Chapter 4

Epigenetic Mechanisms in Autism Spectrum Disorders

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Additional information is available at the end of the chapter

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1. Introduction

Autism spectrum disorders (ASDs) are neurodevelopmental diseases characterized by repetitive, stereotyped behavior and maldevelopment of social and language skills. Classical autism, Asperger syndrome and pervasive development disorder-not otherwise specified (PDD-NOS) are the most commonly diagnosed ASDs. There are also a significant number of cases that are considered idiopathic because the etiology is unclear (Gillis and Rouleau, 2011). The incidence of ASDs has increased substantially in the past twenty years, growing by as much as 5-10 fold; although, the approximate 4:1 ratio of affected males to females has been relatively constant (Kadesjö et al., 1999). Some of this increase has likely been driven by shifting diagnostic criteria, heightened awareness and improved diagnostic techniques (Levy et al., 2007). However, some of this increase could be attributable to an authentic increase in the frequency of ASDs. The factors fueling this increase remain unclear and the precise causes of ASDs remain unknown (Stoltenberg et al., 2010). Although its basis is thought to be multifactorial, ASDs are known to have a heritable component (Hu et al., 2006). The inheritance patterns can to some extent be explained by typical genetic processes but, especially in light of studies noting discordance among monozygotic twins, this is unlikely to be the whole story (Ptak and Petronis, 2010). Substantial scientific evidence is emerging suggesting that epigenetic influences may be partly responsible for the development of ASDs.

2. Epigenetic protein-DNA interactions: proteins mediating epigenetic signaling

2.1. Mecp2 and the frontal cortex

Methyl CpG binding protein 2 (Mecp2) is an epigenetic regulator required for development of neuronal synaptic contacts (Luikenhuis et al., 2004) that is thought to function mainly as a gene silencing molecule (Chahrour et al., 2008). The Mecp2 gene is located on the q arm of the

X chromosome and is subject to X-inactivation (Cohen et al., 2008). The Mecp2 mechanism involves binding to methylated DNA (Yasui et al., 2007) and, after binding, complexing with histone deacetylase-1 (HDAC1). HDAC1 is responsible for removing acetyl groups from histones; this removal causes the chromatin to condense and this suppresses gene transcription at the gene promoter site. Mecp2 is also purported to be an activator of some genes (Mehler, 2008) and, although it is not clear whether the role of Mecp2 in ASDs is dependent on its role as a gene silencer or promoter, there appear to be strong associations between decreased expression of Mecp2 and ASDs.

Decreased expression of Mecp2 in frontal cortex and fusiform gyrus (Nagarajan et al., 2006) - the latter associated with neural processing of face recognition (LaSalle et al., 2001; Pelphrey et al., 2004) - has been reported. The Mecp2 gene on the X chromosome and all males actively express one X chromosome. Hence, aberrant methylation patterns of autistic males (the predominant sex affected by ASDs) cannot be counteracted by a second X chromosome, as occurs in females. Aberrant methylation of the 5' portion of the Mecp2 regulatory region has been reported for brain samples from males affected by ASDs and, as expected, there is an inverse correlation between promoter region methylation and Mecp2 expression. There is a well-established relationship between a missense mutation of Mecp2 and Rett syndrome, a pervasive developmental disorder of females exhibiting ASD-like behaviors (O'Conner et al., 2009).

3. Epigenetic DNA-protein interactions

3.1. Protein kinase c beta and the temporal lobe

Downregulation of the gene for protein kinase C beta (PRKCB1) in the temporal lobe is reported to be correlated with ASDs (Lintas et al., 2009). Phosphorylation of histone H3 at threonine 6 (H3T6) by PRKCB1 appears to prevent lysine-specific demethylase-1 (LSD1) from demethylating H3K4 during androgen receptor-dependent gene activation (Metzger et al., 2010). Consequently, downregulation of PRKCB1 could play a role in the predominance of male ASD patients. Further, this downregulation may impact deep temporal lobe (limbic system) synaptogenesis and consequently preferentially sensitize males (who are generally exposed to higher fetal androgen concentrations than females) to the environmental stressors thought to be involved in the development of ASDs.

3.2. The oxytocin receptor and hypermethylation

Generally, females lack androgen-facilitated arousal inputs to the amygdala and might, therefore, be protected from the development of ASDs by high fetal levels of estrogens and oxytocin (Pfaff et al., 2011). A role for an oxytocin receptor polymorphism in ASDs is reported (Wu et al., 2005; Liu et al., 2010) and aberrant DNA methylation in the promoter region of the oxytocin receptor gene has been observed after acute psychosocial stress (Unternaehrer et al., 2012). Epigenetic dysregulation of the oxytocin receptor gene (OXTR)

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has been implicated in the etiology of ASDs (Jacob et al., 2007) and oxytocin, together with vasopressin, has also been shown to have socialization functions (Gouin et al., 2010). A link between oxytocin and ASDs in humans (Insel, 1992) and in knockout mice is also suggested by the decreased memory and face recognition abilities which are commonly found among ASD patients (Ferguson et al., 2000; Takayanagi et al., 2005) and also adversely impact socialization. It is noteworthy that OXTR-knockout decrements in social interactions appear to be sex-specific (Sun et al., 2008).

These findings suggest that any defect of the oxytocin pathway, including a deficiency of oxytocin receptors, has the potential to contribute to the development of ASDs. A diminished number of oxytocin receptors can have a variety of causes, including both genomic and epigenetic. A study involving a family in which the mother had a hemizygous deletion of the OXTR gene, noted that the mother passed down the deletion to one of her sons, but not the other; however, both sons were diagnosed with autism and it turned out that the promoter region of the OXTR gene of the affected sibling without the deletion was hypermethylated (Gregory et al., 2009). Prior studies had identified two CpG island regions of the OXTR gene that, as a consequence of variable methylation, seem to be associated with differential OXTR expression in liver and myometrium (Kimura et al., 2003). The first CpG island overlaps with exons 1, 2 and 3 of the OXTR gene and the second CpG island was localized to the third intron. The second CpG island, within intron 3, was found to be heavily methylated in all three family members studied. The other CpG island -- overlapping exons 1,2 and 3 -- was found to be methylated differently in each of the family members; specifically, the affected sibling without the deletion showed significantly more methylation than his brother or mother at three sites within the intron. This hypermethylation occurred at locations that have previously been shown to impact OXTR expression. Since both siblings were autistic in spite of the fact that one had a genomic deletion and the other displayed hypermethylated promoter regions, this study demonstrates that epigenetic and genetic mechanisms can have equivalent effects on phenotype. Furthermore, this study showed that five differentially methylated CpG islands which were examined in a group of 20 autistic and 20 phenotypically normal individuals exhibited significantly greater methylation at several of the examined loci. These observations were made in both blood and cerebral cortex samples. Additionally, low levels of OXTR expression were found to be associated with increased methylation at a statistically significant level. This finding strengthens the idea that promoter region methylation causes gene silencing. Moreover, when the data were stratified by sex, two of the loci showed significant differences in methylation for males only, implying that the different frequencies of autism in males and females might be driven by epigenetic mechanisms.

3.3. Bcl-2 and apoptosis

Bcl-2 (B-cell lymphoma 2) is a protein regulator of apoptosis (Tsujimo et al., 1984) and it s gene has been linked to ASDs (Glantz et al., 2006). Decreased expression of Bcl-2 has been shown in cerebellum and frontal cortex of subjects diagnosed with ASDs (Fatemi et al., 2001; Fatemi

and Halt, 2001), and lymphoblastoid cell lines from sets of monozygotic twins discordant for ASDs.

3.4. RORA and oxidative stress

A novel hypothesis suggests that ASDs may develop from epigenetic dysregulation of the retinoic acid-related orphan receptor alpha (RORA), a regulator of circadian rhythm and neuroprotection from oxidative stress and inflammation, is reported (Akashi and Takumi, 2005; Boukhtouche et al., 2006). The Bcl-2 and RORA hypotheses are similar in that both depend on the idea that oxidative stress plays a key role in some instances of apoptosis. The RORA hypothesis is supported by reports that oxidative stress and inflammation are increased in patients diagnosed with ASDs (Pardo et al., 2005; Chauhan and Chauhan, 2006). Differences in RORA gene promoter region methylation and protein product expression is reported for some subjects diagnosed with ASDs and their (non-twin) unaffected siblings (Nguyen et al., 2010). Furthermore, decreased RORA expression was found only in the ASD subjects with severe language impairment.

3.5. Beta-catenin, estradiol and lithium

Estrogens are important for sex-related differentiation of the brain and it is likely that brain estrogen levels are increased in subjects diagnosed with ASDs (MacLusky et al., 1987). Estrogens, being steroid hormones, and their receptors - including estrogen receptor alpha (ERα) - are located in the nucleus and in the cytosol of target cells. One of the targets of cytosolic ERα is GSK3B, which is known to complex with β-catenin in the degradation of β-catenin. ERα activation by estradiol is reported to release β-catenin from this complex, thereby increasing β-catenin availability (Cardona-Gomez et al., 2004). An increase in the cytosolic concentration of estrogens is thought to result in increases in cytosolic and nuclear β-catenin during critical periods of prenatal and neonatal development during which β-catenin binding to the LEC/TCF promoter has positive effects on Wnt pathway gene transcription. Such increased transcription in the Wnt pathway is strongly associated with the development of ASDs. The effect of ERα is to cause the dissociation of β-catenin from a complex whose integral members include the proteins GSK3β, axin and adenomatous polyposis coli tumor suppressor (APC). GSK3β, axin and APC are negative regulators of the Wnt signaling pathway and the complex requires all of these constituents to initiate the destruction of β-catenin. The absence or downregulation of any of these components may increase the availability of cytosolic β-catenin, and consequently the various knock-in effects discussed previously - increased nuclear β-catenin with greater Wnt pathway transcription. Lithium, used mostly as a mood stabilizing drug, exerts an inhibitory effect on GSK3β both directly and indirectly, by interrupting the dephosphorylation of phospo-GSK3β (Jope, 2003). In either case, the effect is the same, and also the same as that of increased estrogen levels; i.e., the complex responsible for initiating the degradation of β-catenin is made nonfunctional, and the concentration of cytosolic β-catenin increases.

3.6. SHANK3 and the neurexin-neuroligin pathway

SHANK3 is a scaffolding protein in the neurexin-neuroligin pathway that interacts with synaptic proteins. Recent research suggests that copy number variations or mutations of either of these proteins may be associated with the development of ASDs (Liu et al., 2013). It appears that epigenetic mechanisms are used to control the expression of this gene. Five CpG islands in the SHANK3 gene (the post-translational methylation of which determines gene expression) and one specific locus - CpG island 2 - appear to particularly impact tissue SHANK expression (Beri et al., 2007). The SHANK3 gene is well conserved between humans and rodents and neonatal expression of certain SHANK3 transcripts in mice is known to temporarily decrease methylation of CpG island 2 after birth (Uchino and Waga, 2013). These findings suggest that the expression of SHANK3 (and thus its effect on the development of ASDs) is regulated by epigenetic mechanisms, though this connection has yet to be directly established in humans. Additionally, two genes responsible for the production of cell adhesion molecules in this pathway, NLGN3 and NLGN4, have also been associated with the development of ASDs (Liu et al., 2013). However, epigenetic regulation of these genes are, at this time, unproven (Yasuda et al., 2011).

3.7. IGF-2 and the cerebellum

Disruption of cerebellum cytoarchitecture with loss of Purkinje cells, effects that could have a negative impact on cerebellum development and postmortem cerebellum weight, has been described in subjects diagnosed with ASDs (Whitney et al., 2009). Insulin like growth factor-2 (IGF-2) is the product of a paternally imprinted gene, the allele-specific expression of which is regulated by DNA methylation. DNA methylation at a key transcriptional repressor (CTCF2) is shown to be correlated with cerebellum weight. Paradoxically, DNA methylation at CTCF3 of the maternally-inherited allele also appears to be associated with an increase in cerebellum weight (Pidsley et al., 2012).

3.8. Neurotrophins

Contemporary thinking has seized upon neurotrophins (proteins tasked with promoting the development, and, later, survival of neurons) as potentially important factors in the later development of ASDs. Neutrophins are growth factors whose prenatal presence (or absence) might affect neurogenesis in such a way as to make the later development more or less likely. In particular, a recent investigation focused on the role of three neurotrophic factors -- brain derived neurotrophic factor (BDNF), neurotophin-4 (NT4) and the immunosuppressive cytokine transforming growth factor beta (TGF-β) - in the development of ASDs (Nickl-Jockschat and Michel, 2011). The results, which were based on analysis of dried blood spot samples of neonates later diagnosed with ASDs and frequency-matched controls, suggest a role for each of studied neurotrophins: neonates with BDNF and TGF-β concentrations in the bottom decile were more likely to be diagnosed with ASDs, and eventual ASD neonates were less likely to have a NT4 concentration in the top decile (Abdallah et al., 2013). In spite of the authors' conspicuously cautious concluding remarks, their finding may have far reaching implications (Pareja-Galeano et al., 2013). BDNF has also been implicated in the etiology of a

variety of neurological and psychiatric conditions - such as Alzheimer's and Huntington - that are posited to be involved in dysfunctional CNS synapse development. The results of the neurotrophin study are in line with this idea. The BDNF gene has been linked to early life stressors and their concomitant brain responses and behavioral outcomes, which suggests than an aberrant BDNF gene - or aberrant epigenetic regulation of that gene - might compromise the brain's advanced paternal capacity to mount a robust response to early life stressors, including those that were linked to ASDs in our earlier discussion. Furthermore, BDNF has been proposed as a link mediating interactions between genes, environmental conditions, synaptic plasticity and apoptosis and some research suggests that environmental and social conditions early in life may affect the epigenetic regulation of BDNF (Balaratnasingam and Janca, 2012). This points to a (somewhat speculative) mechanism for the development of ASDs: Adverse perinatal conditions alter the epigenetic regulation of BDNF which in turn limits the young brain's ability to properly respond to its adverse environment which then makes the development of ASDs more likely. These ideas may also be the first steps towards a strategy to reduce the ASD frequency. Since the higher concentrations of BDNF (as well as NT4 and TGF-β) are associated with decreased frequency of ASDs, promoting the upregulation of those proteins may be protective. Mouse models have shown that music exposure can increase the brain BDNF concentration in mice, however this finding is yet to be extended to the perinatal human context (Angelucci et al., 2007). Similarly, controlled physical exercise demonstrably increases BDNF concentrations in rats (Lee et al., 2013). Either, or both, of these approaches may be useful in encouraging a perinatal environment that minimizes the eventual development of ASDs.

3.9. The locus coeruleus-noradrenergic system and fever

The study of epigenetics encompasses more than differential methylation and acetylation of gene promoter regions. Indeed, any mechanism that affects gene expression, without altering the genetic code, may be considered an epigenetic mechanism. This broader view of epigenetics can help explain a curious, but common, observation: Autistic children consistently exhibit diminished ASD symptoms when febrile (Curran et al., 2007). This effect of fever appears to have nothing to do with methylation, gene silencing or promotion. Furthermore, there is no reason to suspect that the appearance of fever would result in the addition of methyl groups to particular portions of the genome. However, one hypothesis argues that febrile episodes temporarily modify the functional integrity of the locus coeruleus-norandrenergic (LC-NA) system, the dysregulation of which has been implicated as a proximate cause of ASD symptoms (Mehler and Purpura, 2009). This hypothesis is plausible, particularly in view of the functions of the LC-NA system. LC is in the pontine tegmentum and is notable for containing the most widely spread efferent projections of any nucleus in the brain (Foote et al., 1983). Most of the norepinephrine in the brain is transported by these neurons. This property of the LC-NA system is significant because distributed neural networks - those that are associated with the ability to make behavorial adaptations to environmental changes and that tend to be disrupted in ASD patients - are modulated by the LC-NA system. Indeed, the LC-NA system is also implicated in several

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other neurodegenerative and psychiatric disorders. The key point is that autism is frequently associated with dysregulation of the LC-NA system and fever also affects the LC-NA system. Moreover, febrile symptoms are typically induced by bacterial lipopolysaccharide and symptoms in the brain are, in turn, mediated by norepinephrine (Linthorst et al., 1995). Because most of the brain's norepinephrine is released from LC neurons, it is reasonable to think that the LC-NA system is somehow altered in the febrile state, especially since LC neurons are known to exhibit extremely synchronized activity. Consequently, fever may induce a change in LC-NA system functions that diminishes ASD symptoms. The discovery of this relationship between ASD symptoms and LC-NA functions implies that the neural networks of autistic patients are not irreparably damaged but, rather, are functionally intact. Theoretically, these findings suggest that fever-related changes in the brain might provide a useful experimental model for the development of drugs and other targeted therapies for ASDs. Moreover, the existence of this relationship between fever and LC-NA system functions points to a broader view of the concept of epigenetic mechanisms. Indeed, epigenetic effects may be thought of as relatively static, such as in the case of DNA methylation, or dynamic such as in the case represented by the connection between fever and the LC-NA system. This difference is roughly analogous to that between chronic and acute symptoms of a disease. A fully effective treatment of ASDs might require targeting both of the aforementioned types of epigenetic causative mechanisms.

4. Role of maternal hypomethylation

Development of the LC-NA system seems to be strongly impacted by prenatal events, reflecting the importance of environment-gene interactions. Maternal hypomethylation is an epigenetic mechanism that has effects on the intrauterine environment and which, in turn, may predispose fetuses to developing ASDs. Greater prevalence of ASDs is reported in children whose mothers were exposed to hurricanes or tropical storms during gestation (Beversdorf et al., 2005) and if the accompanying stress causes dysregulation in a distributed neural network such as the LC-NA system, it could result in a predilection for ASDs. Thus, epigenetic influences on the maternal genome could alter the intrauterine environment such that the probability of the offspring developing ASDs is increased or decreased. DNA hypomethylation linked to variants in the maternal folate pathway have been linked to aberrant fetal development (Foote et al., 1983; Linthorst et al., 1995). Because folate is the primary one carbon donor in methylation reactions, epigenetic dysregulation of the folate pathway should provide insight into the availability of methylation precursors and also the extent of genomic methylation in mothers of unaffected children compared to mothers of children diagnosed with ASDs.

Indeed, mothers of children diagnosed with ASDs often exhibit aberrant DNA methylation (James et al., 2008). Moreover, mothers of children diagnosed with ASDs also exhibit significantly lower levels of methylfolate and methionine - essential precursors for DNA methylation - than their non-ASD counterparts. In addition, levels of the methylation-inhibiting proteins S-adenosylmethoinine, adenosine and homocysteine were all elevated in autism mothers. S-adenosylmethonine (SAM) is the primary methyl donor for the DNA methyltransferase

reaction, which produces S-adenosylhomocysteine (SAH) and methylated DNA. Because SAM and SAH are linked by the transferase reaction the SAM/SAH ratio is generally considered to be a good indicator of DNA methylation potential. Mothers of children diagnosed with ASDs often exhibit lower SAM/SAH ratios than control groups, indicative of a diminished capacity for methylation. Furthermore, the DNA of the mothers of children diagnosed with ASDs appears to be less methylated than that of mothers of unaffected children; the ratio of 5-methylcytosine to total cytosine - a measure of overall genomic methylation - was significantly lower in the mothers of autistic children (James et al., 2008). Taken together, this evidence strongly suggests that hypomethylation of maternal DNA may be linked to ASDs. However, the significance of these findings about the influences of epigenetics on the development of autism is not entirely clear.

5. Role of advanced paternal age in autism

Advanced paternal age is strongly associated with a variety of childhood conditions (Goriely et al., 2013). The relationship between advanced paternal age and autism is especially well-established. Large-scale studies have shown that advanced paternal age may increase the offspring's risk of developing an ASDs by as many as six fold. Despite such a striking effect, the specific mechanism underlying the relationship between older fathers and autistic children is not precisely clear. It seems likely that the link is at least partly explained by the effect of paternal age on germ cell integrity; a greater number of sperm divisions (and therefore opportunities for duplication errors) occur as paternal age increases, and the disparity between paternal and maternal germ line divisions widens. Of course, advanced paternal age may covary with a number other factors that may exert an effect on ASD frequency, not the least of which is maternal age. It's worth noting that none of these proposed mechanisms are mutually exclusive; in fact, it is entirely possible that the observed effects are the consequence of combination of a number of factors.

Some of these factors may have an epigenetic nexus. Rat model studies have demonstrated the germ cells of older rats tended to have changes in chromatin packaging, and consequently decreased gene integrity. In one notable study, sensitivity to oxidative treatment was used as a proxy for resilience and integrity of chromosomal packaging. Four-month-old and 21-month-old Brown Norway rats were systematically exposed to L-buthionine-[S,R]-sulphoximine (BSO) (a drug with well-known glutathione depleting properties) and hydrogen peroxide. The chromatin from spermatozoa of the younger rats demonstrated less nuclear chromomycin A3 penetration, decreased thiol oxidation, fewer DNA breaks and reduced tendency to dissociate from acridine orange - all indications of more fragile genetic material (Zubkova and Robaire, 2006). Researchers have also conducted systematic searches for age-related global and gene-specific methylation patterns. Using restriction landmark genomic scanning to determine methylation patterns of CpG islands, one study detected a ribosomal DNA locus the methylation of which is correlated with age; interestingly, the methylation of all studied single copy CpG island sequences were independent of age (Oakes et al., 2003). Together, these results suggest the presence of a direct effect of paternal age on the epigenome.

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The science underlying the effect paternal age on genetic integrity is well-known and relatively straightforward; the hundreds of replications that spermatogonial undergo allow for greater numbers of point mutations, chromosomal breakages and copy number alterations than are typically observed in maternal cell lines (Perrin et al., 2007). However, the pervasive nature of the *genetic* effects of advanced paternal age somewhat complicates the evaluation of its *epigenetic* impacts. Consequently, separating the genetic and epigenetic effects can be delicate work. The evidence required to make such a determination must be biochemical; merely noting an association between increased paternal age and frequency of ASDs is insufficient to account for possibly confounding genetic effects. On the other hand, we know that dysregulation of imprinted genes, which as noted earlier often occur in clusters that are regulated by imprinting centers, is associated with altered brain development, and, notably, neurocognitive conditions such as autism. Recent studies have bridged this gap by demonstrating that advanced paternal age may increase the likelihood of the epigenetic dysregulation of the imprinting centers. Specifically, mouse models have indicated that the epigenetic loss of suppression of particular genes occurs more frequently in older mice than younger mice. Two examples of specific genes are illustrative (Bennett-Baker et al., 2003). The first, copper-transporting ATPase 1 (Atp7a), is located on the X chromosome and is consequently subject to X-linked inactivation. Quantitative comparison of RNA transcripts from young and old mice (2 and 24 months of age, respectively) have shown significantly less consistent X-inactivation among the transcripts from the older mice. This, in turn, suggests that, to some extent, the Atp7a gene of the older mice is hypomethylated as compared to the younger cohort. When we recall that the methylation resulting in gene inactivation is heritable, it follows that the offspring of older mice are more likely to inherit hypomethylated, and thus not inactivated, paternal alleles. A similar epigenetic mechanism has been demonstrated for the insulin growth factor 2 gene (Igf2). Unlike Atp7a, Igf2 is subject to genomic imprinting. As we've noted previously, epigenetic effects may manifest through manipulation of the imprinting process. It has also been demonstrated that older mice cohorts produce RNA transcripts from silenced alleles more frequently than their younger counterparts. Here again, this alteration of the imprinting process - mediated through changes in methylation patterns - is a heritable epigenetic change that results as a consequence of advanced paternal age. Further, we're beginning to see similar results in studies of human subjects. Most notably, a study of the cord blood of a group of Chinese Han newborns (approximately 20% of whom had non-imprinted Igf2 genes) found that although no measured maternal factor was statistically correlated with loss of imprinting, this epigenetic alteration was associated with increased paternal age (Dai et al., 2007). These studies comprise fairly compelling evidence of the effect exerted by advanced paternal age on the epigenome. It follows closely that these mechanisms have the potential to result in the passing of genetic material to ASD-susceptible offspring.

6. Nutritional factors

The ultimate effect of nutritional imbalances depends greatly on the developmental period during which the imbalance occurs. Different organs have critical developmental stages, and

the time point at which they are compromised will predispose individuals to specific diseases. Also, depending on the function of the gene, epigenetic modifications that occur during development may not be expressed until later in life. While the majority of studies implicate prenatal and perinatal periods as critical time windows, some research has shown that nutritional intake during adulthood can also affect the epigenome.

Genetic polymorphisms of cytochrome P450 enzymes have been linked to ASDs, specifically the cytochrome P450 family 27 subfamily B gene (CYP27B1), which is essential for proper vitamin D metabolism. Epigenetic regulation of cytochrome P450 genes for hydroxylation and activation of vitamin D in has been shown in prostate cancer cells (Luo et al., 2010). Vitamin D is important for neuronal growth and neurodevelopment, and defects in its metabolism or deficiency have also been implicated in ASDs (Currenti, 2010). Mutations of Mecp2 associated with impaired methylation are known to be associated with autism and a related neurological disorder, Rett syndrome. One component of Rett syndrome is abnormal bone formation caused by abnormal vitamin D metabolism, which is associated with epigenetic dysregulation of cytochrome P450 genes (O'Connor et al., 2009) which could be a conceptual model for epigenetic interactions between Mecp2, vitamin D and cytochrome P450 genes (Currenti et al., 2010). As noted earlier, abnormal folic acid metabolism may play a role in the decreased capacity for methylation and DNA hypomethylation associated with significantly higher-than-normal levels of plasma homocysteine, adenosine, and SAH in mothers of subjects diagnosed with ASDs (James et al., 2010). Indeed, nutritional factors such as folic acid appear to be protectors against the epigenetic dysregulation associated with ASDs. DNA methylation, the most established epigenetic gene regulation, is a one-carbon transfer dependent mechanism requiring folate, choline, betaine and other B vitamins (Anderson et al., 2012) and it is, therefore, not surprising that folic acid supplementation in pregnancy appears to be protective against the development of ASDs (Surén et al., 2013). Changes in autism-related behaviors are reported to be strongly associated with vitamin-supplementation associated changes in plasma levels of biotin and vitamin K (Adams et al., 2011). Although biotin is a known cofactor in bioavailability of methyl groups for DNA methylation, a vitamin K-related epigenetic mechanism has not been described.

7. Toxic factors

7.1. Exposure to valproic acid

Valproic acid (VPA) is a therapeutic anticonvulsant and mood stabilizing drug that gained attention in the 1980s as a potential teratogen. VPA exposure is highly correlated with autism; as many as 60% of infants who exhibit the suite of symptoms associated with VPA teratogenicity also display two or more autistic characteristics (Moore et al., 2000). Autism has also been shown to occur in 9% of cases of prenatal exposure to VPA (Rasalam et. al., 2005). The mechanisms underlying the pharmacological actions of VPA are also suggestive of a correlation between VPA and ASDs (Shimshoni et al., 2007). VPA is responsible for inhibiting two enzymes: myo-inositol-1-phosphate (MIP) synthase and the class 1 and 2 histone deacetylase

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(HDAC). HDAC1 is an important inhibitor of DNA transcription that works by associating with the LEC/TCF transcription factor. When HDAC1 is removed from the LEC/TCF complex, it leaves behind a primed (but inactive) promoter of gene transcription. The primed promoter then forms a complex with β-catenin, thus activating the promoter, and increasing transcription rates of a variety of genes in the wingless-type, mouse mammary tumor virus (MMTV) integration site family, in the type 1 (Wnt) signaling pathway including cyclin D1, as well as in those required for the transition from the G1 to S phases of mitosis. Additionally, the removal of HDAC1 causes the an increase in the transcription rate of MYC, which is a transcription enhancer for many genes throughout the genome (Billin et al., 2000). Accordingly, the consequence of VPA-mediated inhibition of HDAC1 is to upregulate the transcription of Wnt pathway genes. In addition, VPA increases cellular levels of β-catenin, presumably in response to the increased availability of primed LEC/TCF promoters (Wang et al., 2010). The effect of VPA on Wnt gene transcription is well understood but fails to explain the connection between VPA and autism. In order to complete this link, it is necessary to note that an increase in the number of neocortical minicolumns is highly correlated with autism (Williams and Casanova, 2010). This observation is supported by fMRI studies that report differences in how autism brains coordinate the processing of information (Minshew and Williams, 2007). It is reasonable to assume that processes which upregulate genes of the Wnt signaling pathway - such as prenatal exposure to VPA - may result in poorly regulated mitosis and cellular proliferation, one manifestation of which could be an increase in the number of neocortical minicolumns and macrocephaly. This mechanism has been observed at work in a slightly different context. Recall that Mecp2 inactivates genes by forming complexes with a variety of different molecules. One of these molecules is HDAC1 (Nan et al., 1998) and in the absence of HDAC1, or even when HDAC1 has merely been downregulated, the gene inactivating properties of Mecp2 will be expected to have a diminished effect. One of the promoters on which Mecp2 typically exerts its regulatory effect is the LEC/TCF promoter, which, as mentioned previously, ultimately regulates the transcription rates of the Wnt signaling pathway. Although Mecp2 has effects on gene methylation, the function of HDAC1 concerns acetylation of histones. However, in order for a gene to be transcriptionally deactivated, they must often be both methylated and deacetylated. Thus, VPA-induced inhibition of HDAC1 interferes with the functionality of Mecp2 which appears to increase the risk of developing ASD.

7.2. Exposure to sex steroids and antidepressants

Endocrine disruption early in pregnancy during sex-related differentiation of the brain suggests several mechanisms for the development of ASD. Sex steroids are known to be potent inhibitors of 3(17) alpha-hydroxysteroid dehydrogenase (AKR1C1), a bifunctional enzyme that catalyzes the oxidoreduction of the 2- and 17-hydroxy/keto groups of sex steroid hormones, the main metabolite of which is epitestosterone. Epitestosterone is a sex steroid produced in the mammalian brain, including that of humans. Epitestosterone is also shown to occur in higher concentrations in females than in males and could be an important central nervous system epigenetic regulator of gene expression that might help explain some of the male predominance of ASDs. Epitestosterone metabolism may also be linked to the features of ASDs associated with prenatal exposure to citrapolam, estradiol and valproic acid (Sanders,

2012). Several in vitro studies show that sex steroids can also alter serotonin homeostasis and mice expressing the common serotonin reuptake transporter (SERT) polymorphism, SERT Ala56 – which is associated with increased serotonin clearance rates and plasma serotonin concentrations and found in children with ASDs, (Dufour-Rainfray et al., 2010; Simpson et al., 2011; Veenstra-Vanderweele et al., 2012). The serotonin reuptake inhibitor citrapolam (Celexa), which increases plasma serotonin levels, is also implicated in heightened risk of ASDs in the children born to mothers who were prescribed the drug in the early stages of their pregnancies (Rai et al., 2013). These connections between sex steroids and serotonin homeostasis appear to be complex, as evidenced by the finding that citrapolam exposure modulates both cerebral cortical and noradrenergic locus coeruleus functions (previously discussed in relation to ASDs) as well as produces autistic-like behaviors in male rats (Darling et al., 2011).

7.3. Prenatal exposure to organic environmental pollutants

ASDs have also been linked to perinatal exposure to a flame retardant chemical, pentabromodiphenyl ether, BDE-47 (Woods et al., 2012). BDE-47 is one of a group of chemicals whose industrial production is required to be halted under the Stockholm Convention, a treaty intended to protect against environment exposures to persistent organic pollutants. Exposure to BDE-47 is known to negatively impact Mecp-2. Mecp2 has been discussed previously in terms of the X chromosome-linked mutation that produces Rett syndrome, a pervasive autistic-like neurodevelopmental disorder in females. Mecp2(308/+) mice are knock-in animal models shown to exhibit global hypomethylation of adult brain DNA, specifically in female offspring following perinatal exposure to BDE-47. This hypomethylation is associated with decreased social interactions and decreased expression of DNMT3a, a DNA methyltransferase required for learning and memory in hippocampus, specifically in BDE-47-exposed Mecp2(308/+) offspring (Woods et al., 2012). Collectively, these results demonstrate that some chemical environment-gene interactions relevant to social and cognitive behaviors exhibit sexual dimorphism and epigenetic dysregulation that are likely relevant to the pathogenesis ASDs.

8. Other ASD-like diseases

8.1. Angelman syndrome

Epigenetic effects may also manifest through aberrant methylation patterns of imprinted genes. The expression of imprinted genes, which are mostly found in clusters on chromosomes 6, 7, 11, 14 and 15, is controlled by a series of DNA methylations and histone modifications. Imprinting defects may be primary or secondary. Primary imprinting defects cause changes in observed methylation patterns, but leaves the DNA sequence unaltered, and thus may be classified as an epigenetic mechanism (Gos, 2013). Angelman syndrome, which is caused by an absence of active maternal genes in the 15q11-1q13 region, may result from a primary imprinting defect, although the syndrome is more commonly caused by a deletion on the maternal chromosome or a paternal uniparental disomy. There is some basis to suspect a link

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between Angelman syndrome and autism. For instance, in one particular study, though the frequency of Angelman syndrome was only found to be 4 out of approximately 49,000, each of those four children were found to demonstrate autistic behaviors. (Steffenburg et al., 1996). However, other studies place the rate of co-occurrence of autism and Angelman syndrome at a rate of as low as 2%. It seems reasonable to assert that, to the extent that Angelman syndrome and autism are linked, the condition of some percentage of these patients will be related to an epigenetic primary imprinting defect. However, the available evidence does not establish whether the epigenetic defect causing Angelman syndrome leads directly to autistic symptoms or whether the relationship between Angelman syndrome and autism are merely correlative, and not causative.

8.2. Prader-Willi syndrome

On the other hand, secondary imprinting defects occur when a gene mutation results in improper epigenetic regulation. Such a defect may occur in Prader-Willi syndrome, which is characterized by the lack of a paternal contribution at the 15q11-q13 locus. The specific mutation most commonly responsible for secondary imprinting in Prader-Willi syndrome is a cis-acting defect of the imprinting regulatory center of the Prader-Willi syndrome gene (Dykens, and Shah, 2003). Prader-Willi patients present with autistic behavior more frequently than Angelman syndrome patients; studies suggest the frequency of autism co-occurrence with Prader-Willi syndrome is between 18% and 38% (Veltman et al., 2004), though the causative nature of this relationship has not been established.

8.3. Fragile X syndrome

Fragile X syndrome is the leading single-gene cause of autism accounting for as many at 5% of all cases (McLennan et al., 2011). As with Prader-Willi and Angelman syndromes, epigenetic mechanisms can contribute to the development of the Fragile X, which is characterized by the presence of 200 or more CGG repeats in the 5' untranslated region of the FMR1 gene (Willemsen et al., 2011). The resulting increased concentration of cytosine and guanine nucleotides causes the global methylation of not only the CGG-repeat region but also adjacent regions, which happen to include FMR1 promoter elements.

8.4. Rett syndrome

A missense mutation of the protein Mecp2 is known to produce Rett syndrome, a disease formerly termed cerebral atrophic hyperammonemia, the signs and symptoms of which are often confused with Angelman syndrome. Interestingly, hyperammonemia can also be produced by urea cycle disorders and exposure to valproic acid (the latter being a known risk factor for ASDs). Damage to the locus coeruleus-noradrenergic and midbrain dopaminergic systems have been demonstrated and the etiology of Rett syndrome appears to be confirmed by a study demonstrating that restoration of Mecp2 activity may be achieved by an IGF-1 treatment (Tropea et al., 2009).

9. Role of epigenetic drift in autism

Earlier, we discussed investigations involving identical twins to elucidate the effects of the intrauterine environment on the epigenome. As it happens, twin studies are also valuable because they allow us to explore the concept of epigenetic drift. The idea of epigenetic drift closely parallels the more familiar notion of genetic drift: Beginning with identical starting conditions, epigenetic drift is the divergence of two formerly identical epigenomes in response to external pressures. An investigation of global histone H3 and H4 acetylation, 5 methyl cytosine methylation and X chromosome inactivation concluded that despite very few differences between the epigenomes of monozygotic twins at birth, significant differences between the epigenomes develop as the twins age (Fraga et al., 2005). Some studies have indicated that epigenomic drift may occur on a scale two orders of magnitude more substantial than genetic drift (Martin, 2012). Clearly, any number of factors - smoking, diet and physical activity among them - may be responsible for these different changes. But it might also be the case that an intermittent failure to properly transfer epigenetic information during the gene replication process may play a role as well. In any event, regardless of the specific etiology of the epigenomic changes, these are the sorts of heritable changes in methylation and acetylation that we've previously seen to be linked to the development of ASDs in offspring.

10. Summary and conclusions

Figure 1 summarizes the main epigenetic mechanisms that appear to play roles in ASDs include low activity of Mecp2 at CpG islands in genes of frontal cortex is shown to reduce the capacity for inhibiting HDAC1 and chromatin condensation for gene silencing. HDAC1 inhibition by valproic acid and GSK3β inhibition by lithium are shown to upregulate the Wnt signaling pathway, which in turn causes accumulation of β-catenin in the cytoplasm and its translocation to the nucleus. In the nucleus β-catenin can act as an activator of transcription and cause macrocephaly and the attendant increase in the number of cerebral cortical columns. DNMT is shown to methylate the oxytocin receptor gene and silence it, resulting in the low oxytocin and estrogen activity necessary for androgen receptor mediation of high arousal inputs to the amygdyla. This defect is associated with antisocial behaviors after exposure to environmental stressors. Histone H3 phosphorylation by protein kinase C beta is shown to activate the LSD1, an HMT that prevents demethylation of H3K4, which is also necessary for androgen receptor mediation of high arousal inputs to the amygdyla. Hypomethylation by decreased availability of S-adenosyl methionine (SAM) is shown to occur in mothers of autistic children. Environmental and nutritional conditions acting as pro- or anti-autism factors by epigenetic mechanisms suggest strategies for decreasing the prevalence of ASDs. This knowledge of putative epigenetic targets should motivate clinical practitioners and educators to develop novel treatment strategies based on the environment-gene interactions which could contribute to the core symptoms of ASDs.

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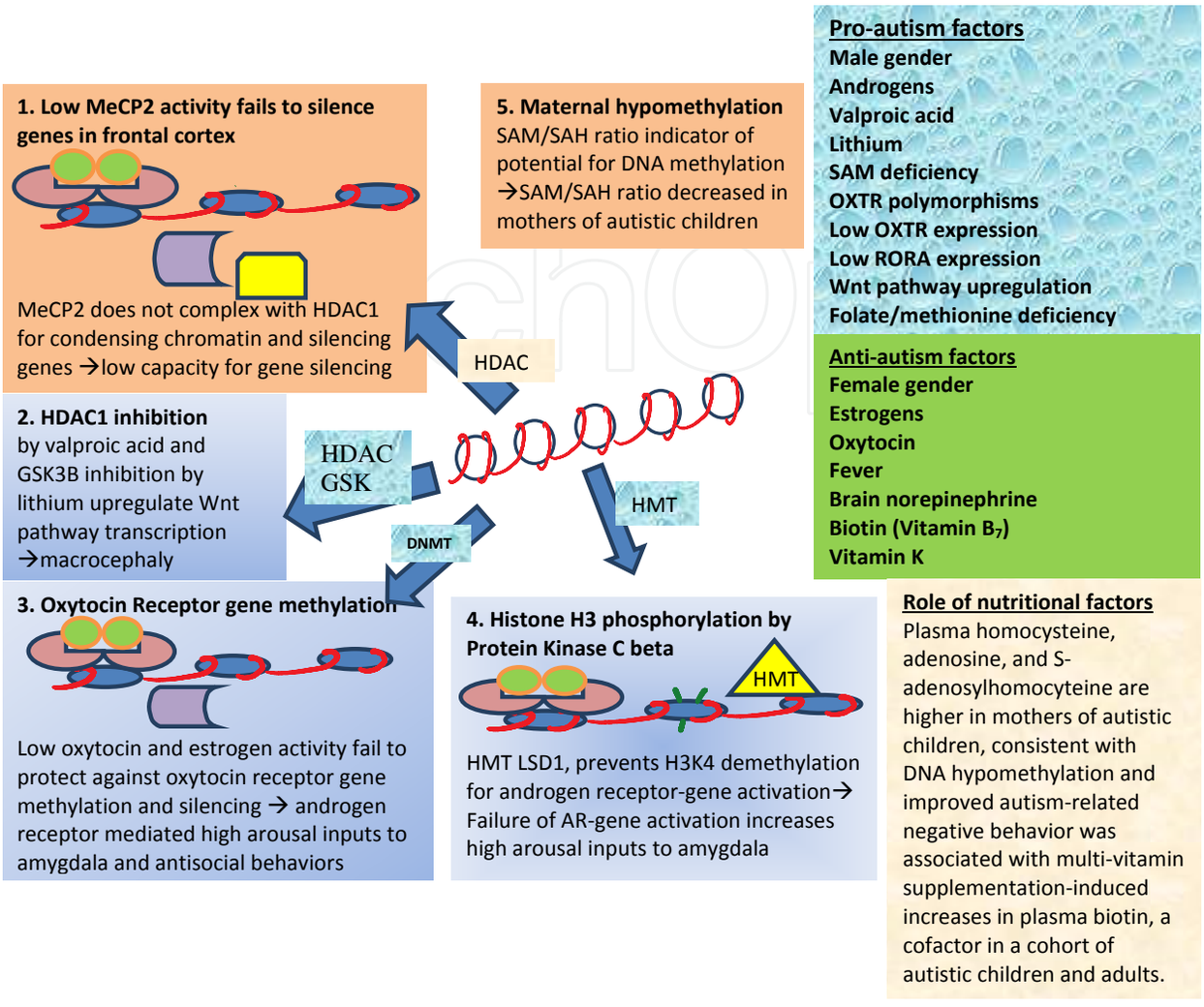

Figure 1. Main mechanisms of epigenetic alterations in autism. Each alteration involves many enzymes but the main players to cause methylation or acetylation are shown by arrows. These are not separate mechanisms and the enzymes do not act alone. Several enzymes act at a promoter simultaneously. 1. Low methyl CpG binding protein-2 (Mecp2) at CpG islands of frontal cortex reduces capacity for complexing with histone deacetylase 1 (HDAC1) for gene silencing. 2. HDAC1 inhibition by valproic acid exposure and glycogen synthetase kinase-3B (GSK3B) inhibition by lithium upregulate Wnt signaling pathway, activates transcription, associated with macrocephaly with increased number of cerebral cortical column. 3. DNA methyltransferase (DNMT) methylates oxytocin receptor gene produces low oxytocin and estrogen activity necessary for androgen receptor mediated high-arousal inputs to amygdala. 4. Histone H3 phosphorylation by protein kinase c beta activates the histone methyltransferase (HMT) lysine demethylase 1 (LSD1) which prevents demethylation of lysine-4 site of histone-3 (H3K4) also necessary for androgen receptor (AR) mediation of high arousal inputs to amygdala. 5. Maternal hypomethylation by dietary folic acid deficiency decreases availability of S-adenosyl methionine (SAM), associated with abnormal intrauterine growth.

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