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REVIEW PAPER

Understanding and Determining the Etiology of Autism

Salvatore A. Currenti

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Abstract Worldwide, the rate of autism has been steadily rising. There are several environmental factors in concert with genetic susceptibilities that are contributing to this rise. Impaired methylation and mutations of *mecp2* have been associated with autistic spectrum disorders, and related Rett syndrome. Genetic polymorphisms of cytochrome P450 enzymes have also been linked to autism, specifically CYP27B1 that is essential for proper vitamin D metabolism. Vitamin D is important for neuronal growth and neurodevelopment, and defects in metabolism or deficiency have been implicated in autistic individuals. Other factors that have been considered include: maternally derived antibodies, maternal infection, heavy metal exposure, folic acid supplementation, epigenetics, measles, mumps, rubella vaccination, and even electromagnetic radiation. In each case, the consequences, whether direct or indirect, negatively affect the nervous system, neurodevelopment, and environmental responsive genes. The etiology of autism is a topic of controversial debate, while researchers strive to achieve a common objective. The goal is to identify the cause(s) of autism to understand the complex interplay between environment and gene regulation. There is optimism that specific causes and risk factors will be identified. The results of future investigations will facilitate enhanced screening, prevention, and therapy for "at risk" and autistic patients.

Keywords Autism · Vitamin D · Methylation · Folic acid · Methyl-CpG-binding protein (mecp2) · Methylenetetrahydrofolate reductase (MTHFR) · Calcitriol · Autoantibodies · Glucocorticoids · CYP27B1 · MMR vaccine · Heavy metals

S. A. Currenti (✉)
Center for Nanoscale Science and Engineering (CNSE), State University of New York (SUNY), Albany, NY, USA
e-mail: currenti10@aol.com

Introduction

Autism is defined as a neurodevelopmental disorder, characterized by repetitive behaviors, social withdrawal, and communication deficits. The disease has variable cognitive manifestations, ranging from a non-verbal child with mental retardation to a high-functioning college student with above average IQ with inadequate social skills (Gillberg and Coleman 2000). It is also recognized as the second most common developmental disability after mental retardation, among children (Centers for Disease Control and Prevention 2009). Autism occurs predominantly in males, with a male:female ratio of 4 to 1. There is also an increased prevalence in African-Americans. The occurrence of autism varies among nations, and is on the increase in most countries. Strikingly, it is estimated that autism affected ~3 in 10,000 people in 1970 (Deth et al. 2008), while today ~20 in 10,000 people are diagnosed with the disorder. The number of affected people is a subject of debate, with some researchers claiming an incidence of up to 66 in 10,000 people (Rice et al. 2007). No definite cause of autism has been identified to date. However, there are multiple areas that are currently being researched to obtain a better understanding of this disease. Genetic factors alone likely cannot account for an epidemic that developed in a short period of ~20 years (Herbert et al. 2006). Environmental factors must be considered as a primary cause. Environmental perturbations may have a large effect on global and/or localized genetic regulation. Environmental factors are likely to play a major role in the increased prevalence of autism (Deth et al. 2008), even

though genetic factors remain important, as it is indicated by high concordance rates among twins and siblings (Smalley et al. 1988; Bohm and Stewart 2009; Bailey et al. 1995; Steffenburg et al. 1989). Recent reviews have provided further insight into the genetic basis of autism and related disorders (Abrahams and Geschwind 2008; Kelleher and Bear 2008). Abrahams and Geschwind (2008) explore issues regarding the identification of autism susceptibility genes along with de novo and inherited copy number variation (CNV). They explain systems biology approaches, such as array-based genetic profiling, which holds great potential and promise to identify common genetic risk variants among autistic individuals. A systems biology approach is a great method of investigation, and Kelleher and Bear (2008) show there are many genes involved in the etiology of this disorder. In addition, they elucidate several single-gene disorders that correspond with high rates of autism, and that these molecular defects disrupt pathways of synaptic protein synthesis leading to cognitive and neuronal impairment. Overall, awareness and diagnosis of autism and autism spectrum disorders has improved recently, however, it is important to address the need to identify and narrow the list of potential causes that are strongly entrenched within genetic, epigenetic, nutritional, and environmental causes.

Genetic Alterations in Autism

The genetic studies are highly focused on mutations involving methyl-CpG-binding protein *mecp2*. Rare mutations in *mecp2* have been identified in autistic individuals and have implications as a major causative factor in a related disease, Rett syndrome (Loat et al. 2008). CpG islands are simply genomic regions containing a high frequency of CG dinucleotides, and are present in a majority of human promoters. Protein MeCP2 binds to methylated CpGs to repress the transcription of downstream genes (Chen et al. 2003). MeCP2 is highly expressed in neurons, and CpG binding sites are hotspots for mutations (Bird 2008). MeCP2 primarily functions as a transcriptional repressor by recruiting histone deacetylases to DNA containing markings for methylated cytosines (Nan et al. 1998). There are potentially three ways in which DNA methylation can cause gene silencing: (1) altering chromatin, changing DNA accessibility; (2) interfering with a DNA-binding protein; (3) attracting proteins with affinity for methylated DNA (Bird 2008). MeCP2 target genes, which are efficiently repressed in non-affected individuals, escape repression in autistic individuals. In addition, *mecp2* loss of function mutations and duplications, as well as modest changes of MeCP2 protein levels result in neurodevelopmental deficits (Samaco et al. 2008).

Methyl-CpG-binding protein has been associated with risk of autism. Its gene product, MeCP2 is capable of binding methylation sites on DNA. This DNA-binding protein is capable of interpreting biochemical signals to bring about transcriptional repression, primarily in the brain. As known, mammalian DNA is compacted by use of histone proteins. Histones are positively charged, and attract negatively charged DNA to wrap around the protein. It is estimated that ~47 base pairs wrap around histones. These regions of highly wrapped DNA known as nucleosomes are segments of genetically inactive chromatin (heterochromatin), as opposed to active sites that are termed euchromatin. The binding of MeCP2 to methylated DNA is also capable of recruiting histone deacetylases, and other chromatin remodeling proteins to form compact, inactive chromatin (Bird 2008). Loss of MeCP2 has been implicated in autism and analogous diseases, such as Rett syndrome.

Mecp2 is X-linked, subject to X-inactivation, and gene mutations may cause neurodevelopmental disorders such as autism (NCBI 2008). X chromosome inactivation (XCI) reduces the number of actively transcribed X chromosomes to one per diploid set of autosomes, allowing for dosage equality between the sexes (Starmer and Magnuson 2009). The prevalence among males suggests X-linkage, and genome-wide screens have found evidence of X-chromosome linkage (Shao et al. 2002; Liu et al. 2001). Human proteins MeCP2, MBD1, MBD2, and MBD4 each have a methyl-CpG-binding domain, and are capable of repressing transcription from gene promoters (NCBI 2008). A schematic from Bird (2008) elucidates this theory, in Fig. 1.

This figure represents a segment of nucleosomal DNA. It is simply a region of highly condensed heterochromatin. In each case, the DNA-binding proteins: MeCP2, MBD1, MBD2, are binding to methylation sites on DNA and recruit different corepressors. As seen in both prokaryotes and

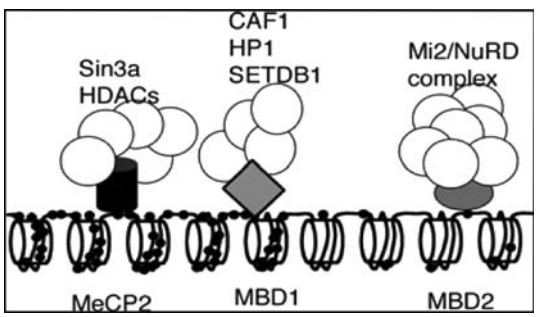

Fig. 1 Protein complexes are bound to methylated sites on nucleosomal DNA. Each protein shown is bound by a corepressor, which assists with transcriptional repression. In this state DNA is heterochromatin, and thus, inactive. Normal transcriptional repression (Bird 2008)

eukaryotes, the structure of multiprotein complexes at gene promoters mediates positive and negative transcriptional regulation. In the case of MeCP2, a histone deacetylase complex is recruited, hence assisting repression. On the contrary, a mutation(s) in gene *mecp2* alters the protein product, affecting its DNA-binding affinity and precluding the occurrence of transcriptional repression. This type of histone modification does not result in any changes in DNA sequence, but methylation and de-acetylation represent a major feature of epigenetic silencing (Fukushige et al. 2008). It has been shown that mutation in MBD1, a methylated CpG-binding protein was found in autistic individuals (Li et al. 2005). Gene function may be changed by either a modification in the DNA sequence or a change in epigenetic programming of a gene in the absence of a sequence change (Szyf 2009). Epigenetics defines all mitotically and meiotically inherited changes in gene expression not coded in the DNA sequence itself, which may alter phenotype without changing genotype (Egger et al. 2004). Epigenetic modulations, such as DNA methylation within CpG islands, could result in heritable silencing of genes without changing DNA sequence (Zhao et al. 2007). Due to the complexity of autism, and importance of DNA methylation, a strong epigenetic link may be present. Most human diseases are related in some manner to the gain or loss in gene functions, however, irregularities in chromatin remodeling are associated with genetically and environmentally related diseases (Liu et al. 2008). Rassoulzadegan et al. (2007) contend that although epigenetic modifications do not result in changes of the gene structure, a progressive accumulation of epigenetic inheritance occurs in organisms ranging from mice to humans. This represents a novel approach to study the causes of many forms of genetic, hereditary diseases. It is important to realize that this form of modification appears to be heritable, and has an effect on subsequent generations of progeny. Then, the following mysterious question is sound: if there is no change in DNA sequence, how is it affecting progeny?

Martinowich et al. (2003), identified the first mammalian target gene of MeCP2. This gene encodes BDNF, which is one of several proteins synthesized in response to neuronal activity, and is essential for converting stimuli into changes in brain activity. BDNF levels are reduced in *mecp2*-null mice (Chang et al. 2006). The research team is unsure of the precise way why this occurs. There may be a second transcriptional repressor protein, capable of repressing BDNF in the absence of MeCP2. However, this team concludes that BDNF has a downstream role of MeCP2. In this case, there may be a "pleiotropic effect" which is responsible for this regulation implying an indirect effect on BDNF regulation. A pleiotropic effect simply occurs when one gene has the capability of directly or indirectly affecting many other genes. Microarray experiments have shown glucocorticoid-responsive genes to be upregulated in the brains of *mecp2*-null mice (Nuber et al. 2005). Glucocorticoids are steroid hormones that can inhibit glucose uptake and promote lipolysis, among other outcomes. In fact, excessive glucocorticoid circulation has negative effects on the brain, and postnatal treatment of children with glucocorticoids display poor motor skills and coordination as well as low IQ scores (Abraham et al. 2001). Nuber et al. showed SGK1 (glucocorticoid-inducible kinase) and FKBP5 (proline isomerase associated with glucocorticoid receptor) are both upregulated in the absence of *mecp2*. These are genes normally repressed by MeCP2, and are good examples of how MeCP2 absence directly affects gene expression.

Samaco et al. (2008) characterized a conditional mouse allele of *mecp2* that expressed half of the wild type level of MeCP2. Figure 2 displays the down-regulation of MeCP2 in wild type versus mutant mice.

It is evident that *mecp2* mutant mice (2D) show decreased immunofluorescent signal of MeCP2, as opposed to wild type mice. The researchers determine that mice harboring the mutant allele display abnormalities, such as learning and motor deficits, and altered social behavior patterns. They conclude that control of MeCP2 is critical for neurodevelopment, and that neurodevelopmental disorders arise from even a slight attenuation in MeCP2 expression levels. A study conducted by Nagarajan et al.

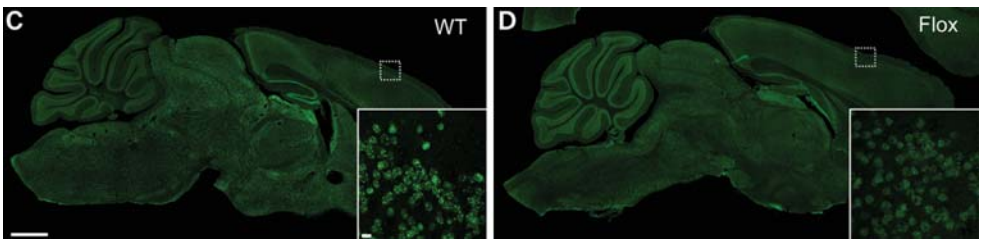

Fig. 2 A section of the cortical region of the brains was taken and magnified to reveal the level of fluorescent signal, which correlates with expression of MeCP protein levels. Although the cellular distribution of MeCP2 is similar, it is clear that the fluorescent signal in 2D (*mecp2* mutant) is decreased as opposed to the wild type *mecp2* (2C). The mutant mice exhibited decreased motor performance, which was not seen in the wild type mice (Samaco et al. 2008)

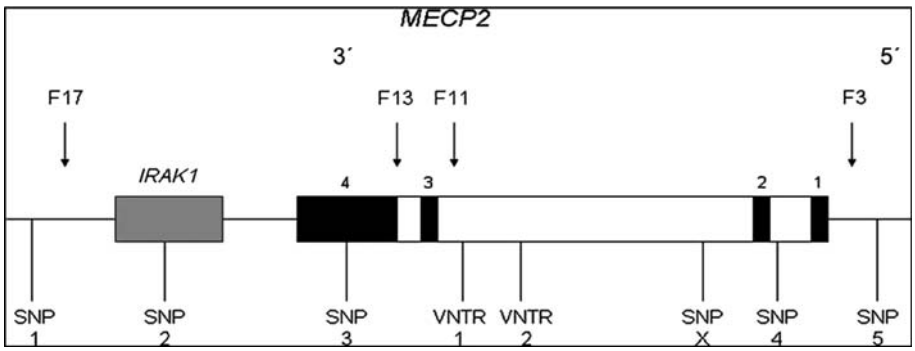

Fig. 3 Gene *mecp2* and neighboring gene *irak1*, with exons labeled in *black* (numbered 1–4) from 5′ to 3′. Single nucleotide polymorphisms and variable number of tandem repeats are labeled as SNP and VNTR, respectively. The "F" designation indicates regulatory regions: F3 and F13 are silencers and F11 and F17 are enhancers (Loat et al. 2008)

(2006) found that in 80% of autistic people the brain tissue has a reduced MeCP2 level of expression. Reduced MeCP2 expression was also found in many tissue samples from Prader-Willi, Angelman, and Down's syndromes patients (Zhao et al. 2007).

Mecp2 compromises four exons (Fig. 3), with the coding sequence shared among exons 2–4, and highly conserved 3′ UTR of 8.5 kb (Coy et al. 1999). Increases and decreases in *mecp2* expression have been implicated in several pervasive developmental disorders including autism, suggesting a common pathway involved in these disorders (Samaco et al. 2004).

This figure diagrams the *mecp2* gene, along with neighboring gene *irak1*, and presence of various single nucleotide polymorphisms (SNP's). Loat et al. (2008) concluded that variations in both coding and non-coding regions of *mecp2*, as well as flanking regions are important in the development of autism. The study investigates polymorphic variants, including flanking and intronic regions (non-coding) to locate potential genetic markers for autism.

In sum, there is compelling evidence supporting the involvement of MeCP2 in the risk of autism. Simply, MeCP2 is a transcriptional repressor protein involved in gene silencing, primarily in the brain. It has been shown that mutations of *mecp2* negatively affect neuronal structure and integrity.

Immune System and Autism

In utero, children are readily supplied with maternal antibodies. This is essential to supply the developing fetus with humoral immune system proteins. Other studies show, however, that in mothers with autistic children there is maternal immunoglobulin reactivity against fetal brain proteins (Singer et al. 2008; Braunschweig et al. 2008). This hypothesis asserts that placental transfer of maternal antibodies will interfere with fetal brain development (Singer et al. 2008), even though there have been no case-based studies explaining the direct relationship between maternal autoantibodies and human fetal neuronal proteins and subsequently the development of autism spectrum disorders (Braunschweig et al. 2008). Additional studies conducted on mice demonstrate that maternal infections alter pro-inflammatory cytokines in the brain with a significant impact on their brain development (Urakubo et al. 2001). Furthermore, the maternal use of acetaminophen has been suggested to have a negative effect on the fetal brain. Acetaminophen allegedly interferes with interleukin IL-6, which is important in the development, differentiation, and even degeneration of neurons in the central nervous system (Marz et al. 1999).

Moreover, the immune system may have a definite role in autism. Families with autism show clustering of autoimmune disorders (Croen et al. 2005). Autistic children have serum antibody reactivity against human cortical and cerebellar brain regions of the brain (Silva et al. 2004). It is suggested that this process begins in utero and is associated with placental transfer of maternal autoantibodies with a negative effect on fetal brain development (Singer et al. 2008). Western blot experiments were conducted (Braunschweig et al. 2008; Singer et al. 2008) using fetal and adult brain tissues to identify reactivity against fetal brain proteins. These latter studies show autoantibody reactivity to proteins of 37 and 73 kDa, and this peculiar phenomenon occurs more frequently in mothers with autistic children than mothers with non-affected children. It is interesting to note that these protein bands are not found in some individuals with autistic children and, therefore, it emphasizes that a variety of etiological mechanisms is likely to take place (Hertz-Piciotto et al. 2006). Braunschweig et al. (2008) state that the presence of anti-fetal brain antibodies in the blood circulation of childbearing mothers, in combination with genetic susceptibility, may trigger an effect on neurodevelopment leading to autism. Their data suggest that maternal autoantibodies reactive to

fetal brain proteins of 37 and 73 kDa molecular weight confer an increased risk for autism. Some weaknesses of the above described include: (1) selection of mothers skewed toward having offspring with developmental regression; (2) evaluation of data at a single time point; (3) exclusion of mothers of autistic children with recognized metabolic causes of autism. However, the studies display a promise linking the maternal immune system to increased risk of autism, while work is currently being conducted in a larger cohort to determine protein–antibody targets.

Maternal infections have also been identified as a potential risk factor in the cause of autism. Fever is the body's response to microbial attack, and is induced to destroy pathogens. Most infections, either bacterial or viral usually result in fever. Since virus cannot be treated with antibiotics, low-grade fever may persist for longer periods of time. In addition, fever reducers are used for extended periods of time in viral infections. The therapy of fever with anti-pyretics, such as acetaminophen, interferes with normal immunological development in the brain, leading to neurodevelopmental disorders such as autism (Torres 2003). Goetzl et al. (2002) show that the treatment of epidural fever with acetaminophen decrease maternal and fetal IL-6 levels at birth. IL-6 is a pro/anti-inflammatory cytokine, an important mediator of fever, and its receptor IL-6R is expressed on neurons (Torres 2003). There is evidence that IL-6 is an important mediator of development and differentiation of neurons in the central nervous system (Marz et al. 1999). It has also been reported that 43% of mothers with an autistic child experienced a respiratory, influenza, urinary, or vaginal infection(s) during pregnancy as opposed to only 26% of control mothers (Comi et al. 1999). There has also been much speculation of the measles–mumps–rubella (MMR) vaccine as a cause of autism. After vaccination, approximately 50–60% of children develop fever with the presence of antibodies as a result of the immune system response to the MMR virus (Stuck et al. 2002). Nevertheless, this effect is relatively benign, and, to date, there is no definitive evidence linking MMR vaccine to autism.

Folic Acid and Vitamin D (Calcitriol)

Approximately 20 years ago, health agencies recommended that childbearing women supplement their diet with folic acid to reduce the risk of neural tube defects (NTD's) and to facilitate proper neural tube closure, preventing Spina Bifida. Research has shown that folate can reduce the incidence of neural tube defects by about 70% and can also decrease the severity of these defects when they occur (Holmes 1988; Milunsky et al. 1989; Mulinare et al. 1988). Genetic polymorphisms of enzymes required to metabolize folate are common with autism and display up to 50% reduction in activity, hence attenuating the ability of folate to function in cellular processes (Scriver et al. 2000). The enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) is required to convert folic acid into a form that can be utilized for methylation (Rogers 2008). By increasing maternal folic acid intake, there is an increased survival rate of children possessing a mutated MTHFR enzyme, C677T mutation. As a result, these children have a decreased enzymatic activity and, if not supplemented with folic acid during development, will eventually experience neurological regression and show an increased risk of autism. Thus, mutations in MTHFR have been associated with the development of autism.

As mentioned, health agencies made recommendations to childbearing women that they should supplement their

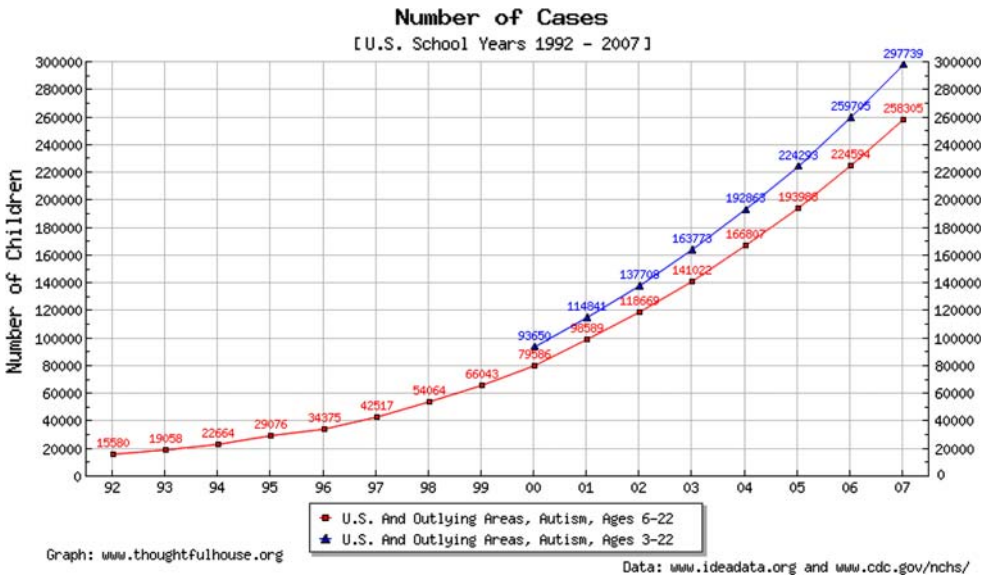

Fig. 4 This graph clearly illustrates the near exponential increase in the number of cases of autism in the US between the years 1992 and 2007. Plots are presented for age groups 3–22 upper curve (*triangles*) and 6–22 lower curve (*squares*) years of age (http://www.fightingautism.org/idea/autism.php)

diet with folic acid to prevent neural tube defects (NTD's). The recommended dose remains 400–1,000 μg. Although this supplement has decreased the rate of NTD's, many feel that, as a result, autism is on the rise. Rogers (2008) investigates this hypothesis, and Fig. 4 displays the cases of autism from 1992 to 2007.

Interestingly, the US Public Health Service has acknowledged a link between inadequate folic acid intake and neural tube defects, and in September 1992, recommended folic acid supplementation (Rogers 2008). It seems that genetic polymorphisms of folic acid metabolism enzymes are common, and attenuate the ability of folate to function (Scriver et al. 2000). A key enzyme—methylenetetrahydrofolate reductase (MTHFR)—is the enzyme required for folate metabolism, whereas the presence of C677T mutant form has been linked to autism (Boris et al. 2004). The hypothesis by Rogers (2008) affirms that by increasing folic acid supplementation, birth rates of children containing MTHFR mutation(s) have increased. A normal or decreased folic acid status in fetuses with attenuated MTHFR activity results in increased risk of miscarriage, and, after birth, the children still require folic acid supplementation to compensate for reduced enzymatic activity. Folic acid plays, hence, an important role in proper methylation, which is important for gene silencing. The children who possess MTHFR mutations and do not receive folic acid supplementation are at risk for developing autism. Figure 5 displays the pathways involved in this hypothesis.

This hypothesis explains how increasing folic acid supplementation alters the natural selection in favor of an adverse gene polymorphism, MTHFR C677T, which is found in high frequency in autism (Rogers 2008). A requirement of folic acid supplementation in children is, therefore, recommended for normal methylation and to promote a proper neurodevelopment. Moreover, hypotheses involving *MTHFR* implication with autism state that as a result of increased folic acid supplementation, natural selection has shifted in favor of progeny possessing a specific mutation(s) that would otherwise increase the risk of miscarriage in absence of folic acid supplementation. Simply, *MTHFR* enzyme metabolizes and activates folic acid for methylation, among other cellular functions, and attenuated phenotypes have negative effects on neuronal integrity. To overview the folic acid hypothesis, there is strong evidence that suggests this phenomenon is not coincidental.

The environment influences responsive genes and, subsequently the genome. Vitamin D is a neurosteroid (McGrath et al. 2001) and follows this type of genetic organization (Cannell 2008). Calcitriol (activated vitamin D) deficiency in mice produces offspring with abnormal cell proliferation, and reduced expression of neuronal structure genes (Feron et al. 2005). Experimental studies showed that vitamin D deficiency dysregulates 36 proteins involved in mammalian brain development (Almeras et al. 2007). Importantly, vitamin D has also been shown to: (1) down-regulate neurologically harmful cytokines in the brain (Moore et al. 2005); (2) partially reverse brain damage (Burne et al. 2004); (3) increase cellular levels of the anti-oxidant glutathione (Garcion et al. 2002), which is capable of removing free radicals and also chelating heavy metals, including mercury (Kern and Jones 2006).

Cui et al. (2007) have determined that vitamin D is important for neural development and its deficiency negatively alters the brain structure and function. As mentioned, vitamin D, a neurosteroid, is considered to be an important regulator of cell proliferation in the developing brain, while

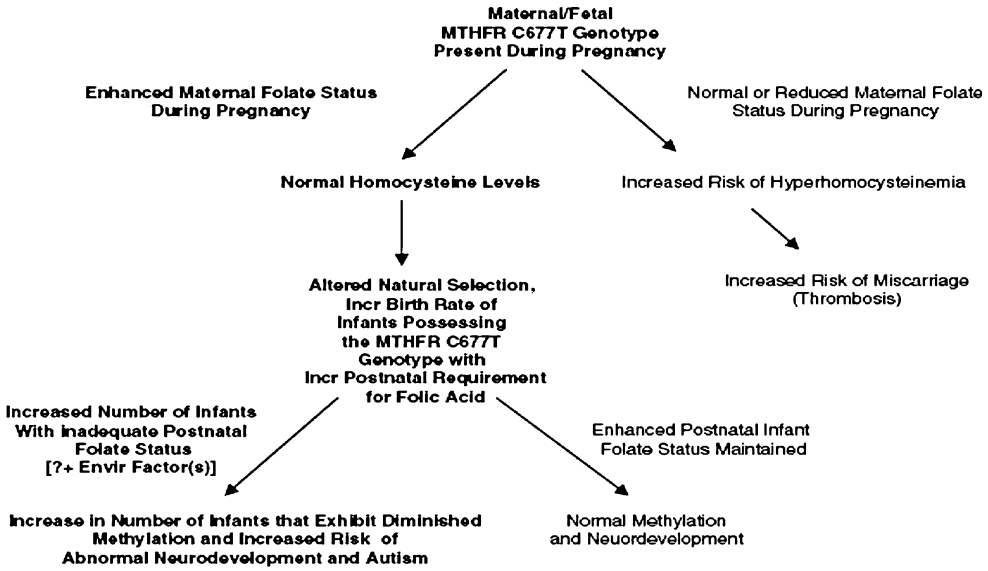

Fig. 5 This pathway illustrates hypothetical neurological outcomes of a fetus possessing MTHFR C677T mutations. In children possessing the mutation with decreased folic acid, results in an increased risk of miscarriage. In those possessing the mutation, with enhanced folic acid supplementation, there is greater likelihood of normal neurodevelopment as opposed to infants born and lack postnatal supplementation will have an increased risk of autism (Rogers 2008)

calcitriol, an activated form of vitamin D, is an up-regulator of nerve growth factor and its receptor is found in a variety of brain tissues early in embryogenesis (McGrath et al. 2001). A deficiency of vitamin D may result from inheritance of polymorphisms in cytochrome P450 gene, CYP27B1, which is required for vitamin D activation (Cannell 2008). CYP27B1 is an enzyme of interest in the study of autism, but, to date, no research has been conducted and/or published analyzing this potential correlation.

Approximately 0.5% of the human genome, which represents only 200 genes, is targeted by calcitriol (Kalueff et al. 2006). It is remarkable that 90.0% of the human vitamin D supply comes from skin production and not from oral intake (Holick 1987). Taking into consideration that sun avoidance has become more common in recent times due to the fear of skin cancer, the increase in autism is steadily increasing, specifically after 1989, when the American Medical Association (1989) warned about the risks of sun exposure and advised mothers to keep infants out of the sun as much as possible. See Fig. 4 for details. In fact, abnormalities in immune responses have been implicated in both autism and vitamin D deficiency (Cannell 2008). Autistic individuals have T cell abnormalities and cytokine excess, which is similar to immune functions affected by vitamin D (Ashwood et al. 2006). Another interesting function of vitamin D (calcitriol) is its ability to increase cellular glutathione levels (Garcion et al. 2002).

Other investigators discovered that estrogen has the capability of increasing neural and cellular calcitriol levels (Epstein and Schneider 2005), while testosterone does not. It is, indeed, well documented that autism occurs much for frequently in males than females. Cannell (2008) concludes that estrogen shields female brains from calcitriol deficiencies, providing strong evidence to clarify this discrepancy. Moreover, as previously mentioned, autism occurs more frequently in African-Americans as opposed to Caucasians (Bhasin and Scendel 2007). African-Americans have a darker pigmented skin color and, therefore, require an elevated exposure to sunlight in order to compensate for the deficiency of vitamin D. Bodnar et al. (2007) also finds that only 4.0% of black women and 37.0% of white women were vitamin D sufficient during gestation. This is very significant in light of the fact that experimental research demonstrates that vitamin D functions as a potent regulator of neuroprogenitor formation in the developing brain (Cui et al. 2007).

The potential involvement of vitamin D and folic acid supplementation, therefore, strongly supports the interest of gene–environment interplay. The results of investigations on the role of vitamin D are very interesting because they may explain specific discrepancies regarding occurrence of autism, such as the higher occurrence in African-Americans and males. Most African-Americans, especially those living in colder climates with less exposure to sunlight, are vitamin D deficient. In general, African-Americans, due to their dark pigmentation of the skin, require more sun to satisfy their vitamin D requirements.

Furthermore, CYP27B1 mutants have been linked to the inability of efficiently metabolizing vitamin D, potentially leading to neurological regression. Vitamin D may be directly involved in the gender disparity between males and females, as autism has a 4 to 1 male to female occurrence. Estrogen has the capability to increase neural calcitriol (active vitamin D) levels, as opposed to testosterone that does not. Both of these facts present compelling evidence that CYP27B1 and vitamin D levels are important mediators of normal neurological functions, and may explain these discrepancies. In light of the fact that polymorphisms of CYP27B1 have been implicated in other neurodevelopmental diseases, they may prove to play a major or principal role in the development of autism. Emerging research strongly suggests environmental changes that have occurred within the past 30 years are influencing our genetic makeup, leading to the conception of children with an increased risk of developing neurological disorders. Alterations in MTHFR and CYP27B1 follow this conjecture.

Heavy Metals

Autism has also been associated with neurotoxic exposures to heavy metals, such as mercury. Mercury exposure in the US constitutes a major problem. It is debatable that exposure to heavy metals, mainly mercury (recognized as a potent neurotoxin), is a causative factor of autism.

The Food and Drug Administration (FDA) estimates that 16% of women have mercury levels, capable of inducing neurological damage to their children (Mahaffey et al. 2004). A decreased ability to excrete mercury has been implicated in autism. Glutathione, an anti-oxidant, is capable of removing free radicals and heavy metals, such as mercury, because it acts as a chelating agent, binding mercury and excreting it via bile (Kern and Jones 2006; Ballatori and Clarkson 1985). Glutathione concentration has been shown to be lower in autistic children versus unaffected children (James et al. 2004). The use of oral antibiotics, in mice, has been shown to decrease the capacity for mercury excretion (Rowland et al. 1980). Additional research has discovered that the incidence rate of autism is high in areas containing coal-burning plants, which emits increased amounts of lead and mercury into the air (Zhang and Wong 2006).

Bernard et al. (2001) have reported symptoms of autism in cases of infantile mercury poisoning, hence suggesting a direct correlation between infantile mercury exposure and autism. Most mercury exposure is from environmental contamination, seafood, and maternal dental fillings. A

common source of environmental mercury exposure is a byproduct of industrial combustion and coal burning. Inorganic mercury (Hg^{2+}) is nephrotoxic, and can damage muscle tissue. The most potent form of mercury toxicity is mediated by methylmercury (CH_3Hg^+), which has capability of crossing the blood–brain barrier; its lipophilic nature allows binding to neurons acting as a potent neurotoxin. Methylmercury may bio-accumulate in aquatic life, primarily sport fish. Microorganisms and algae in waterways have the capability to convert elemental mercury (Hg^0) into methylmercury (CH_3Hg^+). Mercury toxicity may result from either decreased ability to excrete mercury or high exposure, with the former seeming to be the primary concern in autism (Adams and Romdalvik 2007). Excreting mercury involves binding to glutathione, whose levels are much lower in autistic children (James et al. 2004). Adams et al. (2003) found that autistic children tend to use large doses of antibiotics and it is demonstrated in mice that this behavior results in a decreased ability to excrete mercury (Rowland et al. 1980). Since mercury has a half life of only several weeks in the blood, Adams and Romdalvik (2007) have conducted a study to analyze levels of mercury, lead, and zinc in baby teeth of autistic and non-autistic children. Baby teeth are used to represent a long-term exposure. Table 1 displays their findings.

They confirm that autistic children have, on average, a twofold higher level of mercury than non-autistic children. The degree of difference of lead concentration between the two groups is not statistically significant, while the levels of zinc are nearly identical. The increased mercury level suggests a higher burden of mercury during prenatal development and, since mercury is a neurodevelopmental toxin, it may be responsible for the development of autism in some children participating in this study (Bernard et al. 2001). It is interesting that the research team identify that the major factor in the medical history of autistic children is the higher use of antibiotics. In addition to the inability to excrete mercury, the researchers find that the use of oral antibiotics reduces the amount of normal gut flora, while causing an increase in the number of yeast and *E. coli* cells. They also reveal the capability of gut flora to de-methylate methylmercury, whereas yeast and *E. coli* methylate organic mercury. In brief, methylmercury is the most toxic form of mercury exposure, and is responsible for high absorption and decreased excretion of mercury. The results of this study call, indeed, for further research in a larger study to corroborate the research team's findings.

Table 1 This table shows the concentrations of three forms of heavy metals (mercury, lead, and zing) in baby teeth of autistic and non-autistic children (Adams and Romdalvik 2007)

Baby tooth analysis in children with autism versus typical control children		
Component	Autism	Controls
Hg, mean	0.15 ± 0.11*	0.07 ± 0.06
Hg, median	0.14	0.05
Pb, mean	0.38 ± 0.32	0.29 ± 0.14
Pb, median	0.3	0.26
Zn, mean	100 ± 20	98 ± 16
Zn, median	93	96

Note Units are Hg g

* Significant difference from control ($P < .05$)

The Redox/Methylation Hypothesis

Deth et al. (2008) present this hypothesis, and explain how genetic and environmental factors combine to define risks of developing autism. They postulate that most autism cases reflect environmental exposures (heavy metals, xenobiotics), but genetic factors still define the at-risk population via polymorphisms (SNPs). A chart of this hypothesis is represented in Fig. 6.

Environmental factors can create oxidative stress in genetically susceptible individuals, which may potentially initiate a cascade of events. Inhibition of methionine synthase affects methylation activity, and reduced DNA methylation interferes with normal development, and proper gene silencing. Impaired phospholipid methylation leads to disruption of neuronal networks, consequently leading to attention and cognitive deficits. Deth et al. (2008) conclude that the risk of autism can be influenced by polymorphisms (SNPs) at any level in neuro-anatomic or metabolic

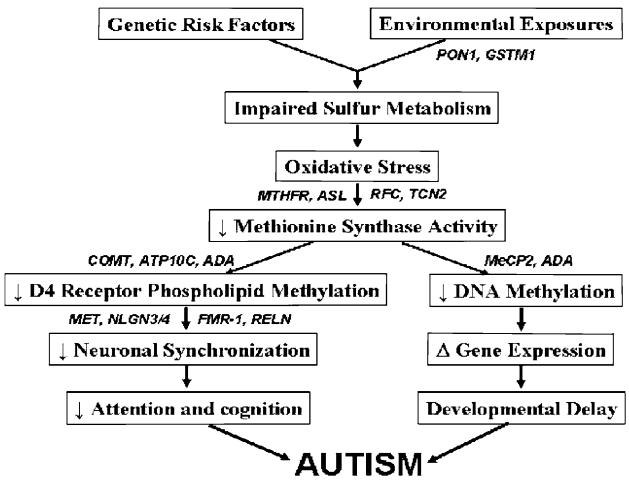

Fig. 6 This hypothesis illustrates many risk factors associated with increased risk of autism. Genetic susceptibilities and environmental exposures combine displaying multiple levels of alterations that may lead to autism and poor neurological development (Deth et al. 2008)

pathways associated with neuronal development. The importance of this hypothesis is that neither genetic nor environmental exposure alone causes autism. The risk factors involve a combination of genetic susceptibilities and environmental exposures. Interestingly, cytogenetic aberrations in autistic individuals have been located in nearly every chromosome (Gillberg 1998). In summary, the convincing evidence elucidates that multiple genetic mutations in concert with environmental perturbations appear to influence neurodevelopment and an increased risk of autism.

Other Hypotheses

Research groups have evaluated the potential role of electromagnetic radiation (EMR) in the etiology of autism (Mariea and Carlo 2007; Thornton 2006). EMR has been shown to disrupt cellular transport and integrity, and trap heavy metals in cells, hence leading to symptoms of heavy metal toxicity. In light of the fact that some individuals have a better ability to excrete heavy metals, genetically susceptible individuals are at greater risk. The investigators have assessed that, between 1998 and 2007, wireless technology usage has increased from 200 million worldwide to 3 billion. Looking back at Fig. 4, it is evident that the cases of autism increase more rapidly around this time frame. Even though there is no consensus that EMR is a direct cause of autism, it appears that the EMR hypothesis may constitute a compelling factor. Also, low intensity magnetic fields (EMF) have been implicated in several neurological diseases, including: Alzheimer's disease, motor neuron disease, and Parkinson's disease (Hardell and Sage 2008). There is substantial evidence to necessitate the need for reduction in EMF exposures given the wide range of negative health outcomes of exposure, especially in vulnerable groups such as young children (Otto and von Mühlendahl 2007). MMR vaccination was also hypothesized by Wakefield et al. (1998) to contribute to the pathogenesis of autism. In 1999, Fombonne (1999) reviews several epidemiological studies of autism and uncovers no association between autism and live MMR vaccination. This review contradicts a previous publication by Wakefield et al. (1998). A more recent review by Miller (2003) asserts no epidemiological evidence has been produced that suggests an association between MMR vaccine and autism. In sum, both MMR and EMR have been hypothesized to be potential causes of neurological damage with regard to autism, however, these theories have not been indisputably verified and should be further investigated.

Summary and Concluding Remarks

Genetic analyses have shown mutations of *mecp2* to be directly associated with autism. It is documented in Bird (2008) that MeCP2 binds methylated DNA sites to bring about gene silencing primarily in the brain, and when mutated, genes that are normally silenced escape repression. However, no genomic search of MeCP2 targets in vivo has been conducted. This finding will likely give insight into identifying other gene-related diseases. The enzyme MTHFR, required for folic acid metabolism, directs metabolites for methylation. No search has been conducted to study the relationship between MeCP2 binding/regulation and MTHFR/folic acid. Two experiments that would be of great benefit are Chromatin-Immunoprecipitation followed by microarray hybridization (ChIP-chip), and transcriptional profiling (hybridization of cDNA to a microarray). Both of these techniques are systems biology approach to study aspects of genomic organization and regulation. The ChIP-chip method involves locating specific protein–DNA interactions, whether direct or indirect, on a genome-wide scale. This would buttress the identification of all MeCP2 target genes, in vivo. Aside from this specific technique, it is important to clarify that systems biology or simply, large scale genomic approaches need to be utilized in order to make progress in determining the cause(s) of autism.

Autism has become a worldwide concern, and is a very common neurodevelopmental disorder among children. It has become a very prevalent disease among children within a short period of time. Affected individuals display normal development early in life, followed by neurological regression, along with social and behavioral deficits. Many researchers have implicated various environmental factors and genetic susceptibilities in the increased risk of autism. It is important to understand that all hypotheses are currently areas of active research, even though some theories appear more tangible than others.

The long-term goal is, indeed, the gain of insight into the genetic versus environmental role to better understand the etiology of autism. If the cause(s) of autism can be identified, enhanced screening and therapies must be developed to potentially prevent, treat, or cure the disease's symptoms. Extensive research has shown that early diagnosis and intervention greatly improve a child's long-term outcome (Filipek et al. 2000; Moore and Goodson 2003). Currently, autism has a large public health impact and may persist for quite some time, until tests, treatments, or cures are identified. There are many researchers hypothesizing specific forms of causation, but the reality is simple. This is a complex disorder with multiple environmental and genetic causes, and hopefully novel research developments will lead to a major discovery in the prevention and treatment of individuals with autism.

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