Epigenetics of Autism Spectrum Disorder

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Michelle T. Siu and Rosanna Weksberg

Abstract

Autism spectrum disorder (ASD), one of the most common childhood neurodevelopmental disorders (NDDs), is diagnosed in 1 of every 68 children. ASD is incredibly heterogeneous both clinically and aetiologically. The etiopathogenesis of ASD is known to be complex, including genetic, environmental and epigenetic factors. Normal epigenetic marks modifiable by both genetics and environmental exposures can result in epigenetic alterations that disrupt the regulation of gene expression, negatively impacting biological pathways important for brain development. In this chapter we aim to summarize some of the important literature that supports a role for epigenetics in the underlying molecular mechanism of ASD. We provide evidence from work in genetics, from environmental exposures and finally from more recent studies aimed at directly determining ASD-specific epigenetic patterns, focusing mainly on DNA methylation (DNAm). Finally, we briefly discuss some of the implications of current research on potential epigenetic targets for therapeutics and novel avenues for future work.

M.T. Siu, Ph.D.
Program in Genetics and Genome Biology, The Hospital for Sick Children,
555 University Ave, Toronto, ON M5G 1X8, Canada
e-mail: michelle.siu@sickkids.ca

R. Weksberg, M.D., Ph.D. (✉)
Program in Genetics and Genome Biology, The Hospital for Sick Children,
555 University Ave, Toronto, ON M5G 1X8, Canada

Division of Clinical and Metabolic Genetics, The Hospital for Sick Children,
555 University Ave, Toronto, ON M5G 1X8, Canada

Department of Paediatrics, University of Toronto, Toronto, ON M5S 1A1, Canada

Institute of Medical Science, University of Toronto, Toronto, ON M5S 1A8, Canada
e-mail: rweksb@sickkids.ca

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Keywords
Autism spectrum disorder • Heterogeneity • Aetiology • Molecular mechanisms • Genetics • Epigenetics • DNAmethylation

Abbreviations

5-hmC	5-Hydroxymethylcytosine
5-mC	5-Methylcytosine
Δβ	Difference in DNA methylation
ADHD	Attention deficit and hyperactivity disorder
ARID1B	AT-rich interaction domain 1B
ART	Assisted reproductive technologies
AS	Angelman syndrome
ASD	Autism spectrum disorder
ATP	Adenosine triphosphate
BA10	Brodmann area 10
BA19	Brodmann area 19
BA24	Brodmann area 24
BBB	Blood–brain barrier
BCL-2	BCL2, apoptosis regulator
BPA	Bisphenol A
BWS	Beckwith-Wiedemann syndrome
C11orf21	Chromosome 11 open reading frame 21
C1Q	Complement subcomponent C1q
CBL	Cerebellum
CDKL5	Cyclin-dependent kinase-like 5
CHARGE	Coloboma of the eye, heart defects, atresia of the nasal choanae, retardation of growth and/or development, genital and/or urinary abnormalities and ear abnormalities/deafness
CHD7	Chromodomain helicase DNA-binding protein 7
CHD8	Chromodomain helicase DNA-binding protein 8
CNV	Copy number variant
DGCR6	DiGeorge critical region 6
DGCR8	DiGeorge critical region 8
DLGAP1	DLG associated protein 1
DLGAP2	DLG associated protein 2
DNAm	DNA methylation
DNMT	DNA methyl transferases
FDR	False discovery rate
FMR1	Fragile X mental retardation 1
GAD65	Glutamic acid decarboxylase 65
GDM	Gestational diabetes mellitus
GOM	Gain of methylation

GRIN2B	Glutamate ionotropic receptor NMDA type subunit 2B
GxE	Gene by environment interactions
H3K27ac	Histone 3 lysine 27 acetylation
H3K4me3	Histone 3 lysine 4 trimethylation
HDAC	Histone deacetylase
HUWE1	HECT, UBA and WWE domain-containing 1, E3 ubiquitin protein
IC	Imprinting centre
ID	Intellectual disability
KDM6A	Lysine demethylase 6A
KMT2D	Lysine methyltransferase 2D
lncRNA	Long noncoding RNA
LOF	Loss of function
LOM	Loss of methylation
MBDs	Methyl-CpG-binding proteins
MECP2	Methyl-CpG-binding protein 2
miRNA	Micro-RNA
MZ	Monozygotic
NDD	Neurodevelopmental disorder
NRXN1	Neurexin 1
NSD1	Nuclear receptor SET (su(var)3–9, enhancer-of-zeste, trithorax) domain-containing protein-1 gene
OCM	One carbon metabolism
OR	Odds ratio
OXTR	Oxytocin receptor
PDD-NOS	Pervasive developmental disorder – not otherwise specified
PFC	Prefrontal cortex
PRRT1	Proline-rich transmembrane protein 1
PTEN	Phosphatase and tensin homolog
RELN	Reelin
RORA	Retinoic acid-related orphan receptor
RTT	Rett syndrome
SAM	*S*-Adenosyl methionine
SHANK3	SH3 and multiple ankyrin repeat domains 3
SNP	Single-nucleotide polymorphism
SNRPN	Small nuclear ribonucleoprotein polypeptide N
TC	Temporal cortex
TNF-α	Tumour necrosis factor alpha
TSPAN32	Tetraspanin 32
UBE3A	Ubiquitin protein ligase
UBE3A	Ubiquitin protein ligase E3A
UPD	Uniparental disomy
VPA	Valproic acid
VUS	Variant of unknown significance
WGS	Whole genome sequencing
ZFP57	ZFP57 zinc finger protein

4.1 Autism Spectrum Disorder (ASD) and Proposed Aetiologies

Autism spectrum disorder (ASD), one of the most common neurodevelopmental disorders (NDDs), is diagnosed in 1 of 68 children in the United States [1] with a 4:1 male-to-female sex ratio. ASD is comprised of a group of complex NDDs characterized by impaired social communication and repetitive behaviours (DSM-5). ASD also presents with a range of other features including morphological (e.g. macrocephaly), physiological (e.g. gastrointestinal, sleep problems) and psychiatric comorbidities (e.g. anxiety) [2]. Variable neuropathological features consistently described in some, but not all, cases of ASD include decreased size and number of Purkinje cells, abnormal neuronal migration, neurite outgrowth and branching and axonal guidance [3, 4]. These additional features may or may not be observed as part of the profile of syndromic cases (Sect. 4.4.1), where ASD is associated with either single-gene mutations or defined chromosomal/cytogenetic abnormalities. In May 2013, the clinical criteria for the diagnosis of ASD were redefined by the DSM-5 diagnostic manual. Several subtypes of ASD previously considered as distinct disorders (autistic disorder, childhood disintegrative disorder, pervasive developmental disorder—not otherwise specified (PDD-NOS) and Asperger syndrome) were then merged under a single umbrella diagnosis, ASD. The clinical criteria for the diagnosis of ASD are still evolving due in part to phenotypic variability and to clinical and aetiologic overlap with other NDDs (e.g. ~30% of ASD cases are comorbid with attention deficit and hyperactivity disorder [ADHD] symptoms) [5, 6]. Therefore, different NDDs are best represented not as distinct categories but as entities along a continuum with some convergence in the underlying genes and pathways. One of the greatest challenges in improving diagnosis and treatment for ASD is the degree of heterogeneity both clinically and aetiologically. It is therefore unsurprising that there are multiple proposed aetiologies and risk factors (Fig. 4.1) identified.

Commonly proposed physiological and metabolic causes of ASD consist of immune, oxidative stress and mitochondrial dysfunction. In some ASD cases, a pro-inflammatory state [8–12] is suggested by alterations in immune and inflammatory markers (e.g. cells of the innate and adaptive [cytokines, interleukins] immune system and abnormalities in microglia [immune cells of the brain]). Furthermore, it has been shown that immune changes in both peripheral tissues and brain can result in anxiety and impaired social behaviour [13–16]. Studies in humans and mouse models suggest that dysbiosis of the intestinal microbiome is a novel physiological contributor to ASD risk, impacting immune function and subsequently learning, memory and behaviour [17–20]. Further, there is emerging evidence, mostly in animal models, that this gut-brain axis is, in part, regulated by epigenetic mechanisms [21–23]. Enhanced oxidative stress, impaired antioxidative capacity and mitochondrial dysfunction have also been extensively reviewed [12, 24]. A number of independent studies support claims that the degree of immune disruption (e.g. cytokines) and mitochondrial (e.g. phosphocreatine) dysfunction are positively correlated with ASD symptom severity [12, 24, 25]. However, it is not known whether these abnormalities existed prior to ASD diagnosis or if they play a causal role.

Investigations of underlying molecular mechanisms in ASD aetiology include altered genetic and epigenetic regulation. Epigenetics has emerged as a vital

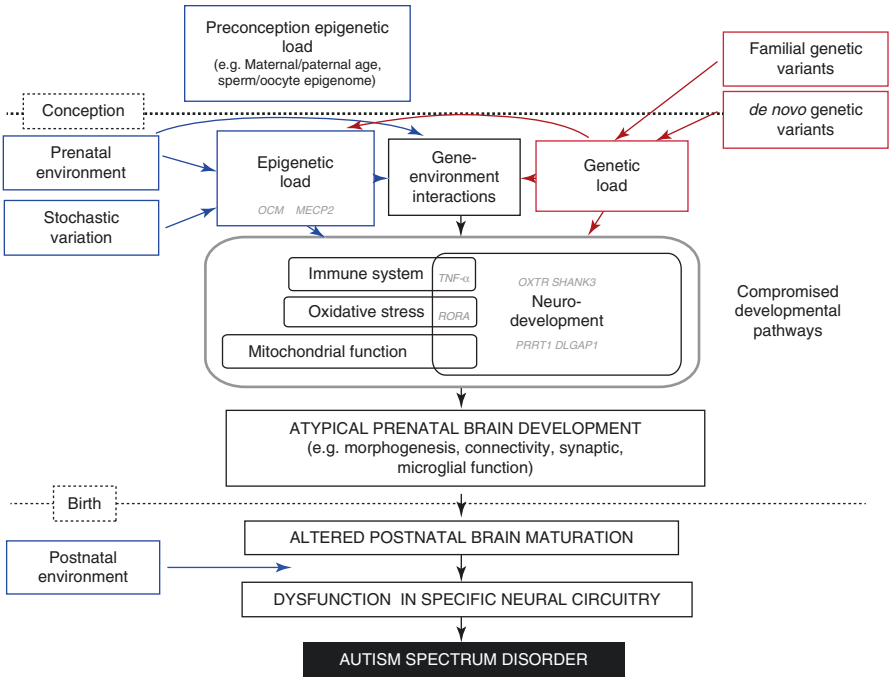

Fig. 4.1 Diagrammatic overview of how genetic, environmental and epigenetic factors interact in the aetiology of ASD. Epigenetic load (from preconception, prenatal environment and stochastic variation) and genetic load (from familial and *de novo* variation) interact to alter neurodevelopmental, immune, oxidative stress and mitochondrial pathways identified through studies of ASD genetics, physiology, expression and/or DNA methylation (targeted and genome-wide). Highlighted in *grey* is the putative involvement of specific genes or pathways mentioned in this review. Over a certain threshold of genetic and epigenetic dysfunction, development is compromised and can lead to adverse neurodevelopmental outcomes such as ASD. Further, postnatal environments may also contribute to severity of symptoms. Abbreviations: *DLGAP1* DLG-associated protein 1, *OCM* one carbon metabolism, *OXTR* oxytocin receptor, *MECP2* methyl-CpG-binding protein 2, *PRRT1* proline-rich transmembrane protein 1, *RORA* retinoic acid receptor-related orphan receptor alpha, *SHANK3* SH3 and multiple ankyrin repeat domains 3, *TNF-α* tumour necrosis factor alpha. Adapted from Fig. 1 in [7]

genome-wide regulatory layer that modulates the transcriptome, impacting transcription initiation, splicing processes and binding of transcription factors. Epigenetic regulation helps to determine the proper spatiotemporal expression of genes via a number of mechanisms including DNA methylation (DNAm), histone modifications and ATP-dependent chromatin remodelling. Epigenetics provides new avenues to investigate and refine risk estimates for NDDs beyond genetic risks alone. The failure to establish proper epigenetic marks may result in aberrant gene expression and, subsequently, various disease phenotypes. Genomic aberrations (e.g. mutations, insertions/deletions, copy number variants [CNVs]) of genes involved in epigenetic regulation ('epigenes') or dysregulation of epigenetically regulated genomic regions (e.g. imprinted genes/regions) can lead to epigenetic disruptions and, ultimately, NDDs. There are >600 confirmed and putative human

epigenes [26], many of which are associated with NDDs such as intellectual disability (ID) and ASD (e.g. chromodomain helicase DNA-binding protein 8 [*CHD8*], DNA methyltransferase 3A [*DNMT3A*], HECT, UBA and WWE domain-containing 1, E3 ubiquitin protein [*HUWE1*]) [27, 28]. There is a growing body of evidence to show that there is substantial genetic overlap between risks for neuropsychiatric disorders and NDDs [29–32]. Many of these genes encode proteins involved in neuronal and synaptic pathways, while others are relevant to molecular pathways involved in epigenetic regulation [28, 32, 33] (Sects. 4.2, 4.3 and 4.4).

What is clear is that there is no single underlying cause of ASD. In order to understand the multifactorial aetiologies of ASD, we must better understand the natural history of molecular events and their regulation during critical periods of human development. ASD more likely arises as an interaction between genetic and environmental risk factors (GxE) mediated by epigenetic mechanisms. Ultimately, a better understanding of how genetics, epigenetics and environment collectively interact and contribute to ASD risk will allow us to better classify and diagnose the disorder and facilitate the application of precision-based medicine.

4.2 Genetics of ASD

The aetiology of ASD is known to have a strong genetic component. Early twin studies of heritability reported estimates of up to 90% heritability [34, 35]. In contrast, current estimates are closer to 10–30% according to data from more recent twin and family studies [36–38]. Next generation sequencing has significantly accelerated our understanding of genetic variability in individuals with ASD compared to the general population. ASD genomic risk variants are comprised of rare, *de novo* variants of large effect, independently of or in combination with more common and/or inherited variants of small effect [39]. Interestingly, it has recently been shown, using whole genome sequencing (WGS) of 85 ASD quartet families (parents and two affected siblings), that although ASD-relevant mutations were found in 42% of individuals with ASD, only 31% of sibling pairs carried the same variants, emphasizing the genetic heterogeneity of the disorder even within families [40]. The considerable variability of ASD necessitates genetic testing of large cohorts of patients on whole genome technologies (whole exome sequencing and WGS) which are becoming more affordable.

Despite the advantages of WGS, sequence variant classification (e.g. variants of unknown significance [VUS]) still poses a significant challenge. Such VUS are being reported at a faster rate than we are able to characterize them with respect to disease relevance. These studies underscore the aforementioned heterogeneity of the disorder; >200 ASD-risk genes have been identified [41–46]; SFARI gene: https://gene.sfari.org]. However, genomic aberrations are detected in only 25–40% of cases [40, 47, 48]. Further, no single mutation or CNV accounts for >1% of ASD cases and is variably penetrant with respect to the ASD phenotype. In addition to risk variants, six risk loci (1q21.1, 3q29, 7q11.23, 16p11.2, 15q11.2-13, 22q11.2) and several genetic syndromes (Sect. 4.4.1) are well known to be associated with ASD [47, 49, 50]. Unsurprisingly, few strong genotype-phenotype relationships have yet to be

uncovered. Many genomic variants are recurrent, but rare (e.g. *CHD8* mutations; Sect. 4.4.1), and therefore more patients are required to better establish such relationships.

Several studies have shown that many ASD-risk genes are involved in converging pathways relevant to the biological bases of ASD. These include development and cell proliferation, neural development, synaptic function and, of particular interest to this chapter, chromatin modifiers and transcriptional regulators [48, 49, 51]. Importantly, many of these genes are expressed in the brain during embryonic development. These functional categories relate to the neurocognitive phenotype of ASD (and other NDDs) and can help us to understand the molecular mechanisms, such as epigenetics, that are perturbed in ASD. The study of ASD-associated genetic syndromes caused by mutations in epigenes will also aid in this endeavour (Sect. 4.4.1). It is becoming increasingly apparent that some ASD-risk genes and loci confer an increased risk for other neuropsychiatric and neurological disorders including ID, ADHD, schizophrenia, epilepsy, motor impairment and sleep disturbance [30, 47, 52].

The genomic architecture of ASD is further elucidated through examinations of large numbers of families and individuals with ASD [40, 43–46, 49]. Better genotype-phenotype relationships are being defined; for example, the presence of *de novo* loss-of-function (LOF) mutations or CNVs is associated with lower IQ [43, 49, 53, 54]. Higher mutational burden has also been correlated with certain ASD features such as seizures and head circumference, observed in subsets of individuals [49, 55]. Genomic studies of ASD have identified genomic features relevant to ASD aetiology [49]. For example, *de novo* variants are distributed in a non-random fashion, enriched in epigenetically relevant regions (e.g. simple repeats and DNase I hypersensitivity sites, marks of open chromatin) [56].

Epigenetic mechanisms can help fill the aetiologic knowledge gap where genetic information alone is insufficient to explain the aetiology of all ASD cases. Epigenetic outcomes, much like genetic outcomes, are also expected to be heterogeneous (Sect. 4.4.2). Combining genetic and epigenetic data is likely to provide a more comprehensive understanding of the molecular landscape of the aetiology of ASD. The discovery of consistent molecular (genotype, epigenotype) and biochemical associations with ASD or ASD subtype-specific phenotypes will allow clinicians to better classify individuals, facilitate earlier diagnosis, and improve prognosis. In parallel, gaining molecular insights into the disorder will also help us to identify more homogeneous subgroups of individuals, which will allow for better patient stratification for behavioural and pharmacological treatments.

4.3 Environmental Exposures and ASD Risk

Twin, adoption and sibling studies have defined the heterogeneous and complex etiopathogenic nature of ASD and have supported potential contributions of environmental factors to ASD risk. Results from such studies suggest that ASD aetiology can be attributed to ~50% genetic contribution and ~50% influenced by non-shared environmental factors [37, 57–60]. The epigenome acts as an interface between the genome and the environment, transforming the genome into a regulator of cell type and developmental time-specific transcription. The epigenome is programmed during

embryonic/foetal development by multiple genetic factors including genes that encode DNA methyl transferases (DNMTs), histone deacetylases (HDACs) and chromatin remodelling factors. Epigenetic errors can arise as primary stochastic events or in response to genetic mutations and/or environmental exposures. At critical times during development, typical foetal programming can be dysregulated by gene mutations, environmental exposures or epigenetic errors potentially leading to adverse long-term health outcomes [61–63]. There have been extensive investigations during the critical period of maternal gestation to examine the effects of exposures to both exogenous and endogenous environmental factors, which will be summarized below.

4.3.1 Exogenous Environment

Smoking, alcohol, medications (e.g. valproic acid [VPA], selective serotonin reuptake inhibitors) and environmental chemicals (e.g. pesticides, metals, bisphenol A [BPA]) are the most commonly studied exogenous exposures in relation to adverse foetal neurodevelopmental outcomes. Gestational smoking and alcohol exposure studies are inconsistent with respect to ASD risk [64–67], likely due to differences in study cohorts and methodologies, but also because it is extremely difficult to accurately estimate levels and timing of exposure in the mothers and more critically in the foetus. These challenges are compounded with the fact that the mechanisms of action and effects of maternal vs. foetal metabolism are not well understood enough to directly infer causation. In general, the impact of complex GxE interactions requires further investigation. Integrating genetic, environmental and epigenetic datasets will enable us to better understand the synergistic effects of these interactions. Animal models are critical for such studies, allowing for more precise and quantitative manipulations of environmental exposures. Further, we need to be able to distinguish between the direct effects of the exposure itself (e.g. direct perturbation of neurodevelopmentally important genes by gestational exposure to maternal smoking) and the downstream effects that may result (e.g. the fact that maternal smoking has been linked to decreased birth weight and reduced in utero brain growth). Interestingly, several studies demonstrate that environmental exposures affect epigenetic marks. Altered DNAm or DNMT expression/activity has been reported in various tissues of both human and animal models following exposure to a variety of toxicants including alcohol, cigarette smoking, BPA and VPA [68–76]. Some of these DNAm alterations have been further associated with ASD-relevant endpoints (e.g. behavioural outcomes, neurite outgrowth, axon formation and in ASD-relevant brain regions) (Table 2 in [65]).

4.3.2 Endogenous Environment

Preconception environmental risks include maternal [37, 77] and paternal [77, 78] age, which have been positively associated with an increase in ASD risk (relative

risk [RR] of 1.16 to >1.5), both independently and with a joint effect. Interestingly, genetics may partially explain this finding; a greater number of *de novo* mutations in ASD probands have been found as a function of paternal age [43, 46]. Age-related epigenetic changes such as altered DNAm observed in both sperm and oocytes [79–81] may also contribute to this association. The mechanisms by which these factors introduce enhanced ASD risk still need to be further explored.

Epidemiologic studies show that preterm birth, due to various causes, significantly increases (3–14-fold) the risk of developing ASD [82–84]. A recent study [85] tested for DNAm differences between preterm and term foetal placental tissues at preselected ASD candidate genes. These include *OXTR*, *SHANK3*, BCL2, apoptosis regulator (*BCL-2*) and *RORA* that are known to have altered DNAm in some ASD cases. A significant gain of methylation (GOM) was found only in *OXTR*. More studies are needed to understand whether this DNAm mark at *OXTR* has a functional impact on ASD risk.

There have been inconsistent reports regarding the risk of ASD following the use of assisted reproductive technologies (ART; e.g. *in vitro* fertilization, intracytoplasmic sperm injection) [86–88]. ART currently account for ~1.6% of live births in the United States and rates of use are increasing [89, 90]. Although results are controversial, epidemiological studies have shown a possible increase in the incidence of ASD in offspring conceived with ART [86, 87]. There are several reasons why ART should be carefully considered. First, there are inherent risks of ART including preterm labour, multiple births and low birth weight that, independently of ART, already confer an increased risk for ASD [91–93]. Second, ART are used during critical windows of gametogenesis and early embryogenesis, when epigenetic reprogramming is occurring [92, 94]. Previous studies in the aetiologically heterogeneous paediatric imprinting disorders (Beckwith-Wiedemann [BWS] and Angelman syndromes [AS]) demonstrate that ART have a significant impact on epigenetic outcomes. These disorders are caused by loss of methylation (LOM) at critical imprinted sites on chromosomes 11p15 and 15q11 in individuals with BWS or AS, respectively. Most molecular alterations identified in these subjects following ART arose from epigenetic rather than genetic alterations; LOM is increased in frequency following the use of ART [95–100]. Currently, it is unclear if a parallel effect of ART occurs in the context of the development of ASD.

The endogenous maternal gestational environment has been studied extensively with respect to ASD risk [101–103]. Modest increases in ASD risk have been found to be associated with certain perinatal complications. Two of the main hypotheses relate back to two proposed underlying aetiologies of ASD (Sect. 4.2)—oxidative stress and immune function. Epidemiological data have shown that hypoxia-related obstetric complications pose a significant increase in ASD risk (effect estimate >1.4) [91, 101]. The maternal immune system is also central to several associations, although they are yet to be well established or replicated. Infection during pregnancy [82, 104–106] (OR: 1.24–1.37) and autoimmune disorders [107] (OR: 1.34) are linked to an increased risk for ASD. Recent studies report the maternal production of antibodies against circulating foetal

brain proteins, detected in ~20% of mothers of children with ASD, but only in 1% of mothers of neurotypical children [108, 109]. Replication and further studies are required to solidify the potential role of these antibodies as biomarkers and their functional effect on foetal outcome. Maternal metabolic factors such as obesity and gestational diabetes mellitus (GDM) have both been associated with an increased risk for ASD [110–113]. There are convincing data demonstrating a role for epigenetic regulation of these metabolic processes. One study of women of South Asian origin, who have a high risk of GDM, showed statistically significant DNAm differences in cord blood and placental tissue, identifying differentially methylated genes involved in embryonic development and intracellular metabolic processes [114]. Other studies have reported DNAm changes in general immune, metabolic and endocrine pathways in placenta and cord blood of infants exposed to GDM [115–117]. There are no existing data available yet to validate these individual studies in cohorts of ASD patients exposed to GDM or maternal obesity.

Prenatal maternal stress has been identified as a small but robust risk factor for ADHD and ASD [118]. Maternal stress has been correlated with offspring autistic traits [119]. General and social communication scores were associated with altered DNAm of *OXTR*, a recurring ASD-risk gene of interest (Sect. 4.4.2). Further, specific single-nucleotide polymorphism (SNP) genotypes of *OXTR* were found to be predictive of methylation outcomes. However, this study was unable to find a GxE interaction, where associations between maternal stress and autistic traits were not related to *OXTR* methylation or genotype. Maternal prenatal nutrition, specifically folate supplementation, has been shown to have a protective effect, showing a reduced risk of ASD (OR: 0.61) [120–122]. Folate is well known for its role in preventing neural tube defects. Its protective effect for ASD may derive from the role of folate in OCM. OCM recycles homocysteine to generate cysteine and methionine for the process of methylation and antioxidative capacity through the formation of *S*-adenosyl methionine (SAM), supporting epigenetic processes important for typical neurodevelopment.

In summary, it will be important to more thoroughly explore whether the associations between environment and ASD outcome may be reflected in stable epigenetic marks detectable in the foetus or neonate. This would require large sample sizes to achieve sufficient power and well-annotated exposure data. Ultimately, the goal is to discover replicable GxE effects associated with ASD, perhaps in parallel with prenatal genetic testing, that are not only statistically but also biologically significant.

4.4 The Direct Role of Epigenetics in ASD

4.4.1 Genetic Syndromes Involving Epigenes and ASD

Syndromic ASD accounts for ~10–15% of cases [123–125]. A significant number of such genetic syndromes involve mutations in epigenes and are associated with increased risk for ASD (Table 4.1). Some of these epigenes function as

Table 4.1 Genetic syndromes with known/putative epigenetic aetiology comorbid with ASD and/or ID

Syndrome	Aetiology	Epigenetic type	Epigenetic mechanism involved in disorder	Risk for ASD	Degree of ID
Rett	Mutations in *MECP2* and *CDKL5*	Reader	Improper reading and establishment of epigenetic marks by MECP2	>50% Rett females with ASD symptoms [141]. Mutations in *MECP2* found in ASD patients[127–129, 142, 143]	Severe to profound
Fragile X	CGG repeat expansion and subsequent DNA methylation of *FMR1* gene	Indirect; non-coding RNA	Reduced *FMR1* expression due to DNAm; *FMR1* involvement in RNA processing	60–67% in males, 23% in females[123, 144]	Severe to mild, majority in moderate range
22q11.2 deletion (DiGeorge)	1.5–3 Mb hemizygous deletion	Indirect, not well-defined; non-coding RNA	*DGCR8* found in 22q11.2del region involved in miRNA processing; *DGCR6*, also in 22q11.2del region, is imprinted	20–40% (based on DSM-IV criteria) [145, 146], <20% if using both clinical criteria and parental report [147]	Mild to moderate
Prader-Willi	Paternal deletions, maternal UPD at 15q11–13, deletions and mutations of IC, translocations disrupting *SNRPN*	Imprinted region	Lack of expression of paternally expressed genes from imprinted cluster at 15q11–13, due to GOM at paternal IC	19–36.5% [148–150]	Mild to moderate
Angelman	Maternal deletion, paternal UPD, deletions and epimutations at IC, mutations of *UBE3A*	Imprinted gene	Lack of expression of maternally expressed gene *UBE3A* in brain due to LOM at maternal IC	Not conclusive due to severe intellectual disability	Severe to profound
15q11–13 maternal duplication	Maternal duplications of 15q11–13 region	Unknown	Unknown	>85% [151]	Variable

(continued)

Table 4.1 (continued)

Syndrome	Aetiology	Epigenetic type	Epigenetic mechanism involved in disorder	Risk for ASD	Degree of ID
Sotos	Mutations in *NSD1*	Writer; specific histone modifications	*NSD1* encodes histone H3K36 methyltransferase, important for normal embryonic development	No clear estimate for risk, but >80% demonstrate some ASD clinical features [138]	Mild to severe
Kabuki	Mutations in *KMT2D*, *KDM6A*	Writer; specific histone modifications	*KMT2D* encodes histone H3K4 methyltransferase, *KDM6A* encodes a tri-/dimethylated histone H3 demethylase. Interaction with members of WAR complex (WDR5, RBBP5 and ASH2L), shown to be involved in histone methylation	Autism or autistic-like behaviour reported in several cases [152, 153], no risk estimate	Mild to severe
CHARGE	Mutations/ deletions in *CHD7*	Chromatin remodeller	Alters CHD7 binding of active chromatin, also interacts with members of WAR complex	15–50% [133–135]	Normal to severe
CHD8 mutations with ASD	Mutations/ deletions in *CHD8*	Chromatin remodeller	Alters CHD8 binding of active chromatin, regulates transcription through CTCF binding	>85% [131, 132]	Normal to profound
Turner syndrome	Monosomy for chromosome X	Potential imprinted gene(s)	Potential imprinted gene(s) on chromosome X	3% [154]	Usually no ID

Note: Epigenetic marks or mechanisms associated with each syndrome are described; there may be additional known/unknown mechanisms. Abbreviations: *ASH2L* ASH2 like histone lysine methyltransferase complex subunit, *CDKL5* cyclin-dependent kinase-like 5, *CHARGE* coloboma of the eye, heart defects, atresia of the nasal choanae, retardation of growth and/or development, genital and/or urinary abnormalities and ear abnormalities/deafness, *CHD7* chromodomain helicase DNA-binding protein 7, *CHD8* chromodomain helicase DNA-binding protein 8, *CTCF* CCCTC-binding factor, *DGCR6* DiGeorge critical region 6, *DGCR8* DiGeorge critical region 8, *FMR1* fragile X mental retardation 1, *GOM* gain of methylation, *IC* imprinting centre, *ID* intellectual disability, *KDM6A* lysine demethylase 6A, *KMT2D* lysine methyltransferase 2D, *LOM* loss of methylation, *MECP2* methyl-CpG-binding protein 2, *miRNA* micro-RNA, *NSD1* nuclear receptor SET (su(var)3–9, enhancer-of-zeste, trithorax) domain-containing protein-1, *RBBP5* RB binding protein 5, histone lysine methyltransferase complex subunit, *SNRPN* small nuclear ribonucleoprotein polypeptide N, *UBE3A* ubiquitin protein ligase E3A, *UPD* uniparental disomy, *WDR5* WD repeat domain 5

epigenetic writers (DNMTs, histone methyltransferases and acetyltransferases), erasers (HDACs, lysine demethylases), readers (proteins containing bromo-, chromo- or Tudor domains), chromatin remodelling factors (e.g. *CHD8*) and epigenetic regulators of imprinted regions (ZFP57 zinc finger protein). Others exert more indirect effects through OCM, noncoding RNA processing or recruitment of methyl-CpG-binding proteins (MBDs) to modify histones and regulate transcription. Genetic syndromes caused by mutations in epigenes are also highly comorbid with ID, sometimes making it difficult to estimate ASD risk. As data for WGS of larger numbers of well-phenotyped ASD cases become available, additional genetic syndromes involving epigenes may be identified.

Rett syndrome (RTT; OMIM 312750) has been described in detail in Chaps. 1 and 2 and therefore will not be discussed in detail here. ASD symptoms can appear in early infancy, but the clinical phenotype becomes more distinct as RTT features (e.g. loss of hand skills, deceleration of head growth) develop with age. Interestingly, a large proportion of patients (>70%) with milder RTT variants exhibit ASD-like features [126]. Rare *MECP2* mutations associated with ASD but not RTT have also been identified [127–130]. Typically, these mutations are found to be intronic and located in the 3′ untranslated region of the gene as opposed to LOF mutations which lead to RTT. The functional role that *MECP2* may play in ASD pathogenesis has yet to be identified.

Mutations (single-nucleotide variants and small indels) in the chromatin modifier gene *CHD8* have recently been described as a novel genetic syndrome with a strong association (>87%) with an ASD phenotype, amongst other common features such as macrocephaly (>80%), tall stature (86%) and gastrointestinal problems (80%) [131, 132]. Individuals with mutations in a related gene, chromodomain helicase DNA-binding protein 7 (*CHD7*; CHARGE syndrome, OMIM 214800), have a lesser but still significant risk (40%) for ASD [133–135]. The two genes have different interacting proteins and target binding sites [136], explaining at least in part the differences in phenotype.

Sotos syndrome (SS; OMIM 117550) is a congenital overgrowth disorder caused primarily (90%) by mutations in the nuclear receptor SET (su(var)3–9, enhancer-of-zeste, trithorax) domain-containing protein-1 gene (*NSD1*), a developmentally important histone methyltransferase. SS presents an example of a genetic syndrome with robust functionally relevant genome-wide epigenetic alterations [137]. Notably, reports show that >55% of SS patients display ASD symptomatology above clinical cutoffs [138–140]. It has recently been shown that individuals with SS have a specific blood DNAm signature that distinguishes individuals with pathogenic *NDS1* mutations from controls [137]. Further, this DNAm signature is able to classify *NSD1* VUS, which holds great potential for clinical application and molecular diagnostics. Examining a genetically homogeneous group of individuals as an approach for the study of ASD may eliminate some of the resultant epigenetic heterogeneity. The ability to refine and make more consistent molecular and/or phenotypic observations within subsets of individuals with ASD will help to establish causal roles for aetiologic factors.

4.4.2 Direct Assessment of Epigenetic Marks in ASD

Specific genomic alterations (e.g. mutations, CNVs) are known to confer increased risks for ASD, but these risks often are fairly broad ranging. Given the anticipated role of epigenetic dysregulation in ASD aetiology, multiple studies of different epigenetic marks in ASD cases have investigated stable epigenetic biomarkers either with or without an underlying genomic change. Stable biomarkers found in easily accessible, peripheral tissues such as blood would have a profound impact in the clinical diagnostic arena, especially if blood biomarkers were confirmed to reflect biomarkers in the brain. Examining cross-tissue markers, specifically brain vs. peripheral tissues, would help to further elucidate the underlying biological pathways involved in the aetiology of ASD. This section will focus mainly on assessments of the most stable and commonly studied epigenetic mark, DNAm (Table 4.2). We will also review data for epigenetic marks that are less frequently examined that will complement DNAm data in the future.

Table 4.2 Genome-wide DNA methylation studies in ASD

Reference	Sample population (*n*)	Tissue	Method	Findings
[155]	MZ twins discordant for ASD (3, male-male), unaffected siblings (2).	Lymphoblastoid cell lines	8.1 K CpG island microarray	**GOM** in *BCL-2* and *RORA*
[156]	MZ twins discordant and concordant for ASD/ASD severity (50 twin pairs)	Whole blood	Illumina 27 K	No global differences. Multiple DMVs (**GOM and LOM**) identified b/w discordant twin pairs, including *MBD4*
[157]	ASD cases (47), controls (48) born to mothers >35y.o.	Buccal epithelium	Illumina 450 K	Only 1 DMR passing FDR correction (*OR2L13*), **LOM** at promoter
[158]	Biological fathers of existing ASD child, collected in 1st or 2nd trimester of second pregnancy (44)	Sperm	CHARM 3.0 array, Illumina 450 K	**193** DMRs; overlapped sperm CHARM data with 450 K (75/193 probes) and post-mortem brain (18/75) data from [160]
[159]	ASD cases (9), unrelated controls (9)	Post-mortem brain (BA19)	Illumina 27 K	**No** significant DMVs; downregulation of expression of genes of mitochondrial phosphorylation, protein translation

Table 4.2 (continued)

Reference	Sample population (*n*)	Tissue	Method	Findings
[160]	ASD cases (19), unrelated controls (21)	Post-mortem brain (TC, PFC, CBL)	Illumina 450 K	4 significant DMRs (TC, CBL), Δβ range from 6.6% **LOM** to 15.8% **LOM**. 3/4 DMRs validated in multiple brain regions of independent ASD cases
[161]	ASD cases (23), unrelated controls (23)	Post-mortem brain (BA10, BA24)	Illumina 450 K	>5000 DMVs in BA10, >10,000 DMVs in BA24 ($q < 0.05$, $\Delta\beta > 5\%$): **LOM** in BA10 at sites related to immune function (e.g. *C1Q*, *TNF-α*), **GOM** at sites related to synaptic membrane (e.g. *DLGAP1*, *DLGAP2*)

Abbreviations: *Δβ* difference in DNAm, *BA10* Brodmann area 10, *BA19* Brodmann area 19, *BA24* Brodmann area 24, *BCL-2* BCL2, apoptosis regulator, *C1Q* complement C1q A chain, *CBL* cerebellum, *DLGAP1* and *DLGAP2* DLG-associated proteins 1 and 2, *DMR* differentially methylated region, *DMV* differentially methylated variant, *GOM* gain of methylation, *Illumina 27 K* Illumina Infinium HumanMethylation27 BeadChip array, *Illumina 450 K* Illumina Infinium HumanMethylation450 BeadChip array, *LOM* loss of methylation, *MBD4* methyl-binding domain 4, *MZ* monozygotic, *OR2L13* olfactory receptor family 2 subfamily L member 13, *PFC* prefrontal cortex, *RORA* retinoic acid-related orphan receptor, *TC* temporal cortex, *TNF-α* tumour necrosis factor alpha, *y.o.* years old

Although there is a solid rationale supporting a role for epigenetics in ASD molecular aetiology, there are relatively few studies that have directly measured epigenetic marks in ASD patients, especially when compared with the number of genetic studies available. Differentially methylated variants (DMVs) at specific CpG sites or differentially methylated regions (DMRs) spanning multiple CpGs have been measured in a variety of tissue types: lymphoblastoid cell lines [155], whole blood [156], buccal [157], sperm [158] and post-mortem brain [159–161]. The epigenome is characterized by cell-, tissue- and brain region-specific methylation patterns [161–167], making it impossible to directly compare data across these studies. However, as previously mentioned, from a biomarker and pathophysiological standpoint, it will be important to define intersecting ASD-specific DMVs/DMRs and pathways across cell types and tissues.

Genome-wide studies performed primarily using DNAm microarrays have yielded variable results for several reasons: differences in tissue type, ASD cohorts, methods (single site vs. region specific, i.e. DMVs vs. DMRs) and limited sample size (<50 cases) will affect the epigenetic output. These results emphasize the need for increased power in genome-wide DNAm studies focused on discovery of ASD-specific DNAm alterations across heterogeneous ASD groups. Most findings in ASD are reported with overwhelmingly modest effect sizes (<10% absolute difference), and some are statistically unreliable (e.g. without correction for multiple testing). Other possible confounding variables have yet to be addressed for their potential impact on DNAm outcome, including sex, age, post-mortem interval

and cause of death (for post-mortem brain samples) and brain cell type-specific DNAm patterns, to name a few. Replication of these results in larger cohorts of ASD patients will strengthen the support for a role for dysregulation of DNAm in ASD neuropathology. Only two studies [160, 161] have shown replication of differentially methylated sites that were hypomethylated in the 3′ untranslated region of *PRRT1*, tetraspanin 32 (*TSPAN32*) and *C11orf21* in brains of ASD patients when compared with controls. For many of the other identified differentially methylated genes across all studies, there is no known function in the context of ASD. Others appear to be functionally relevant, with potential roles in brain electrophysiological function (e.g. *PRRT1*), immunity (e.g. *C1Q*, *TNF*-α) and/or involving known ASD-risk genes (e.g. AT-rich interaction domain 1B [*ARID1B*], glutamate ionotropic receptor NMDA type subunit 2B [*GRIN2B*], neurexin 1 [*NRXN1*], phosphatase and tensin homolog [*PTEN*]).

Several studies have focused on the targeted quantification of DNAm in promoters of ASD candidate genes (glutamic acid decarboxylase 65 [*GAD65*], *OXTR*, *SHANK3*, reelin (*RELN*), *UBE3A* and *MECP2*) [168–173] in different tissues (blood, specific regions in post-mortem brain). No differences between ASD and controls were found for DNAm of *GAD65* or *RELN* [173] nor for one of the *OXTR* studies [168]. The latter result did not agree with an earlier study covering an overlapping region of *OXTR* [169], where significant GOM was found at specific CpG sites overall and in a sex-specific manner. In summary, significant ASD-specific DMVs at *OXTR*, *SHANK3*, *UBE3A* and *MECP2* were identified at specific promoter CpG sites in each gene (i.e. not across all sites analysed) [168–172]. Absolute differences were found to be modest (~twofold) on average and did not affect all ASD cases equally. Overall, the variability in results observed in the genome-wide DNAm studies is also reflected in these targeted studies, for many of the same reasons (tissue type, unselected ASD cases examined, methods, sample size).

Only two studies to date have looked at differences in histone marks between individuals with ASD and neurotypical controls [174, 175]. The two studies are difficult to compare since each study differed in the histone marks examined (H3K4me3 vs. H3K27ac), brain regions (prefrontal cortex vs. prefrontal cortex, temporal cortex and cerebellum), patient cohorts and methodology. However, each study independently found ASD-specific patterns of histone mark methylation to varying degrees. Shulha *et al.* (2012) report that there were no global alterations of H3K4me3, but rather an expansion in the presence of H3K4me3 at specific genomic regions in ASD. Sun *et al.* (2016) describe brain region- and ASD-specific differentially acetylated regions. Differences were found to correspond to functionally relevant genes involved in synaptic transmission, neuronal connectivity, immunity and behaviour. Researchers are also just beginning to look at differential miRNA and long noncoding RNA (lncRNA) expression in ASD [176–179]. Thus far, there is a lack of consistent findings across these studies.

In spite of current limitations, the studies cited above will act as a catalyst for the study of ASD to identify epigenetic biomarkers for prediction or classification of individuals with ASD. Additionally, they have brought to light the many critical variables that need to be considered to improve study design and interpretation of data going forward.

4.5 Therapeutics

Identifying epigenetic targets with therapeutic potential exploits the dynamic and modifiable nature of epigenetic pathways, allowing for new approaches to ameliorate ASD symptoms. Pharmacologic agents could be used to target direct epigenetic regulators such as histone acetylation (HDACs) or to target indirect and/or downstream pathways (e.g. OCM). Some of these targets already have existing therapies/drugs for testing in clinical trials.

There are several significant challenges to this endeavour. Will general inhibition/activation of epigenetic processes be too disruptive of other mechanisms? Conversely, how can we develop more targeted (e.g. tissue and cell type, enzyme isoform-specific) epigenetic drugs? One of the most critical obstacles to overcome for the treatment of ASD neurobehavioural deficits is ensuring that a drug is able to pass the blood–brain barrier (BBB). Currently, HDAC and DNAm inhibitors have poor brain penetrance and potency, although some recent work is showing improvement in this area. Improved delivery systems are being tested with a novel HDAC inhibitor analogue [180] and image-guided (positron emission tomography) radiolabelled drug delivery [181]. However, the BBB issue may be bypassed by harnessing the therapeutic potential of the microbiome to effect downstream neurobehavioural outcomes.

Indirect targets and pathways that affect epigenetic mechanisms may also have pharmacologic potential. The importance of folate in maintaining proper SAM levels and therefore methyl donors (Sect. 4.4) is demonstrated in its apparent protective effect on ASD risk. The neuropeptide hormone oxytocin, which binds to *OXTR*, has been highlighted as a promising pharmacological agent in several clinical trials [182–185] for the treatment of certain neuropsychiatric disorders including ASD. Although results have been mixed, some positive results [186–188] support the use of oxytocin for improving specific deficits seen in ASD such as emotion recognition and eye gaze, as well as for its prosocial and anxiolytic properties.

The exploration of epigenetic therapeutic targets for ASD is in its infancy since researchers are still uncovering the molecular conundrum by which epigenetic mechanisms are perturbed in ASD. However, genomic and epigenomic insights are uncovering potential biological pathways that may be targeted for therapeutics. With more comprehensive classifications of ASD patients, we may identify subgroups of individuals that will be candidates for more precision-based therapies (e.g. pathways susceptible to environmental influences, immune, metabolic, chromatin modifiers, etc.).

4.6 Future Directions/Summary

There is still much to learn about ASD in the context of epigenetics. As technology advances, we may interrogate the genome and epigenome with higher resolution. This will allow researchers to refine DNAm studies with increased genomic coverage, to expand on our knowledge of ASD-specific histone marks and to explore the role of noncoding regions (e.g. enhancers, intergenic regions, noncoding RNAs).

Beyond CpG methylation, non-CpG methylation (CpH where H = A, C, or T) and 5-hydroxymethylcytosine (5-hmC) should not be overlooked. Both of these alternative types of methylation have been found to be important during neurogenesis [163, 189, 190] and thus could play a role in the pathogenesis of ASD. 5-hmC, an intermediate in the process of oxidative demethylation, is highly abundant in the brain relative to 5-methylcytosine (5mC) [191] and could reveal important regulatory brain region-specific epigenetic patterns.

One emerging method of better defining these mechanisms is to tackle the issue of heterogeneity in ASD by examining more homogeneous subsets of ASD patients based on various factors. Presented in this chapter were examples of environmental (preterm labour) and genetic (*NSD1* mutations in SS) stratification, which demonstrate the strength of this approach and are paving the road for the future of research into the aetiologies of ASD. It is clear from the current literature that many roads lead back to epigenetics; from genetics to structural, physiological and biochemical hallmarks of the disorder to environment, epigenetic mechanisms are intimately involved in interfacing with and regulating these aetiologic factors. The complexity of epigenetic mechanisms, its intermediary role bridging multifactorial risk factors through GxE interactions and its malleable nature underscore both the challenges of studying ASD in the context of epigenetics and the exciting potential for this area of research.

Although we were unable to touch upon these areas of research and knowledge in this chapter, there are several important additional topics to consider in the context of ASD aetiology and epigenetics.

Additional Reading
Sex bias: [192–194].
Animal models of ASD: [195–199].
Noncoding RNA and ASD: [200–203].

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