic drift. This may begin to explain the stochastic nature of the epigenomes and the divergence from their identical starting conditions [33].

There are three main molecular epigenetic mechanisms involved in the regulation of gene expression: covalent modification of DNA through methylation, chromatin modifiers, and ATP-dependent chromatin remodelers [35].

13.2.1 DNA methylation and hydroxymethylation in autism

The majority of studies that have investigated the epigenetic architecture of autism have studied DNA methylation, as it acts at the interface between genetic and environmental factors and may hold an answer to autism's complex etiology [36]. Previous studies have identified perinatal environmental exposures to various environmental factors and their link to impaired methylation in children with autism [37–40]. DNA methylation is the covalent binding of a methyl group (-CH_3) to a cytosine C5 side chain. This chemical modification is catalyzed by DNA methyltransferases (DNMTs), a class of enzymes that transfer a methyl group specifically to cytosines that are usually followed by a guanine, forming the CpG dinucleotide sequences, or followed by any other base, forming a CpH. More than two-thirds of the CpG dinucleotides in the genome are methylated, whereas the remaining occur in CpG islands (small clusters). The methylated CpG dinucleotides are found mainly in intra- and intergenic sequences within DNA repeats, whereas the unmethylated CpGs occur in regions that actively regulate gene transcription [35]. Although Wyatt and Cohen reported the presence of 5-hydroxymethylcytosine (5-hmC) in the early 1950s, their findings were not recognized until 2009 when the dynamic role of 5-hmC in DNA demethylation was demonstrated [41].

The novel ten–eleven translocation (TET) protein family acts as an "eraser" and is responsible for the conversion of 5-methylcytosine (5-mC) to 5-hmC followed by oxidization to produce 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC) in the demethylation process [42]. Furthermore, 5-mC and 5-hmC can be directly converted to thymine and 5-hydroxymethyluracil, respectively. This is mediated by activation-induced cytidine deaminase and apolipoprotein B mRNA editing enzyme, a catalytic polypeptide-like (APOBEC) family. Thymine DNA glycosylase then recognizes and removes the mismatched thymine, 5-hmU, 5-fC, or 5-caC from the genome and replaces them with unmodified cytosine to initiate base excision repair [43].

Several lines of evidence suggest an important role for epigenetic regulation in the risk for autism. One of the earliest genes implicated in autism is *MECP2*, which encodes the methyl-CpG-binding protein causing the Rett syndrome [44] (see also Chapter 9), and *de novo* frameshift mutations in the transcriptional activator CREB-binding protein that cause the Rubinstein–Taybi syndrome (RSTS). Missense mutations in the Tenascin C (TNC) gene, insulin-like growth factor–binding protein, and acid-labile subunit (IGFALS) gene have been reported in whole-exome sequencing of a patient with RSTS presenting with an autism phenotype [45]. Duplications of *MECP2* cause MECP2 duplication syndrome, which also has similarities to autism [46]. Canonically, MECP2 is thought to bind to methylated DNA to condense chromatin, repressing transcription. However, studies suggest that MECP2 might also act as an enhancer, indicating a more complex role for the protein during neurodevelopment [47].

Studies have associated TET and DNMT mutations with intellectual disability and autism [29]. The Angelman syndrome and the Prader–Willi syndrome are closely related to the autism spectrum. They result from the loss of maternal and paternal alleles, respectively, of 15q11.2–q13.3. In some rare cases, the imprinting control region is unmethylated in the Angelman syndrome and hemimethylated in the Prader–Willi syndrome. So although this locus is deleted in both syndromes, it is duplicated in autism [48,49].

13.2.1.1 Candidate gene methylation studies in humans

Akin to candidate gene association studies, a few studies have investigated methylation modifications in candidate genes in autism. One such gene is *SHANK3*, which encodes a synaptic scaffolding protein, involved in the formation and maturation of dendritic spines. Mutations in this gene have been associated with autism [50]. Zhu and colleagues [51] conducted a DNA methylation profiling of five CpG islands (CGI-1 to CGI-5) in the *SHANK3* gene in postmortem brain tissues from 54 cases of ASCs and 43 controls. This study was the first to report an altered methylation pattern. Specifically, a significant increase in overall DNA methylation of three intragenic CGIs (CGI-2, CGI-3, and CGI-4) of *SHANK3* in ASC brain tissues was observed. Notably, 15% of ASC brain samples revealed an increase in DNA methylation specifically in CpG-2 and CpG-4 of *SHANK3*. Moreover, there was an altered splicing pattern of *SHANK3* isoforms, with concurrent increase in methylation of specific CGIs in ASC brain tissues. The findings suggest that *SHANK3* has an important role in causing ASCs and may serve as a valuable biomarker to dissect the contributions of genetic and environmental components.

Oxytocin receptor (*OXTR*), which encodes a G-protein-coupled receptor that binds to the neurotransmitter peptide hormone oxytocin, is another important gene implicated in ASC etiology [52]. Gregory and colleagues [53] performed a high-resolution genome-wide microarray and comparative genomic hybridization on 119 probands from multiplex ASC families to identify copy number variants in the *OXTR* gene. The results from this study showed a genomic deletion in the proband and his mother, although the affected sibling did not harbor a deletion but displayed aberrant gene silencing in the region of *OXTR* promoter by DNA methylation, with the highest increase in methylation at a single CpG being 37%. They also carried out a DNA methylation analysis on peripheral blood and temporal cortex from ASC cases and matched controls from independent data sets. The methylation status of several CGIs in *OXTR* was found to be significantly increased by 23% in DNA extracted from peripheral blood in 20 sex-matched ASC cases when compared with controls. Further analysis on DNA methylation of temporal cortex brain tissues in independent data sets from 10 ASC cases and sex-matched controls revealed up to 41% higher methylation at specific CGIs in males with ASCs. This study provided the first evidence supporting the role of epigenetic regulation of OXTR in causing ASCs.

Glutamate decarboxylase (*GAD*), which encodes an enzyme that catalyzes the conversion of the excitatory neurotransmitter glutamate to inhibitory neurotransmitter γ-aminobutyric acid (GABA) and thereby plays a pivotal role in maintaining the excitatory—inhibitory balance, is considered to be another relevant gene in causing ASCs [54,55].

MECP2 is an important epigenetic regulator in human brain development. As a member of the methyl-binding domain family of proteins, it is highly enriched in the central nervous system, especially in GABAergic interneurons. MECP2 binds to methylated CpGs and can act as either a gene activator or a repressor depending on the PTM or methylation status (5-mC or 5-hmC) of the associated target gene. The binding affinity of MECP2 to 5-mC or 5-hmC is an important factor to account for when considering its differential regulatory action. As glutamic acid decarboxylase 67 (GAD1), glutamate decarboxylase 65 (GAD2), and reelin (RELN) have been previously reported to be downregulated in the postmortem brains of ASC cases when compared with controls [56,57], a study aimed to investigate the binding of MECP2 and its role in regulating the expression of those genes. The authors reported a significant increase in MECP2 binding specifically to the promoter regions of *GAD1*

and *RELN* in the brains of patients with ASCs. They used methyl DNA immunoprecipitation (MeDIP) and hydroxymethyl DNA immunoprecipitation (hMeDIP) to measure the total 5-mC and 5-hmC levels, respectively. They reported an enrichment of 5-hmC levels relative to the levels of 5-mC at the regulatory regions of the investigated genes. This is associated with an increased MECP2 binding at the *GAD1* and *RELN* promoters. These results suggest that increased levels of 5-hmC at regulatory gene domains improve MECP2 binding and downregulate the expression of the target genes in ASCs [58].

13.2.1.2 Methylome-wide association studies

In contrast to candidate gene methylation studies, advances in methylation bead arrays have allowed for the investigation of methylation patterns across the genome (called methylome-wide association studies, or, in some instances, epigenome-wide association study (EWAS), but the term epigenome is not restricted to methylation patterns). Methylome-wide association studies are usually conducted following two different approaches: those based on sequencing followed by bisulfite treatment and those based on the affinity for methylcytosine [59]. However, sequencing-based protocols are expensive, time consuming, and require expertise [60,61]. For these reasons, new-generation arrays have been developed to interrogate a wide range of CpG sites throughout the genome [62,63].

One of the challenges of methylation studies is tissue source. Methylation patterns vary widely across tissues; however, some of these signatures are preserved across tissues. For autism, investigating methylation patterns in neural tissue will provide the most accurate mechanistic insights into the biology of the condition. A few studies have investigated differential methylation in autistic brains compared with nonautistic brains using postmortem tissue. Ginsberg and colleagues [64] compared differentially methylated gene networks in DNA isolated from postmortem cerebellar cortex and occipital cortex of nine cases with idiopathic ASC and nine age-matched typically developing controls using HM27 arrays. No significant ASC-specific differentially methylated probes (DMPs) were discovered at a false discovery rate (FDR) of 0.05 but downregulation of genes of mitochondrial oxidative phosphorylation and protein translation was identified. Ladd-Acosta and colleagues [65] investigated methylation profiles in ASC brain samples from the prefrontal cortex (6 cases and 5 controls), temporal cortex (6 cases and 10 controls), and cerebellum (7 cases and 6 controls) using the Illumina 450 K array and were able to identify four significant autism-associated differentially methylated regions (DMRs).

Using the same type of Illumina 450 K array, Nardone and colleagues [66] identified 5329 differentially methylated CpG sites in the prefrontal cortex (12 cases and 12 controls) and 10,745 significant CpGs in the anterior cingulate gyrus (11 cases and 11 controls) between the control and autistic postmortem brain tissues. To understand the effect of methylation on gene expression in autistic brains, they compared the DMRs from this study with transcriptomic data from autistic postmortem brains published by Voineagu and colleagues [67]. Interestingly, the hypomethylated regions overlapped with genes showing higher expression, and the genes with altered CpG methylation and altered expression were enriched for immune-related functions [66,67]. Extending this work, Nardone and colleagues investigated methylation signatures specifically in neurons in postmortem autistic and nonautistic brains. They measured methylation using the Illumina 450 K array in fluorescence-activated cell-sorted neuronal nuclei. Although they did not identify any significant CpGs that differed in methylation levels in autistic and control brains, they identified 58 DMRs [68].

To identify sets of methylated regions that are correlated in their methylation, the authors conducted weighted gene coexpression network analyses and identified eight modules that were significantly correlated with case-control status. Two of these modules were significantly enriched for synaptic processes and one for immune-related genes mirroring gene-set enrichment in transcriptomic studies [67,69]. The two modules enriched for synaptic processes were also enriched for candidate genes implicated in autism and for neurodevelopment-specific methylation signatures.

In parallel to studies on the postmortem brain, a few studies have also investigated methylation in peripheral tissues in autism. Although epigenetic signatures differ across tissues, several studies have demonstrated the use of studying methylation in peripheral tissues. The first methylome-wide study of autism investigated methylation patterns in twins discordant and concordant for autism. Nguyen and colleagues performed global methylation analysis on the lymphoblastoid cell lines derived from three pairs of discordant monozygotic twins for autism and siblings from two of these pairs. At a liberal FDR threshold of 10%, the authors identified 73 differentially methylated CpG islands. This study revealed many candidate genes that were differentially methylated between discordant monozygotic twins as well as between both the twins and unaffected siblings [70]. Genes reported included *BCL-2* and retinoic acid–related orphan receptor alpha (*RORA*). Their methylation status was further confirmed by bisulfite sequencing and methylation-specific polymerase chain reaction. Immunohistochemistry analysis showed decreased expression of both genes in the cerebellum and frontal cortex in the autistic brain when compared with controls.

A similar approach was taken by Wong and colleagues [71], in which they investigated methylation patterns in 100 twins (50 pairs) for both case-control status and quantitative traits related to autism and measured them using the Childhood Autism Symptom Test (CAST). Methylation was measured from blood samples. The authors identified differences in regional methylation in twin pairs discordant for autism. They further examined methylation differences in quantitative traits associated with autism and identified several CpGs that were associated with scores on the CAST.

The largest methylation study [72] of autism to date investigated methylation patterns using neonatal dried blood spots in 1263 individuals in the MINERvA cohort from Denmark, of which approximately half of the participants had an autism diagnosis. Methylation was investigated using the Infinium HumanMethylation450k array. Regression analyses were conducted using polygenic risk score for autism from the iPSYCH autism study as the predictor variable and methylation as the dependent variable. The study identified two epigenome-wide significant CpGs (cg02771117: $P = 3.14 \times 10^{-8}$ and cg27411982: $P = 8.38 \times 10^{-8}$), with several additional CpGs with $P < 1 \times 10^{-5}$. The two significant probes flank a region implicated by GWASs for autism, suggesting a contribution of both genetic and nongenetic effects to methylation in autism. To identify if case status is associated with methylation, the authors meta-analyzed the data from the MINERvA cohort and two additional cohorts (methylation from blood samples), but they did not identify any significant signals in the total sample of 1425 ASD cases and 1492 controls. Further investigating the genetic contribution to methylation in autism, the authors conducted bayesian colocalization using methylation quantitative trait locus (QTL) data from blood. They identified 91 loci that contribute to both autism risk and DNA methylation in autism.

To further understand the role of genetic variation in autism, Andrews and colleagues [73] created methylation QTL maps across multiple tissues including fetal brain, blood, and cord blood. The study identified relatively high concordance in methylation QTLs across blood and cord blood, but to a lesser extent across fetal brain and the two peripheral tissues. GWAS loci associated with

autism at various *P*-value thresholds were enriched for methylation QTLs in the fetal brain and to a lesser extent in blood and cord blood. To understand the biological processes underlying both the polygenic and methylation architecture in autism, the authors conducted gene-set enrichments of methylation QTL targets of the autism-associated SNPs. Several of the processes involved were related to immune regulation.

A few studies have investigated methylation in other tissues. Feinberg et al. [74] tested the relationship paternal sperm DNA methylation with autism risk in offspring. They examined genome-wide DNA methylation in paternal semen from an enriched-risk cohort of fathers of autistic children using CHARM 3.0 array and Illumina Infinium HumanMethylation450K BeadChip array. They also compared it with the methylation profiles previously obtained from postmortem brain in the study from Ladd-Acosta and colleagues [65]. With overlapping CHARM data with 450K data, 193 DMRs were identified. Furthermore, 18 of 75 (24%) 450K array probes showed consistent differences in the cerebellums of autistic individuals when compared with controls. This indicated the role of epigenetic differences in the paternal sperm as a potential risk factor to autism risk in the progeny [74].

While the BeadChip technology focuses on investigating CpG regions, other studies took a more global approach to study methylation not only at CpG regions but also at CpH sites throughout the genome. A study used reduced representation bisulfite sequencing to analyze methylation status in 63 postmortem cortical brain samples (Brodmann area 19) from 29 autism-affected and 34 control individuals. Although no significant DMRs were found in this study, a marked enrichment of CpH methylation in autistic brains was reported [75].

13.2.2 Histone modifications

13.2.2.1 Histone methylation and acetylation

Histone PTMs can be residue and site specific and they are far more diverse than DNA methylation. They include 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 (Y) hydroxylation, and several others.

These modifications are coregulated and occur as a group of histone PTMs affecting chromatin structure and function. For example, histone H3 lysine 4 (H3K4) methylation and acetylation marks are found in many active promoters. The trimethylated forms of H3K9, H3K27, and H4K20 are found to cluster at the same loci in the genome and are known to be repressive PTMs. These are specific examples, but generally, acetylation of histones marks active regions of the genome, while histone methylation marks either active or inactive chromatin region loci depending on the state and site of methylation.

To date, most EWASs in autism have focused on DNA methylation, whereas very little is known about other chromatin modifications such as histone acetylation. Autism has been previously linked to deleterious mutations in genes involved in the regulation of chromatin structure and function, such as histone deacetylase HDAC4, histone H3 lysine 9 (H3K9) methyltransferase EHMT1/KMT1D/GLP, and H3K4 demethylase JARIDIC/KDM5C/SMCX, as reported in Balemans et al. [76] and Adegbola et al. [77] respectively. H3K4me3, the trimethylated form of H3K4, is enriched at the transcriptional start site of genes and has a major role in neuronal health because of its role in chromatin remodeling complexes of genes involved in differentiation, growth, and plasticity required for hippocampal

learning and memory [78]. Shulha and colleagues [79] reported the first neuron-specific study of H3K4me3 in autism. They used prefrontal cortex neurons (sorted and separated from postmortem tissue) from 16 subjects with autism and 16 control subjects ranging in age from 6 months to 70 years to identify autism-associated H3K4me3 changes using anti-H3K4me3-ChIP followed by deep sequencing. Comparing the H3K4me3 epigenomes in the two groups and across a wide range of age revealed that the generalized disruption of developmentally regulated chromatin remodeling in infant prefrontal cortex neurons is only found in the normal cohort and not autism. They also identified an excess (503 loci) and loss (208 loci) of H3K4me3 marks in autistic subjects. Most of these loci represent transcription start sites and overlap with previously annotated autism-risk loci. This reflects a shared genetic and epigenetic risk architecture in autism. To clearly identify and assess the acclaimed role of H3K4me3 abnormalities in autism pathophysiology, additional research with larger cohorts is required.

Sun and colleagues [80] conducted a genome-wide histone acetylation study on 257 postmortem samples (including three brain regions: prefrontal cortex, temporal cortex, and cerebellum) from syndromic and idiopathic ASCs and age-matched controls by using chromatin immunoprecipitation sequencing (ChIP-seq). Genome-wide acetylome profile from this study revealed epigenetic variation in genes involved in synaptic transmission, chemokinesis, ion transport, epilepsy, behavioral abnormality, and immunity.

13.2.2.2 Chromatin modifying and remodeling complexes

Methods such as large-scale exome and whole-genome sequencing have identified the role of chromatin modifiers in causing autism. Modification of histones can occur via two main mechanisms. The first is to directly alter the overall structure of chromatin and the second is to bind to effector molecules such as chromatin-modifying enzymes, or chromatin-remodeling complexes. These complexes affect transcription initiation and/or elongation. Many of the genes identified through whole exome sequencing in autism belong to the family of such complexes. For example, the chromodomain-coding genes *CHD2*, *CHD7*, and *CHD8* are all more frequently mutated in autistic individuals than in nonautistic individuals [29]. All three genes encode ATP-dependent helicases that are involved in chromatin remodeling. These proteins alter the placement of the nucleosome, thereby making the DNA more accessible to transcription factors. One of the most frequently mutated genes in autism is *CHD8* (chromodomain helicase DNA−binding 8). Mutations in *CHD8* are thought to contribute to risk in as much as 0.5% of autistic individuals [81]. Autistic individuals with *CHD8* mutations often have distinct phenotypes including macrocephaly and gastrointestinal difficulties [81]. CHD8 is thought to repress Wnt/β-catenin target genes, and CHD8 targets are significantly enriched for autism-risk genes [82].

The CHD family of chromatin remodelers are not the only genes implicated in autism. *ARID1B* is another gene that is frequently mutated in autism and is a component of the ATP-dependent human SWI/SNF (or BAF) chromatin-remodeling complex [83,84]. Two other genes, *BCL11A* and *ADNPç* identified to be frequently mutated in autism, encode proteins that directly interact with members of the SWI/SNF complex [85]. Mutations in *ADNP* contribute to consistent features in autistic individuals, including attention deficit/hyperactivity disorder and facial dysmorphism such as a prominent forehead and broad nasal bridge [85]. Individuals with intellectual disability also frequently have mutations in other genes in the SWI/SNF complex, highlighting the central role of this complex in typical neural and cognitive development [86].

Chromatin modifiers have also been identified in the list of 78 genes that are enriched for protein-truncating mutations. ASH1L is a histone-lysine N-methyltransferase and contains an SET domain that methylates histones, thereby altering the accessibility of the chromatin-bound DNA. Other examples include the (WW domain-containing adaptor with coiled-coil) also known as WAC; it contains a WW domain protein module found in a wide range of signaling proteins. WAC acts as a linker between histone H2B monoubiquitylation and RNA transcriptional machinery and regulates transcription. Another gene prioritized in a study is *KMT2C*, which is also a methyltransferase that methylates lysine 4 of histone H3 [84].

Statistical evaluation of genes identified using exome sequencing supports the role of chromatin modification in autism. Modeling based on gene coexpression patterns in the human midfetal cortex identified a significant enrichment for genes involved in transcriptional and chromatin regulation [84]. However, this is not unique to autism, or to rare genetic variants. For instance, one study integrating genetic GWAS data for three adult psychiatric conditions (schizophrenia, depression, and bipolar disorder) also identified an enrichment for histone methylation pathways [87]. Another study investigating case-control and trio-based exome sequencing data in schizophrenia identified an enrichment in loss-of-function mutations in *SETD1A* (*KMT2F*), which encodes for a H3K4 histone-lysine N-methyltransferase [88]. In the recent GWAS of autism, there was a nominal enrichment for histone H3 lysine 4 monomethylation marks (H3K4me1), which are histone marks on nucleosomes near enhancers [19,21]. Interestingly, *KMT2C* (*MLL3*), a gene prioritized from exome sequencing and from the recent GWAS of autism, is responsible for the deposition of the H3K4me1 marks, suggesting that rare and common variants may converge on similar pathways. In addition, *KMT2E* (*MLL5*), another SET domain-containing protein but catalytically inactive, was also identified in these GWAS studies.

13.2.3 Risk factors affecting the epigenetics of autism

The GxE (gene × environment) concept represents a combination of genetic susceptibility (G) and environmental factors (E) in understanding complex conditions such as autism. A model has been proposed in which the environmental factors change the gene expression via DNA and histone modifications rather than changes in the DNA sequence [89]. Some environmental factors are known to affect gene expression through epigenetic mechanisms. These changes sometimes persist and can even be transferred to the next cellular generation or furthermore to the next organism generation. This is especially the case during critical periods of embryo formation and development and the fetus is at risk for epigenetic and genomic imprinting disorders such as autism.

Although exposure to environmental factors can be harmful, they cannot cause autism on their own, but they are rather considered as risk factors that in combination with genetic susceptibility increase the risk of developing autism. Many factors have been studied and are divided into prenatal, natal, and postnatal risk factors.

Evidence from epidemiologic and genetic studies showed that increase in parental age (both the father and mother) increases the risk of the offspring developing autism [90]. Studies have yielded conflicting results in determining whether this risk is caused by maternal age, paternal age, or both. Parner and colleagues [91] found that both maternal and paternal ages were associated with a greater risk of ASD in the offspring. The mechanism through which maternal or paternal age impact this risk is different. Whole-genome sequencing studies showed that older fathers have a higher rates of *de novo* mutations. Shelton et al. [92] showed that advancing maternal age increases the risk for autism in the

offspring regardless of the paternal age, while increased risk associated with advanced paternal age is observed among younger mothers. This suggests that maternal age plays a more major role than paternal age as a risk factor for later causing autism [92]. Although maternal age is of major importance, younger age of mothers is also critical, as studies reported that mothers younger than 20 years pose a greater risk of growth retardation of fetus and preterm birth, which are both associated with increased risk of autism [93,94].

Maternal well-being is of crucial importance during pregnancy, both physical and mental health. Bleeding and metabolic syndromes such as diabetes, hypertension, and obesity significantly increase the risk of having a child with autism. This can be explained by the resulting hypoxia in utero that alters normal fetal brain development [95]. Viral infections also pose a risk on the fetus due to the abnormal maternal immune activation and elevated cytokines that interfere with brain development epigenetically [93].

Maternal medication use during pregnancy is associated with 46% increased risk for the fetus to develop autism. Placental crossing of different kinds of drugs increases risks for the fetus to develop autism later. For example, antiepileptic drugs, valproic acid, and acetaminophen cause oxidative stress and affect gene expression resulting in abnormal brain development and delays, deficient motor activities, and deficient social behaviors. Other studies showed that analgesic/antipyretic drugs induce apoptosis and necrosis, which are observed in autistic brains [96]. Other drugs have also been reported (see Karimi et al. [93] for review).

Similar to maternal physical well-being, mental health impact is of major importance especially during pregnancy. Mothers who experience depression, anxiety, and mental stress during pregnancy can have indirect effects on genes involved in neurobiological metabolism and in the physiology of the developing fetus through various epigenetic mechanisms. Longer periods of stress and aggression in pregnant mothers exposes the fetus to elevated steroid hormone levels, which have epigenetic effects on fetal brain development [93].

13.2.3.1 Hormones

Steroid hormones play an important role in epigenetic fetal programming on early brain development [97]. In 2015, Baron-Cohen and colleagues [98] showed that levels of amniotic fluid steroid hormones (delta-4 pathway) and cortisol are elevated in those who were later diagnosed with an ASD. These findings were important to elucidate the importance of prenatal environment in the early development of autism. Sex differences are known to exert influences on brain function and neural development. After the bipotential gonad is differentiated by chromosomal sex, steroid hormones are produced and through them the brain acquires its sexual phenotype [99]. Chromosomal sex directs the differentiation of the bipotential gonad, and it is the steroid hormone production by the gonad that then determines the phenotype of the brain in regard to relative masculinization or feminization [100]. Steroid hormones, also known as gonadal hormones, are androgens, estrogens, and progestagens. They are produced in the gonads, the cortex of the suprarenal gland, the placental tissue, and some peripheral tissues. Their secretion is mediated via the hypothalamic-pituitary-gonadal axis. The hypothalamic neurons synthesize and release the gonadotropin-releasing hormone. This stimulates the anterior pituitary to secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Together, the LH and FSH regulate the level of sex hormones derived from cholesterol found in the male testes or the female ovaries. Cholesterol is converted to the sex steroid hormones via a cascade of enzymatic events, mediated by P450 enzymes [101].

During the perinatal window, the human brain is sensitive to the organization effects of sex steroid hormones. In the male brain, testosterone levels have three distinct surges, the first one is mid-gestational and two postnatal peaks [102]. Hence, the exposure of neural tissue to testosterone and/or estradiol (aromatized form of testosterone) during gestation is necessary for both defeminization and masculinization of the developing male brain [100,102,103]. Estradiol-mediated masculinization of the brain induced male sexual behavior in the adult, while defeminization is the process by which the female specific traits are suppressed. This restricts the release of LH and FSH from the anterior pituitary and thus reduces female sexual behavior in the adult [104].

The preoptic area (POA) is perhaps the most reliable sexually dimorphic brain area. It contains the medial preoptic nucleus, which is critical for control of male sexual behavior. It also includes the anteroventral periventricular nucleus (AVPV), which is important for gonadotropin secretion and LH surge in females during ovulation. In the neonatal rodent brain, estradiol protects the sexually dimorphic nucleus of the POA from apoptosis, while it induces apoptosis in the AVPV to defeminize the brain. In addition to those opposing actions to modulate cell death and survival, estradiol also mediates sexually dimorphic synaptogenesis in the MPN. It triggers a twofold increase in putative excitatory synapses and higher density of dendritic spine synapses when compared with females [103,104]. To determine the role of methylation in the estradiol-mediated masculinization of the POA, Nugent and colleagues [105] measured the Dnmt enzymatic activity and expression in male, female, and estradiol-treated female rat pups. They then inhibited Dnmt activity and performed whole-genome bisulfite sequencing. They reported that the Dnmt inhibition mimics steroids and results in masculinized neuronal markers and male sexual behavior. Also, females showed higher levels of methylation than males. The permanent perinatal organizational effects of steroids on the developing brain is long standing, but what this study aimed to investigate is the involvement of DNA methylation in this process and their results show that the feminization of the brain is maintained through methylation and repression of masculinization [105]. This sheds light on how anomalies of prenatal steroidal exposure might be an important risk factor for brain development in autism.

13.3 Discussion

In this chapter, we have discussed the epigenetic mechanisms, such as DNA methylation and histone modifications, implicated in causing ASCs. However, it is worth mentioning that epigenetic variation can be causal or can arise as a consequence of a neurodevelopmental condition. Examples of epigenetic alterations resulting from a disease state could include alterations of expression in immune cells in autoimmune disorders, somatic mutation—induced epigenetic changes in cancer, etc. [60]. Nevertheless, it is not straightforward to disentangle this from epigenetic mechanisms that could play a causal role in a disease state or mental health condition. Therefore it is crucial to consider whether the changes arise before the symptoms of the condition become apparent. Three key factors in elucidating this could be based on whether the variations are inherited and present in all tissues, emerge stochastically during development in utero and are restricted to specific tissues, or arise postnatally because of environmental or lifestyle-related risk factors [60]. Tissue-specific epigenetic variations pose a challenge to compare and replicate findings on ASC-specific biomarkers from independent studies because of the heterogeneity of biological samples (e.g., postmortem brains, blood, saliva).

Another limitation in all these studies is the low number of cases and controls, which is in most cases less than 100. Another concern is the age of the participants, as ASC-discordant pairs are likely to grow more epigenetically discordant over time due to stochastic or environmental risk factors or progression of the condition and may obscure the causal pathway. The differences in the method of autism diagnosis may also act as a potential confounding factor mainly because DMPs/DMRs are set specific for each diagnostic tool. Diversity in the methods of analyses may also introduce difficulties in comparison across multiple studies. For example, in the majority of the EWAS reports discussed previously, some studies focus on probe-based methods (HM27, HM450), whereas other studies use region-based techniques (MIRA, MeDIP). An ideal way for future studies would be to overcome the analysis biases and confounds by using a minimal effect-size cutoff and multiple comparison adjustment. Besides, the use of independent methods in the same study cohort and replication in independent population may resolve the problem of dealing with confounds. Another effective strategy could be standardization of differentially epigenetically regulated probes and regions with sufficient sample size.

The role of environmental and lifestyle-related risk factors on epigenetic programming, especially during fetal development in ASCs, are some of the areas that have gained scientific attention, and in the future, studies may lead toward integrating information from several independent platforms ranging from genomic, epigenomic, transcriptomic, and proteomic levels to thoroughly elucidating the role of epigenetic biomarkers and gene−environment interactions in ASC diagnostics and therapeutics.

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