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The involvement of epigenetic defects in mental retardation

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ABSTRACT

Mental retardation is a group of cognitive disorders with a significant worldwide prevalence rate. This high rate, together with the considerable familial and societal burden resulting from these disorders, makes it an important focus for prevention and intervention. While the diseases associated with mental retardation are diverse, a significant number are linked with disruptions in epigenetic mechanisms, mainly due to loss-of-function mutations in genes that are key components of the epigenetic machinery. Additionally, several disorders classed as imprinting syndromes are associated with mental retardation. This review will discuss the epigenetic abnormalities associated with mental retardation, and will highlight their importance for diagnosis, treatment, and prevention of these disorders.

1. Introduction

The occurrence rate of many diseases with a suspected genetic basis often does not match predictions from traditional genetics. One of the most striking examples of such discordance is the disease risk of monozygotic twins who, despite having an identical genetic make-up, have notably different susceptibility to diseases (Haque, Gottesman, & Wong, 2009). Although still not well understood or explained, it is now largely recognized that further to the genome, the epigenome is of crucial importance for such differences, and influences an individual's disease risk. The epigenome is different in each individual, and significantly fluctuates across life. It is strongly influenced by the environment, and varies between each individual, including between monozygotic twins, depending on such factors as lifestyle, diet, living conditions, and age (Fraga et al., 2005). The epigenome therefore plays an extremely important role in many diseases ranging from cancer to neurodevelopmental and neurodegenerative disorders. Here we review the current evidence that anomalies in epigenetic marks, and in the components of the epigenetic machinery that recognize and respond to these marks, are strongly associated with neurodevelopmental disorders leading to mental retardation.

Mental retardation is characterized by an impairment of intellectual abilities, and by severe deficits in the capacity to adapt to the environment and the social milieu (American Psychiatric Association, 1994). The high prevalence of mental retardation worldwide (2.3%), and its strong familial and societal impact, make it of extreme importance to investigate its mechanisms and find new avenues towards its potential prevention and treatment. Mental retardation is a feature expressed in several neurodevelopmental disorders including Rett syndrome, Fragile X, and Down syndrome. Although its causes have not been clearly identified, its potential underlying mechanisms have been linked to epigenetic alterations. These alterations of the epigenome may be due to duplications or loss-of-function mutations in key components of the epigenetic machinery, particularly those involved in reading and interpreting the epigenetic code, or to anomalies of the epigenetic marks themselves. These two mechanisms are not mutually exclusive, since impaired functions of core genes that regulate the epigenome can have a dramatic effect on the epigenetic profile. Interestingly, severe mental retardation is one of the major characteristics of imprinting disorders, that result from aberrant chromosomal marks in genes normally expressed monoallelically from either the maternal or paternal copy depending on its epigenetic state. These disorders are thought to result from errors in the erasure, the establishment, or the maintenance of imprints leading to aberrant expression of imprinted genes. Many of the genes involved in mental retardation are located on the X-chromosome (Jensen et al., 2011),

2. Misinterpretation of epigenetic marks

The posttranslational modification (PTM) of histone proteins in the chromatin is an important mechanism for the epigenetic marking of the genome. Histone PTMs are varied and co-occur in complex combinations on individual histones and in different nucleosomes. Because their combinations are not random but

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appear to follow specific rules, it was proposed that they form a "histone code" (Strahl & Allis, 2000). According to this concept, histone PTMs are established in a specific manner through complex cross-talk that form a particular code in individual genes, and determine whether chromatin is in an "on" or "off" state, and whether genes are activated or silenced (Baker, Allis, & Wang, 2008). This code is controlled by a complex machinery of histone-modifying enzymes. In most tissues, including the brain, multiple histone-modifying enzymes have been identified that can add or remove PTMs on specific sites or residues (known as "writers" or "erasers", respectively) (Musselman & Kutateladze, 2009). Factors or complexes known as "readers" interpret the histone code by recognizing different histone PTMs. But through their own intrinsic activity or by recruiting cognate factors, many of these "readers" can also be "writers" or "erasers" and modify PTMs to alter chromatin structure. This combination of "read-write" or "read-erase" mechanisms may favor the spread or the erasure of epigenetic marks over particular stretches of DNA, and participate in the complex mechanisms of transcriptional control.

2.1. PHD domains

The plant homeodomain (PHD) finger is a motif that can recognize unmodified or methylated lysine residues in histone tails. When present in a protein, it confers the ability to act as "reader", and recognize the methylation state of histone lysine residues (Baker et al., 2008). Two unique subclasses of PHD fingers that can recognize either trimethylated H3K4, a PTM commonly associated with actively transcribed genes (Berger, 2007), or unmethylated H3K4 have emerged. PHD domains in BPTF (bromodomain and PHD finger transcription factor) and ING2 (inhibitor of growth 2), preferentially recognize trimethylated H3K4 over mono- and unmodified H3K4, while PHD fingers in DNMT3L and BHC80 recognize unmethylated H3K4 (Lan et al., 2007; Ooi et al., 2007; Shi et al., 2006; Wysocka et al., 2006). Several other PHD fingers are also thought to associate with different methylated or acetylated lysine marks (Baker et al., 2008; Cosgrove, 2006; Musselman & Kutateladze, 2009). Recently, PHD fingers in two other proteins, SMCX and ICBP90, were demonstrated to recognize trimethylated H3K9, a mark associated with transcriptionally inactive genes (Berger, 2007), and tandem PHD fingers in DPF3 were shown to preferentially bind all acetylated lysine residues of H3 or H4 (Iwase et al., 2007; Karagianni, Amazit, Qin, & Wong, 2008). Many more PHD fingers are likely to act as "readers" of the histone code but more research is needed to identify them.

Point mutations, deletions or chromosomal translocations in the PHD fingers of several proteins have been associated with mental retardation, cancer, and immunological diseases. In mental retardation, PHD fingers within proteins such as mental retardation syndrome X-linked (ATR-X), alpha thalassaemia, KDM5C, PHD finger protein 6 (PHF6), nuclear receptor-binding SET domain containing 1 (NSD1), and CREB binding protein (CBP) have been implicated. These mutations are thought to have a strong impact on the activity of the genes and alter their functions in the brain.

2.1.1. ATR-X

ATR-X is a protein coded by the X-linked gene *atrx*, whose mutations can result in ATR-X syndrome, a disorder characterized by severe mental retardation, microcephaly, seizures and delayed growth (Baker et al., 2008). In the mouse brain, ATR-X is necessary for neuronal survival during corticogenesis, and its alteration interferes with neurodevelopmental processes (Berube et al., 2005). Although the impact of *atrx* mutation on the syndrome is not well defined, it has been suggested to involve anomalies in chromatin structure and gene expression. This derives from the property of ATR-X to interact with several heterochromatin-associated proteins such as heterochromatin protein alpha 1 (HP1alpha), histone lysine N-methyltransferase (EZH2), and methyl-CpG binding protein 2 (MeCP2) (Kramer & van Bokhoven, 2009). The N-terminus of *atrx* also contains an ATR-X-DNMT-DNMT3L (ADD) domain, named based on its sequence homology exclusively with members of the DNA methyltransferase family, and an atypical "PHD-like" domain, implying that ATRX most likely interacts directly or indirectly with DNA or chromatin (Kramer & van Bokhoven, 2009). While more than 40 disease-causing *atrx* mutations have been recognized, 26 are within the PHD finger, suggesting that this region plays a particularly important role in the pathogenesis of the disease (Argentaro et al., 2007).

2.1.2. KDM5C

Another X-linked protein associated with mental retardation is KDM5C. KDM5C contains a PHD that recognizes trimethylated H3K9, and a JmjC domain, which catalyses demethylation of H3K4. Since trimethylation of H3K9 represses gene transcription while trimethylation of H3K4 activates it, the binding of KDM5C acts synergistically to repress gene transcription. In patients with X-linked mental retardation, a point mutation in the PHD finger of KDM5C (A388P) reduces the protein's binding to H3K9 and decreases demethylase activity (Iwase et al., 2007; Tzschach et al., 2006). Likewise, PHF6 is another protein that contains 2 PHD fingers, whose gene (also X-linked) is mutated in Borjeson-Forssman-Lehmann syndrome, a disorder characterized by mental retardation(Baker et al., 2008). Currently, it is thought that 23% of all Borjeson-Forssman-Lehmann syndrome mutations lie within the first PHD finger in *phf6,* suggesting that the potential role of PHF6 in chromatin remodeling is of key functional importance (Mangelsdorf, Chevrier, Mustonen, & Picketts, 2009).

2.1.3. NSD1

NSD1 is another protein involved in epigenetic regulation that acts as either a co-repressor or a co-activator. NSD1 contains a su(var)3–9, enhancer-of-zeste, trithorax (SET) domain with histone methyltransferase activity, and multiple PHD domains (Huang et al., 1998). Mutations in the PHD fingers are associated with two overgrowth syndromes, Sotos syndrome and more rarely, Weaver syndrome. These diseases are characterized by both, pre- and postnatal somatic overgrowth, craniofacial abnormalities, advanced bone age, and mild mental retardation. The fifth PHD finger in NSD1 is necessary for the recruitment of the protein to the promoter, however the sequence of this domain does not contain the known H3K4-engaging residues, suggesting that its interaction with chromatin may be via another yet unknown mechanism (Baker et al., 2008).

2.1.4. CBP

CBP is a transcriptional regulator linked to mental retardation in Rubenstein-Taybi syndrome. CBP has HAT activity and its haploinsufficiency alters brain functions. Such insufficiency has been modeled in mice, and was shown to have severe consequences on cognition. Thus, transgenic mice expressing an inducible form of CBP that lacks HAT activity have deficits in long-term spatial and recognition memory, suggesting that HAT activity of CBP is necessary for long-term memory formation (Korzus, Rosenfeld, & Mayford, 2004). Point mutations or internal deletions in the PHD located within the HAT domain have been implicated in this disorder, including one that alters a conserved PHD finger amino acid (E1278 K) and a second that deletes the exon encoding the central region of the PHD finger, exon 22 (Kalkhoven et al., 2003). Mutations in the PHD finger reduce endogenous CBP HAT activity, suggesting that this domain has functional importance for maintaining a normal epigenetic profile (Kalkhoven, Teunissen, Houweling, Verrijzer, & Zantema, 2002; Kalkhoven et al., 2003).

2.2. Methyl-CpG binding protein-2 (MeCP2)

The recognition of methylation patterns is essential for the epigenetic regulation of gene expression. MeCP2 is a methyl-CpG binding domain (MBD) protein that recognizes methylated DNA by selectively binding to methylated CpGs adjacent to A/T sequences (Klose et al., 2005). Traditionally, MeCP2 has been associated with transcriptional repression because it was found to recruit proteins known to be involved in gene silencing (LaSalle, 2007). However, recently it was also associated with transcriptional activation, and was demonstrated to bind to promoters of active genes in association with the transcriptional activator cAMP response element binding protein 1 (CREB1) (Chahrour et al., 2008). Further, in a human neuroblastoma cell line, SH-SQ5Q, ChIP-chip analysis revealed that MeCP2 indeed associates with actively expressed promoter regions (Yasui et al., 2007), corroborating the hypothesis that MeCP2 is functionally linked with both transcriptional repression and activation. Interestingly in some brain areas like the hypothalamus, MeCP2 was even suggested to play a more important role in gene activation than in repression (Ben-Shachar, Chahrour, Thaller, Shaw, & Zoghbi, 2009; Chahrour et al., 2008).

MeCP2 is expressed ubiquitously across human tissues, but is present in high amount in the brain, particularly in neurons (Monteggia & Kavalali, 2009). In mice and humans, its level increases during postnatal neuronal development, suggesting a conserved function in the maturation of existing neurons, rather than in the generation of new neurons (Balmer, Goldstine, Rao, & LaSalle, 2003; Kishi & Macklis, 2004; Matarazzo et al., 2004). In neurons, MeCP2 expression peaks after mitosis, and continues to be high in postmitotic neurons in the adult, suggesting that it is also necessary for neuronal function in mature neurons (Monteggia & Kavalali, 2009).

Mutations in MeCP2 are primarily linked to Rett syndrome, a disorder that mainly affects girls, with a prevalence of approximately 1:10,000 in alive baby girls (Monteggia & Kavalali, 2009). Rett syndrome is characterized by a progressive cognitive decline despite normal early infancy and by the development of hand stereotypy and seizures that appear between 6 and 18 months of age (Gonzales & LaSalle, 2010; LaSalle, 2007). Mutations in the coding region of MeCP2 are responsible for 96% of classic Rett syndrome (Zoghbi, 2005). However, mutations and dysfunctions in MeCP2 have also more rarely been associated with X-linked mental retardation, neonatal encephalopathy, Angelman syndrome, and autism. MeCP2 mutations include full to mild loss-of-function mutations, and a more general group of duplications and other noncoding mutations that result in altered MeCP2 expression (Gonzales & LaSalle, 2010). MeCP2 duplication syndrome is generally inherited, although *de novo* cases have also been reported (Ramocki et al., 2009). In humans, boys with MeCP2 duplication syndrome have mental retardation, similar to what is observed in transgenic mice overexpressing MeCP2, while girls tend to have more psychiatric symptoms including anxiety and depression (Ramocki et al., 2009).

An overall reduction in brain volume has been seen in girls with MeCP2 mutations and Rett syndrome (Carter et al., 2008). This reduction seems to be due to reduced neuronal size in both cortical and subcortical areas, and reduced dendritic complexity in specific sub-populations of neurons. Indeed, samples from girls with Rett syndrome show that basal dendrites of layer three and five pyramidal cells in the motor and frontal cortex, apical dendrites of layer five of the motor cortex, and basal dendrites of layer four of the subiculum have reduced complexity compared to control (Armstrong, Dunn, Antalffy, & Trivedi, 1995; Bauman, Kemper, & Arin, 1995). Such reduction in dendritic arborization supports the hypothesis that MeCP2 is important for the maturation of neurons. Further, the decreased dendritic arborization correlates with an increase in the presence of immature neurons, and this is associated with a deficit in synaptic formation and/or transmission (Gonzales & LaSalle, 2010). Indeed, MeCP2-deficient mice have reduced excitatory neurotransmission due to reduced excitatory synaptic connectivity, but no real deficit in plasticity or long-term potentiation (Dani & Nelson, 2009).

The functions of MeCP2 in the maturation of neurons are not well understood, but have been suggested to be mediated by the property of MeCP2 to be a transcriptional regulator. MeCP2 can regulate the expression of several genes required for synapse formation, including brain-derived neurotrophic factor (*BDNF*), DNA-binding protein inhibitor *ID1*, early growth response protein 2 (*EGR2*), and transcription factor *JUNB* (Gonzales & LaSalle, 2010). However, microarray analyses examining the transcriptional profile of Rett syndrome patients or of mouse models of the disease have provided inconsistent results, and thus makes it difficult to draw conclusions about the actual modes of action of MeCP2 (Chahrour et al., 2008; Colantuoni et al., 2001; Tudor, Akbarian, Chen, & Jaenisch, 2002). Nonetheless, evidence has suggested that MeCP2 may have additional functions independent of transcriptional regulation, that contribute to the pathogenesis of diseases like Rett syndrome. MeCP2 can also regulate the splicing of reporter minigenes by interacting with the RNA-binding protein Y box-binding protein 1 (Young et al., 2005). This role in RNA splicing is thought to underlie the abnormal pattern of alternative splicing detected in a mouse model of Rett syndrome carrying a truncated mutation in MeCP2 (Young et al., 2005). Additionally, MeCP2 may have a role outside the nucleus, since it has also been localized to post- but not pre-synaptic compartment (Aber et al., 2003), but this role is so far unknown.

Despite the relatively low level of MeCP2 in glial cells, recent findings have suggested that altered MeCP2 function in astrocytes may also contribute to Rett syndrome pathogenesis. Astrocytes with a *mecp2* deficiency cannot support normal dendritic morphology when grown with normal neurons or *mecp2*-deficient neurons (Ballas, Lioy, Grunseich, & Mandel, 2009). This suggests that *mecp2*-deficient astrocytes have an abnormal secretion of soluble factors required for proper neuronal morphology. Consistently, *mecp2* deficiency in astrocytes results in increased BDNF release and decreased cytokine production, including interleukin 1β (IL-1β) and IL-6 (Maezawa, Swanberg, Harvey, LaSalle, & Jin, 2009). Interestingly, such deficiency can affect neighboring mosaic MeCP2-/+ astrocytes through gap junctions leading to an amplification of *mecp2* deficiency in astrocytes (Maezawa et al., 2009).

3. Abnormal epigenetic marks

A normal epigenetic profile is necessary for a gene to be correctly transcribed. An altered DNA methylation or an abnormal profile of histones PTM can have profound effects on gene expression and alter cellular functions. Here, we describe how altered epigenetic marks may contribute to the pathogenesis of mental retardation.

3.1. Imprinting disorders

Imprinted genes, many of which are expressed in brain, are genes that are preferentially expressed monoallelically from either the maternal or the paternal copy (Dulac, 2010). It is believed that approximately 1% of all human genes are imprinted, suggesting that imprinting is an important mechanism for gene regulation (Amor & Halliday, 2008). To date, almost 100 imprinted genes have been identified (Dulac, 2010). Approximately 80% of them occur in clusters on particular chromosomes, and many are thought to be under the control of an imprinting center or imprinting control

element (Reik & Walter, 2001). Most genomic imprints are mediated by a CpG-rich differentially methylated region linked with gene repression. Their differential expression may be tissue-specific, or may occur over a specific time window during development. Interestingly, several genes have sex-specific imprinting features in the hypothalamus and the cortex (Gregg, Zhang, Butler, Haig, & Dulac, 2010). In the hypothalamus, the majority of these genes are observed in females, suggesting that the hypothalamic function of females are under parental control (Gregg et al., 2010). Altered imprinting has been linked with mental retardation and with several diseases including different cancers, psychiatric disorders, and obesity.

Prader-Willi and Angelman syndromes are two prominent imprinting disorders characterized by intellectual disability (Amor & Halliday, 2008). Their clinical symptoms are different, but both involve severe mental retardation. In both syndromes, a cluster of imprinted genes on chromosome 15 has been implicated, and epimutations are also involved in a small proportion of patients (Chamberlain & Lalande, 2010). Prader-Willi syndrome affects approximately 1 in 17,500 children, with a large majority carrying a 15q11-q13 deletion, and a small but significant proportion carrying either maternal disomy 15 or defects in the imprinting center that controls gene expression in chromosome 15 region (Amor & Halliday, 2008). Less than 1% have an epimutation, namely a hypomethylation of the paternally-inherited allele. In the case of Angelman syndrome, a disorder that affects approximately 1 in 16,000 children, approximately 3% have a paternal-only pattern of methylation but biparental inheritance of 15q11.2–15q13, mostly as a result of epimutations (Amor & Halliday, 2008).

3.2. Immunodeficiency, centromeric region instability and facial anomalies (ICF) syndrome

DNA methyltransferase 3b (DNMT3b) is an enzyme responsible for *de novo* methylation of CpG dinucleotides, that is extremely important for the establishment of epigenetic marks across the genome. Compound heterozygous hypomorphic missense mutations in the DNMT3b gene result in immunodeficiency, centromeric region instability and facial anomalies (ICF) syndrome, a rare recessive disease characterized by immune system deficits, instability of pericentromeric satellite 2-containing heterochromatin, facial deformation and mental retardation (Jin et al., 2008). The alterations induced by DNMT3b deficiency are not fully understood but likely result from genomic hypomethylation, a feature that has been observed in both ICF tissue and cell lines (Jeanpierre et al., 1993; Tuck-Muller et al., 2000). They may however also result from the disruption of protein complexes involving DNMT3b since DNMT3b has multiple interacting partners including other DNMTs, histone deacetylases, and chromatin remodeling proteins. Nonetheless, the majority of DNMT3b mutations in ICF patients are in the catalytic C-terminal region of the protein, and rarely interfere with protein–protein interactions (Ehrlich et al., 2008; Kramer & van Bokhoven, 2009).

3.3. Coffin–Lowry syndrome

Coffin–Lowry syndrome is a neurological disease associated with severe mental retardation and skeletal abnormalities in males (Kramer & van Bokhoven, 2009). It is caused by loss-of-function mutations in *RSK2*, an X-linked gene that encodes a serine/threonine protein kinase member of the Rsk family (Kramer & van Bokhoven, 2009). Coffin–Lowry syndrome has been classified as an epigenetic disorder because RSK2 can affect chromatin structure either by the direct phosphorylation of histones, or through interactions with CBP that promote histone acetylation (Urdinguio, Sanchez-Mut, & Esteller, 2009). Histone phosphorylation is thought to facilitate gene transcription by promoting the binding of CBP and consequent histone acetylation, thus RSK2 has synergistic functions to promote transcription (Urdinguio et al., 2009). It is still not known whether the cognitive deficits observed in Coffin–Lowry patients are the result of altered transcription due to epigenetic abnormalities (Kramer & van Bokhoven, 2009; Urdinguio et al., 2009). RSK2 can additionally phosphorylate proteins other than histones, including CBP, suggesting that imapired cognitive functions may also be due to altered transcription mediated through the CREB1/CBP complex (Urdinguio et al., 2009). Thus, the contribution of the epigenetic function of RSK2 to mental retardation still requires further investigation.

4. Diagnostic uses, intervention and prevention

4.1. Using DNA methylation patterns to diagnose diseases associated with mental retardation

The use of epigenetic markers has long been applied to cancer, and the number of markers of susceptibility, likelihood of transformation from precancerous lesions to cancerous lesions, prognosis markers, as well as diagnostic markers has increased tremendously (Deng, Liu, & Du, 2010). DNA methylation signatures in cancerous tissue after surgical extraction can be used as epigenetic biomarkers. For example, methylation of the CpG island surrounding exon 4 in *Septin9*, is increased in colorectal cancer (CRC) tissues compared to normal colon tissues (Lofton-Day et al., 2008). Samples requiring non-invasive or less invasive methods have also been useful for early diagnosis. Altered promoter methylation in urine DNA has been associated with bladder cancer, and changes in DNA methylation of *Septin9* in plasma is associated with CRC (Deng et al., 2010; Lofton-Day et al., 2008). This possibility has important implications for the use of these epigenetic biomarkers in neuroscience, as information gained from DNA methylation in plasma samples in humans, and not from post-mortem brain tissue, may also be of use for brain-related disorders.

The use of epigenetic biomarkers is much more recent in the field of memory disorders. Currently, the usage of epigenetic abnormalities in the diagnosis of mental retardation has been best studied for Down syndrome (DS). Trisomy of all or part of human chromosome 21 (HSA21) results in DS, a disorder characterized by significant mental retardation, and other health problems such as cardiovascular and metabolic defects. Determining the differential methylation of HSA21 has been suggested as a means for non-invasive prenatal detection of HSA21 trisomy and DS (Chim et al., 2008; Old, Crea, Puszyk, & Hulten, 2007; Tong et al., 2010). For instance, the promoter of the holocarboxylase synthetase (HLCS) gene is hypermethylated in DS placenta (Tong et al., 2010). This may offer the possibility to compare the dosage of methylation of this locus and another locus on a reference chromosome to reliably identify trisomy 21 (Tong et al., 2010).

4.2. Potential for epigenetic drugs to alleviate symptoms of mental retardation

The use of drugs that target epigenetic mechanisms, in particular DNMT and HDAC inhibitors, is a new and promising possibility in the clinic. While these drugs have been used successfully in the treatment of cancer and, to some extent in psychiatric disease, little has been done clinically for mental retardation. However, many promising studies in animal models have identified therapeutic potential for epigenetic drugs in neurodevelopmental disorders. For instance, in a CBP heterozygous knockout mouse model of Rubinstein-Taybi syndrome, intracerebroventricular administration of an HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA), can correct long-term emotional memory deficits as

assessed by contextual fear conditioning (Alarcon et al., 2004). Further, in transgenic mice expressing a form of CBP without HAT activity, intraperitoneal administration of the HDAC inhibitor trichostatin A (TSA), can correct deficits in long-term recognition memory (Korzus et al., 2004). These examples of memory rescue are thought to be due to a restoration of normal acetylation levels following the correction of the balance of HDAC/HAT activity (HDAC inhibition in a mouse with reduced HAT activity). However, mice lacking the CBP binding domain known to associate with CREB do not show a change from transient early-phase LTP to transcription-dependent long-lasting LTP, as is normally the result of HDAC inhibition by TSA after a single 100 Hz train (Vecsey et al., 2007). Thus, the formation of a complex between CREB and CBP is necessary for the effects of HDAC inhibition, and is not achieved simply by increasing HAT activity (Vecsey et al., 2007). This suggests that the rescue in synaptic plasticity previously observed using mouse models of Rubinstein-Taybi that were either heterozygous or involved expression of a transgene, occurred due to the wild-type CBP still present and able to bind CREB in these models.

Other options include the use of DNMT inhibitors in fragile X syndrome, a disorder associated with hypermethylation of an expanded sequence of CGG repeats in the *FMR1* promoter that results in a lack of *FMR1* expression and subsequent loss of FMR1 protein (Chiurazzi, Pomponi, Willemsen, Oostra, & Neri, 1998). Treating lymphoblastoid cells taken from fragile X patients expressing a full mutation (>200 CGG repeats) with the DNMT inhibitor 5-azadeoxycytidine fully restores *FMR1* expression and protein level. Such gene reactivation is particularly promising from a therapeutic perspective because males carrying the mutation that do not present with hypermethylation have normal IQ (Hagerman et al., 1994; Steyaert, Borghgraef, Legius, & Fryns, 1996).

Clearly, drugs such as DNMT or HDAC inhibitors have great potential for the treatment of mental retardation, specifically for the types of retardation resulting from deficits during postnatal rather than prenatal life. However, these drugs should be used with caution because of their widespread effect on the epigenome. In turn, more specific epigenetic drugs will be required to optimize the full potential of manipulations of the epigenome.

4.3. Identifying risk factors for neurodevelopmental disorders to reduce disease incidence rates

Identifying risk factors for mental retardation is important to potentially prevent or reduce its occurrence in the population. One of these identified risk factors relates to the use of assisted reproduction technologies (ART), which is thought to disrupt the establishment of the epigenetic profiling during gametogenesis and fertilization. Currently, three of the nine known imprinting disorders including Angelman syndrome (discussed in Section 3.1) have been associated with ART. Interestingly, the epimutation associated with these disorders involves a hypomethylation of the differentially methylated region on the maternal allele (Amor & Halliday, 2008). Because of the low frequency of Angelman syndrome (1/16,000), and the low proportion of Angelman syndrome patients with an epimutation (2–3%), in combination with the relative rarity of ART (2–3%), the incidence rate occurring by chance would be approximately 1 in 20,000,000 (Amor & Halliday, 2008). However, five patients conceived by ART carry this epimutation, suggesting a link between ART and abnormal imprinting. This risk is obviously small in comparison to the success of ART, however work is required to investigate this link and minimize the risk.

Another identified risk factor associated with DS is linked to folic acid/one carbon/transsulfuration (1C–TS) metabolism. 1C–TS metabolism is important for the proper establishment and maintenance of DNA methylation patterns, as folate acts as a methyl donor (Patterson, 2009). Many genes on HSA21 are important for this pathway, and diet, particularly folate intake around the date of conception or during pregnancy, was suggested to affect the rate of DS (Patterson, 2008, 2009). Perinatal folate supplementation was previously shown to reduce the rate of births with neural tube defects. This led to the requirement that all wheat flour based products be supplemented in folic acid in many countries including the United States (Patterson, 2008). This also provided a unique opportunity to investigate the occurrence rate of DS pre- and post-supplementation, but no apparent difference in DS birth rate has been observed (Canfield et al., 2005; Ray, Meier, Vermeulen, Cole, & Wyatt, 2003). The conflicting data regarding the contribution of maternal and paternal diet, maternal grandmother dietary factors, and maternal metabolism towards DS risk factors has been discussed in a recent review, and so will not be discussed here (Coppede, 2009). Nonetheless, due to the numerous evidence that does suggest that perinatal folate intake is linked to DS birth rates, research surrounding the possibility of reducing the risk of DS through diet continues.

5. Conclusions

Epigenetic anomalies seem to be a common thread in the pathogenesis of mental retardation. Many syndromes associated with mental retardation appear to be the result of loss-of-function mutations in single genes, suggesting that gene-therapy approaches may be beneficial. However, this type of therapy in humans is not yet feasible. Rather, the potential usage of DNMT and HDAC inhibitors is likely to be a focus for pharmacological interventions used to treat mental retardation, as has been already the case for cancer and psychiatric disease. In particular, drugs with a higher specificity is required, since many of the epigenetic drugs currently available affect all DNMTs or all class I HDACs (HDAC1, 2, 3 and 8) generally (Gravina et al., 2010; Kilgore et al., 2010). Work is currently underway to increase the specificity of these drugs. For instance, a small-molecule inhibitor, BIX-01294, can selectively inhibit histone 3 lysine 9 (H3K9) methyltransferase activity, thereby transiently reducing H3K9 methylation *in vitro* (Kubicek et al., 2007). However, despite the promise that epigenetic drugs may hold for neurodevelopmental disorders, one must stress the importance of better defining the risk factors for epimutations to minimize occurrence rates. Thus, an increased understanding of the environmental factors that may lead to epimutations prenatally is an important feature of the research still required.

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