Genetic Polymorphism Related to Oxidative Stress in Autism

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1 Introduction

Autism is a neurodevelopmental disorder that is characterized by impaired communication and reciprocal social interaction and by repetitive behavior and restricted interest. Strong evidence derived from family and twin studies supports the role of genetic factors in the etiology of this complex disorder (Losh et al. 2008). The mode of inheritance is unknown, but the involvement of both common and rare variants is suggested, and several dozen autism spectrum disorder (ASD) susceptibility genes have been identified in the past decade (Geschwind 2011).

The prevalence of autism and ASD has dramatically increased during the past 3–4 decades, from 3 in 10,000 children in 1970 to approximately 20 (autistic disorder)–30 (pervasive developmental disorder, not otherwise specified, PDD NOS) in 10,000 (Fombonne 2009). Although genetic etiology may be the foremost etiological factor of autism, it is not sufficient to account for the overall changes that have occurred within a few decades. Other factors that play an important role in the etiology of ASDs are an increased rate of detection, a widened range of diagnostic criteria, and environmental factors.

Prevailing evidence supports the involvement of genetic, epigenetic, and environmental factors that negatively affect prenatal and postnatal neurologic development (Folstein and Rosen-Sheidley 2001; James et al. 2006). Oxidative stress and the susceptibility of an individual to oxidative stress disorders are proposed as key elements in mediating the influence of environmental factors and genetic predisposition in the development of autism. These influences have been reported in a wide

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variety of other chronic neurological disorders, including schizophrenia, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, HIV-associated dementia, and fetal alcohol syndrome (Bowers et al. 2011; Cohen-Kerem and Koren 2003; Do et al. 2009; Perry et al. 2004; Zawia et al. 2009).

In addition to the metabolic and biological changes associated with oxidative stress and susceptibility genes, the role of specific oxidative stress genes or endogenous antioxidants in the development of autism has been proposed, and research has been focused on understanding the development of mutations and genetic polymorphisms relevant to oxidative pathways and impaired methylation processes.

This chapter will describe the research findings regarding the following: (1) polymorphisms of various genes that are potentially related to oxidative stress and susceptibility genes to autism and Rett's disorder; (2) implications from animal studies using knockout mice; (3) the possible linkage among environmental factors, oxidative stress, and epigenetic factors in the pathophysiology of autism and related developmental disorders; and (4) clinical and treatment implications.

2 Potential Candidate Genes

2.1 Genes from the Glutathione Pathway

The genes that have been most widely studied are closely linked to *folate-dependent methionine transmethylation and transsulfuration cycles* and are involved in the redox status. Oxidative stress during prenatal and early postnatal development results from the abnormal expression of key antioxidant genes that are primarily involved in methionine transmethylation and transsulfuration pathways in the fetus/infant (James et al. 2006). Under oxidative stress, multiple adaptive pathways shift the flux of sulfur resources toward increased de novo synthesis of cysteine-containing tripeptide glutathione (GSH), the primary intracellular antioxidant (Deth et al. 2008).

The first genetic associations reported were based on observations of metabolic abnormalities in autism regarding the methionine transmethylation and transsulfuration pathways. Most of the research was focused on *glutathione* and its metabolic cofactors, which provide the primary defense against oxidative stress, including directly scavenging free radicals and reducing peroxides and conjugations with toxic electrophilic compounds (Maher 2006). The plasma levels of the transsulfuration metabolites are reportedly abnormal in autistic individuals; for example, cysteine, total glutathione, and free reduced glutathione (GSH) are reduced, whereas cystathionine and the oxidized disulfide form of glutathione are increased. Moreover, the ratios of total glutathione and GSH glutathione to oxidized glutathione disulfide (GSSG) (redox ratios) are reduced (Geier and Geier 2006; James et al. 2006).

The genes associated with this phenomena were allelic variations of genes related to the transsulfuration pathway and glutathione metabolism, such as glutathione S-transferase Mu 1 (GSTM1), glutathione S-transferase Pi 1 (GSTP1), and glutathione peroxidase (GPX1) (Buyske et al. 2006; James et al. 2006; Ming et al. 2010; Serajee et al. 2004).

2.1.1 Glutathione S-transferase Gene

Previous data emphasize the importance of the glutathione S-transferases (GST) as protective factors against reactive oxygen species and the products of oxidative stress. Human cytosolic GST is primarily encoded by 5 loci: GSTA, GSTT1, GSTM1, GSTP1, and GSTM3 (Schilter et al. 1993). GSTM1, which conjugates GSH to toxic electrophiles, has three alleles: GSTM1*0, GSTM1*A, and GSTM1*B. The homozygous deletion (0/0), or null genotype, which leads to copy number variations (CNVs) at either the GSTM1 or the GSTT1 locus, resulted in loss of enzyme function. Originally, it was hypothesized that this locus was associated with the susceptibility to autoimmune conditions, such as lupus erythematosus and various forms of cancer, including oral, gastric, colorectal, prostate, bladder, and hepatic cancer and renal cell carcinoma (Economopoulos and Sergentanis 2010; Gronau et al. 2003; Hayes and Pulford 1995; Simic et al. 2009; Zhang et al. 2010, 2011).

Two studies have reported an association between the null allele and autism (Buyske et al. 2006; James et al. 2006), suggesting that GSTM1 contributes to the risk of oxidative stress and autism. The first study, which was a case–control analysis of 358 children with autism and 201 age-matched control children, revealed a marginal increase in allele frequency with borderline significance for the GSTM1 null genotype (OR, 1.37; CI, 0.98 ~ 1.96) (James et al. 2006). A similar but clearer association was observed in a combined case–control family-based study. Although the sample sizes were small (54 complete case–parent trios and 172 controls, 45 with autistic disorders and 9 with PDD NOS), this study was noted for the use of an analytic method of combining both the traditional case–control analysis and the 1-df likelihood ratio test (utilizing controls and family trio data), and the results supported the association of the homozygous GSTM1 deletion genotype with an increased risk of autism ($p = 0.028$ and $p = 0.046$, respectively) (Buyske et al. 2006).

The pi class of GSTs, represented by a single GST (known as GSTP1, GSTP1-1, GSTP, GSTp, and GSTpi) encoded by a gene on chromosome 11q13, are expressed at the highest levels in most extrahepatic tissues (Lev-Ram et al. 1995). A family-based association study of 196 parent–proband trios using the transmission disequilibrium test (TDT) failed to demonstrate a significant genetic association ($p = 1.00$) (Serajee et al. 2004). GSTP1 attracted attention for possessing potential teratogenic alleles, which might contribute to the phenotype of the affected child in the mother during pregnancy. In one study, Williams et al. (2007) genotyped 137 individuals in 49 families with autistic disorders for the GSTP1*G313A and GSTP1*C341T polymorphism using maternal trios, consisting of the mother of an individual with autistic disorder and her parents. The results revealed that the GSTP1*A haplotype was overtransmitted to the case mothers, and the GSTP1*B and GSTP1*C haplotypes were undertransmitted at almost the same rates (OR = 2.67, 95 % CI = 1.39–5.13), while the individual genotypes were not significantly overtransmitted using the TDT ($p = 0.06 \sim 0.36$) (Williams et al. 2007). Despite limitations, including small sample size and the lack of phenotyping the mothers, this study is one of a few studies that explored the maternal genotype of children with autism, proposing the role of hazardous teratogenic allele in the interactions of the intrauterine environment and the susceptibility of the children. Different mechanisms were derived from studies of the

function of GSTP1, including the regulation of kinases, such as mitogen-activated protein kinase (MAPK) and Jun N-terminal kinase (JNK); however, the precise mechanism of teratogenic action has yet to be clarified (Williams et al. 2007).

2.1.2 Glutathione Peroxidase Gene

Glutathione peroxidase (GPX1) has recently been implicated as a candidate gene in autism. GPX is a major enzyme in the glutathione pathway that catalyzes the reduction of free radicals by glutathione and represents a major enzyme for defense against oxidant molecules (Robertson et al. 2007). Altered GPX1 enzymatic activity has been reported in spina bifida, meningomyelocele, idiosyncratic valproate-induced toxicity, fetal valproate syndrome, and autism (Ghanizadeh 2011; Leonard et al. 2008; Williams et al. 2001). A small family-based association study of a GCG repeat polymorphism of a human GPX1 polyalanine repeat (ALA5, ALA6, and ALA7) in 103 family trios of autistic disorder revealed a significant transmission disequilibrium ($p=0.044$) in the overall transmission of the three alleles. The ALA6 allele was undertransmitted ($p=0.017$), suggesting its protective effect for autistic disorder (Ming et al. 2010), although the precise function of ALA6 has not yet been clearly demonstrated, except for evidence showing that the GPX1 ALA6/198Leu polymorphism decreased enzyme activity by 40 %. These results and indirect evidence of an association with fetal valproate syndrome suggest that the GPX1 gene might be one of the plausible candidates in the development of autism (Hamanishi et al. 2004).

2.2 *Genes from Methionine Transmethylation Pathway and Folate Metabolism*

2.2.1 Methylenetetrahydrofolate Reductase

Methylenetetrahydrofolate reductase (MTHFR) is an important methionine transmethylation pathway-related gene. This enzyme catalyzes DNA methylation using a methyl donor from dietary folate and regulates folate availability (Ulrey et al. 2005). Two common functional polymorphisms (677C>T and 1298A>C) are reported to reduce the enzyme activity of MTHFR, and 677TT is associated with a 60 % reduction of enzymatic activity (Frosst et al. 1995; Schmidt et al. 2011). Disrupting the folate metabolic pathway through the reduced enzymatic activity of MTHFR impedes the conversion of homocysteine to methionine, subsequently reduces the formation of the methyl donor S-adenosyl methionine (SAM) for DNA methylation, and leads to hypomethylation (dos Santos et al. 2010). Several studies have reported the proportions and associations of a specific allele of the MTHFR gene in autism across diverse ethnicities, although these researchers did not report consistent results.

MTHFR is the only replicated gene involved in the B vitamin-dependent folate, methionine, and transmethylation pathways. James et al. (2006) observed an increase in the frequency of the MTHFR 677TT over the 677CT genotype in autistic

cases with borderline significance (OR = 1.45 and 1.36 for each) (James et al. 2006). The role of this gene has been explored in small- to moderate-sized case–control and family-based association studies (39 probands ~512 families). Generally, the low-activity MTHFR 677 T allele and 677TT genotype are observed more frequently in cases of autism than in controls with high ($p < 0.01$) or borderline significance ($p = 0.09$) (D'Amelio et al. 2005; Liu et al. 2011; Mohammad et al. 2009; Pasca et al. 2009). Specifically, the role of the MTHFR T allele is critical, as studies have shown an increased risk of autism of approximately threefold in a dose-dependent manner ($P_{trend} < 0.0001$) and an overtransmission in family-based association studies in simplex families (Liu et al. 2011; Mohammad et al. 2009). However, the risk of the T allele and CT genotypes in autism was not evident in cases involving Brazilian children (dos Santos et al. 2010).

In the case of MTHFR 1298AC (another functional polymorphism related to low enzyme activity), the function according to allelic and genotypic variants tends to be increased with regard to the haplotype or co-segregated genotype. For example, the 677T-1298A haplotype and double homozygous 677TT/1298AA genotype are significantly more frequent to affected individuals, and the co-segregation of MTHFR 677T-1298C variant alleles was associated with an 8.11-fold increased risk for autism (95 % CI: 2.84–22.92) as compared with the MTHFR 677CC/1298AA genotype in another study (Liu et al. 2011; Mohammad et al. 2009).

As the autism has a complex and heterogeneous behavioral phenotype, problematic behaviors from 3 principal domains of autism show a wide spectrum. In 25 % of families affected by autism, multiple family members are affected by clinical or subclinical autistic traits, and within this subset of families, the distribution of autistic traits and symptoms appears highly quantitative (Constantino 2011). Understanding the core social abnormality of autism as a quantitative trait rather than as a categorically defined condition has key implications for understanding the underlying genetic and neurobiological mechanisms (Constantino 2011). Moreover, the analysis of phenotypic–genotypic relationship might be a rational approach to exploring the function of this risk allele, assuming the dose-dependent increase of autism risk according to the number of MTHFR 677T alleles (Goin-Kochel et al. 2009).

In a study of 147 stringently phenotyped subjects from the Autism Genetic Resource Exchange (AGRE) collection (94 % white/Caucasians), the authors analyzed the MTHFR 677CT polymorphisms, the "Restricted, Repetitive, and Stereotyped Patterns of Behavior" composite scores, and other behavioral variables consistent with anecdotal reports of behavioral changes among children with autism spectrum disorders who were treated with folate supplementation. An analysis of language, nonverbal cognitive functioning, and adaptive behavior was also included. The results indicated four ADI-R behaviors (direct gaze, current complex body movements, a history of self-injurious behavior, and current overactivity (ORs = 2.72, 2.33, 2.12, 2.47, respectively)) that were more common and problematic (95 % CI) among those with at least one copy of the T allele as compared with homozygous wild-type individuals among the children with autism, although correction of the multiple tests was not applied (Goin-Kochel et al. 2009). Further studies concerning the phenotypic–genotypic interaction of the MHTFR gene need to be replicated with more careful corrections of the population stratification and a direct measure of the variety of autistic symptoms.

MHTFR and other folate metabolism-related genes might have clinical implications regarding the potential treatment and prevention of autism using vitamin supplements. A recent, relatively large cohort study (278 children with autism, 144 with autism spectrum disorder, and 278 typically developing children) was focused on the association of genetic variants and prenatal vitamin intake with autism susceptibility. In the context of gene–environment interactions, the authors examined the association between autism and maternal vitamin supplement intake during conception and prenatal periods, in combination with common functional maternal and infant gene polymorphisms in the folate, methionine, and transmethylation pathways. The results showed a significant association between the prevalence of the maternal MTHFR 677TT genotype and no preconception maternal prenatal vitamin intake (combined OR = 4.5, CI = 1.4 ~ 14.6, interaction $p = 0.04$) (Schmidt et al. 2011). Despite limitations, such as the retrospective reporting of vitamin and supplement information and the fact that only 9 (4 %) children with autism were included in the MTHFR 677TT/no vitamin supplement group, this type of gene–environment interaction study should be expanded and replicated in the future.

2.2.2 Other Genes Within the Pathway

Other functionally important polymorphisms in folate metabolism genes that show a significant association with ASD include a 19-bp deletion in the dihydrofolate reductase gene (DHFR), transcobalamin II (TCN2 776C>G122), catechol-O-methyltransferase (COMT 472G>A122), and reduced folate carrier (RFC1 80A>G), but studies concerning these genes need to be further replicated; currently, there have only been reports of one or two studies for each gene (Adams et al. 2007; James et al. 2006, 2010). Of those polymorphisms, the functional polymorphism RFC1 gene is a potential mediator of oxidative stress–gene interactions; the results from a large-scale study with 529 case–parent trios and 566 neurotypical controls demonstrated a significant increase in the G allele frequency among mothers of children with autism (James et al. 2010).

2.3 Antioxidant Genes Outside the Methionine and Glutathione Pathway

2.3.1 Paraoxigenase Gene

There is evidence for the possible involvement of antioxidant genes outside the methionine transmethylation/transsulfuration and glutathione pathways. *Paraoxonase 1 (PON1)*, which is associated with organophosphate hydrolysis and plays a role in protection against the oxidative modification of low-density lipoprotein, homocysteine-thiolactone, and bacterial endotoxins, has been implicated in

autism (D'Amelio et al. 2005; Herbert 2010; Pasca et al. 2010; Serajee et al. 2004). A significant association of PON1 with autism was demonstrated in Caucasian-American ($p<0.025$), but not Italian, families in which less organophosphate is used (D'Amelio et al. 2005). The genetic association was not significant in another cohort of Romanian children with autism spectrum disorders in which the bioavailability and the catalytic activity of PON1 were significantly impaired (Pasca et al. 2010).

2.3.2 Neuromodulator Genes

There are two different types of inducible enzymes activated by adverse events in the central nervous system. *Nitric oxide synthase IIA (NOS IIA)* is induced in the microglia and astroglia after perinatal hypoxia or intrauterine infection in the central nervous system (Shen et al. 2007). Nitric oxide (NO) is thought to play an important role in neuroinflammation and elevated NO production, and higher plasma NO levels were observed in children with autism as compared with the control children (Sogut et al. 2003; Sweeten et al. 2004). *Cyclooxygenase-2 (Cox-2 or prostaglandin-endoperoxide synthase 2, PTGS2)* is induced by growth factors, cytokines, and proinflammatory molecules. Elevated activities of Cox-2 isoforms at the cellular and subcellular levels in ischemia and neurodegenerative diseases strongly suggest that these enzymes might play important roles in inducing inflammation and oxidative stress associated with neurodegenerative processes (Phillis et al. 2006). A variety of chronic neurological conditions, such as Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, Creutzfeldt–Jakob disease, and seizure disorders, are associated with increased activities and high levels of Cox-2.

However, few genetic polymorphisms of Cox-2 and NOS IIA in autism have been reported. The results from association studies involving family trios (proband with both biological parents) from 151 Korean children with autism spectrum disorders revealed an SNP from the Cox-2 gene (rs2745557, $p<0.01$) and a haplotype that were significantly associated with ASDs ($p<0.01$) (Yoo et al. 2008). Another study using the same Korean cohort showed weaker but significant associations in 2 SNPs (rs8068149, $p=0.039$ and rs1060826, $p=0.035$) and 2 haplotypes ($p=0.014$ and 0.031, respectively) of NOS IIA gene in ASD (Kim et al. 2009). In both studies, statistically significant associations were demonstrated between each genotype and specific symptom domain scores using ADOS and ADI-R; *communication, qualitative abnormalities in reciprocal social interaction, and overactivity/agitation* were associated with the Cox-2 gene ($p=0.00$), and the *failure to use nonverbal behaviors to regulate social interaction* was associated with the NOS IIA gene (Kim et al. 2009; Yoo et al. 2008). Theoretically, functional polymorphisms in those genes could lead to varying degrees of Cox-2 and NOS IIA production, which could determine the susceptibility of an individual to environmental/endogenous risks for autism. These findings must be replicated using larger and ethnically diverse populations with more genetic markers and genetically informative endophenotypes in the future.

2.3.3 Rett's Syndrome, MeCP Gene, and Oxidative Stress

Rett's syndrome is one of the five conditions included in the category of pervasive developmental disorder and rare form of PDD that exclusively occurs in females. It is characterized by the deceleration of head growth between the ages of 5 and 48 months, the loss of previously acquired purposeful hand skills between the ages of 5 and 30 months with the subsequent development of stereotyped hand movements, the early loss of social engagement, the appearance of poorly coordinated gait or trunk movements, and severely impaired expressive and receptive language development with severe psychomotor retardation following a period of normal development (Percy 2011). In the great majority of cases, Rett's syndrome is an X-linked disorder caused by mutations in the *MeCP2 gene* (Percy 2011). Oxidative stress is suggested as a key modulator of disease expression in Rett's syndrome based on the observation that the MeCP2 mutations that were related to severe phenotypes exhibited higher oxidative stress marker levels than those of milder phenotypes; however, the association of the MeCP2 mutation with oxidative stress remains a major challenge for future research (Leoncini et al. 2011).

3 Single Gene Variation Versus Genetic Pathway

The advent of large protein–protein interaction maps, full genomic expression profiles, and large-scale computing resources, networks, and pathway analyses offers promise for exploring the interaction and connectivity between candidate genes involved in complex autism disorders. For example, recent reports suggest the possibility that the alleles of serpin peptidase inhibitor, clade E (SERPINE), plasminogen activator, urokinase receptor (PLAUR), receptor tyrosine kinase MET (MET), phosphatase and tensin homologue (PTEN), the tuberous sclerosis complex (TSC), fragile X mental retardation 1 (FMR1), and cytoplasmic FMR1 interacting protein 1 (CYFIP1) genes might contribute to autism via epistatic interactions in ASD (Bill and Geschwind 2009). Autism is not a single-gene disease, but rather might be derived from complex interactions between multiple genes; therefore, it would be more informative to obtain more precise information concerning the interactions among the genes involved. However, there has not been many gene–gene interaction studies published regarding autism due to difficulties in experimental methodology concerning the use of genome-wide association data or because of the lack of high-density information concerning certain pathways (Bowers et al. 2011).

While it is possible that the genes involved in the methionine transmethylation/transsulfuration and glutathione pathways might interact among each other, few studies have explored the interrelationship among these candidate genes in the development of autism. One study highlighted gene–gene interactions of MTHFR with borderline significance (OR = 1.78) in the susceptibility of autism, such as homozygous or heterozygous combinations of the RFC1 gene G s and the MTHFR 677T alleles (GA/CT, OR 3.2; GA/TT, OR 4.4; and GG/CT, OR 3.1), MTHFR

677CT/1298AC, and 80G allele of the RFC gene, which encode the enzyme for transporting methylfolate into cells. In addition, the GSTM1 null genotype showed a highly significant interaction with the reduced RFC-1 G allele, rendering a 3.78-fold increase in autism susceptibility in children with combined GSTM1 null and RFC1 heterozygous GA genotypes (James et al. 2006).

However, the comprehensiveness of the genes selected and the density of the markers analyzed limited the interpretation of the results from this study. A recent study reported the occurrence of more systematic multiple gene interactions within the glutathione pathway for 42 genes (308 SNPs) among 1,149 individuals from 318 family trios in the AGRE database (Bowers et al. 2011). This study utilized a carefully designed methodology, such as information-based gene selection, selecting tag SNPs to examine a large amount of genes and flanking regions, acquire standardized genetic data, and evaluate higher-order gene–gene interactions using the logic regression method. A single SNP analysis revealed a significant association ($p < 0.05$) in nine SNPs located in cystathionine gamma lyase (CTH), alcohol dehydrogenase 5 (ADH5), gamma-glutamylcysteine synthetase, catalytic subunit (GCLC), glutaredoxin, and glutaredoxin 3 (GLRX3) genes, which were not previously reported; two SNPs approached nominal statistical significance in independent AGRE samples (rs524553 and rs761141, both located in GCLC). Notably, a three-SNP joint effect was observed for the genotype combinations GLRX3 and CTH (OR = 3.78, 95 % CI: 2.36, 6.04).

Though the function of the associated genes should be validated in future studies, the results indicate that the gene–gene interaction approach might be a promising and more systematic way to explore the contributions of multiple genes to the risk of autism.

4 Functional Genetic Polymorphisms and Animal Model of Autism

Transgenic mouse models are important approaches in the study of human diseases, allowing for the use of a variety of experimental approaches to dissect the contribution of a specific chromosomal or genetic abnormality in human disorders (Robertson and Feng 2011), although a plausible animal model of autism has yet to be established. Most of the attempts at animal models of autism have been targeted to pre-existing monogenic developmental disorders showing autistic-like behaviors and transgenic mouse models that mimic point mutations or null mice for synaptic and signaling molecules (Robertson and Feng 2011). Animal knockout models for genes directly related to oxidative stress have rarely been developed; however, recently, the relationship between the GSTM1 null genotype and autistic behavior was examined using a rodent model based on the hypothesis that genetically altered mice might be more sensitive to toxic exposure early in life, followed by autistic regression (Yochum et al. 2010).

By relating those two ideas, Yochum et al. (2010) examined the sensitivity of the GSTM1 knockout mice to incurring the neural behavioral deficits induced by the postnatal administration of sodium valproate on day 14 (P14). VPA administration induces fetal valproate syndrome, which partially resembles autism due to oxidative stress (Na et al. 2003; Verrotti et al. 2008). Moreover, in this study, VPA treatment resulted in the increase of apoptosis in the hippocampus and cerebellum and social behaviors, such as crawl under and allogrooming, as compared with the saline-treated knockout animals and the wild-type controls, although the effect of the GSTM1 knockout on each social behavior was inconsistent as shown in Fig. 1. A significant genotype x VPA treatment x sex interaction was observed, against VPA-induced neuronal death in female mice (Yochum et al. 2010).

Cerebellar mutant and NOS knockout mice are other proposed models for autism, which are closely related to each other. Behaviorally, the NOS knockout mice showed a significant change in learning behavior through cerebellar long-term depression (Lev-Ram et al. 1997; Yochum et al. 2010). Based on shared cerebellar histopathology and genetic involvement in autism, including cerebellar hypoplasia,

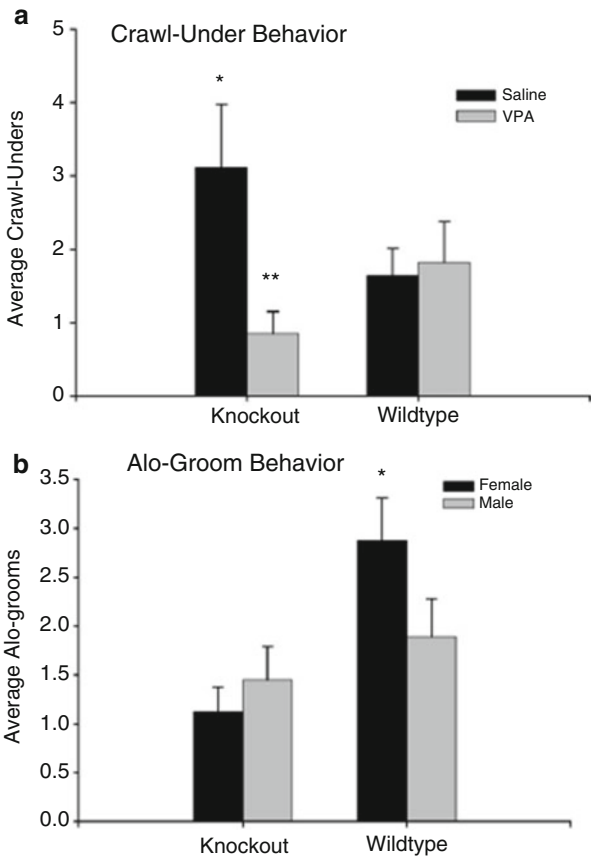

Fig. 1 (**a**) Number of crawl-under behaviors completed by genotype and treatment-matched pairs of GSTM1 wild-type and knockout mice over a 30 min open field trial (run between postnatal days 30–40) following P14 sodium valproate (VPA, 400 mg/kg, s.c.) or saline treatment. (**b**) Number of allogroom behaviors completed by sex and genotype-matched pairs of GSTM1 wild-type and knockout mice over a 30 min open field trial (run between postnatal days 30–40) following P14 sodium valproate (VPA, 400 mg/kg, s.c.) or saline treatment (Reprinted from Yochum et al. 2010, with permission from Elsevier)

Purkinje cell loss or reduced size, decreased blood reelin levels, and the participation of the reelin gene, the cerebellar mutant mice (reeler mice) were proposed as a useful model system to study autism (Lev-Ram et al. 1997; Yochum et al. 2010).

5 What Are the Mechanisms?: Epigenetic Mechanisms and DNA Methylation

Concerning the genetic etiology of autism, the linkage and association studies were rarely replicated, implying that a number of factors including gene–environment interactions and genetic heterogeneity are involved in development of autism, as indicated by the fact that many known genes and genomic regions associated with autism spectrum disorders account for less than 2 % of the cases (Abrahams and Geschwind 2008). Both genetic and environmental factors play causative roles, influencing fetal or early postnatal brain development, directly or via epigenetic modifications (Grafodatskaya et al. 2010). *Epigenetics* is defined as heritable changes that are independent of the genomic sequence, and these changes provide a mechanism for controlling the genome without involving the alteration of the genomic sequence. Epigenetic modifications normally annotate DNA and associated histone proteins and regulate the expression of many genes (Grafodatskaya et al. 2010). The developing mammalian brain is particularly sensitive to epigenetic alterations, and the etiology of a variety of neurodevelopmental disorders are attributed to this process (LaSalle 2011). The epigenetic modulation of gene expression lies at the interface between genes and environmental influences and could potentially provide a molecular explanation for the downregulation of gene expression in the autistic brain (James et al. 2010).

Epigenetics is especially useful to define the molecular mechanism that links environmental effects with gene function in complex diseases, such as schizophrenia, autism, and mental retardation (Zahir and Brown 2011). The interplay among oxidative stress and genetic factors in the pathogenesis of autism could be understood in the context of epigenetic dysregulation, especially in the DNA methylation process (cell-specific gene expression and differentiation). DNA methylation might inhibit gene expression through direct interactions with factors that repress transcription or through the recruitment of methyl-CpG-binding proteins (Grafodatskaya et al. 2010; LaSalle 2011). The functional polymorphisms in genes encoding key enzymes involved in the DNA methylation pathway might disrupt this pathway by decreasing the methyl donors or, conversely, by increasing the demands for more methyl donors (LaSalle 2011). The DNA hypomethylation observed in autism supports the involvement of the DNA methylation pathway in autism (James et al. 2008, 2010).

The roles of oxidative stress and related genetic variants in the development of autism could be explained with two different but biochemically linked processes with regard to DNA methylation dysregulation. The first process involves a decrease in methyl donors in the folate-dependent pathway (one-carbon methylation pathway), involving the remethylation of homocysteine to methionine. This pathway is highly polymorphic, and evidence for the involvement of common functional genetic

polymorphisms of SNPs of MTHFR and related genes in autism supports the hypothesis that alterations in this pathway result in methylation deficits that can cause abnormal brain development (Pasca et al. 2010). As this pathway is dependent on the dietary intake of folic acid and thus gene–nutrient interactions mediated by the dietary intake of folate and vitamin B, amino acid deficiencies and environmental exposure could potentially modify the expression of certain metabolic pathways.

The second process involves the more direct influence of oxidative stress and an increased demand for enhanced glutathione synthesis, which acts as an inhibitor of SAM. When exposed to oxidative stress, the need for glutathione is enhanced to conjugate toxins or as an antioxidant to activate the adaptive response (LaSalle 2011). In autism, if simplified, the functional genetic polymorphisms of the enzymes modulating this pathway, such as the GSTM1 null polymorphism, GST, and GPX, might alter the enzyme activity and inhibit the synthesis of glutathione, rendering genetically susceptible subjects more vulnerable to neuronal damage or developmental aberrations through oxidative stress, especially in the early stages of fetal development. Figure 2 shows a schematic overview of the an integrative genomic model of the major genetic and environmental pathways influencing the human DNA methylation.

In addition, methionine synthase (MS), which converts homocysteine to methionine, is responsive to the cellular oxidative status and is inhibited by oxidative stress (Deplancke and Gaskins 2002). This adaptive process initiates a cascade to increase cysteine availability for glutathione synthesis, while decreasing DNA methylation through a subsequent decrease of SAM as a methyl donor. Thus, the MS gene might be one of the plausible candidate genes related to oxidative stress and neurodevelopmental disorders connecting the two interdependent pathways described above; however, no significant association of MS gene polymorphisms with autism has been reported so far (Adams et al. 2007).

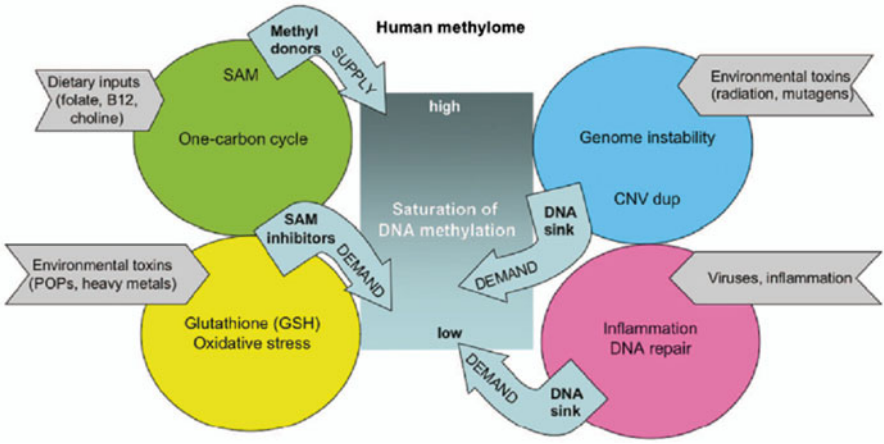

Fig. 2 An integrative genomic model of the major genetic and environmental pathways influencing the human methylome ([Used with permission from LaSalle 2011])

When methionine synthase activity is reduced by oxidative stress, the D4 receptor-mediated dopamine-stimulated phospholipid methylation (PLM) is reduced, as it is absolutely dependent on MS activity (Deth et al. 2008). Impairment in dopamine-dependent PLM limits the frequency-dependent synchronization of the neuronal network, which results in deficits in attention and cognition, which are important features of autistic psychopathology (Deth et al. 2008; Rommelse et al. 2011). As D4 receptor-mediated PLM is synergistic with SNPs affecting dopaminergic functions (e.g., COMT) and/or the neuronal substrates participating in synchronization (e.g., RELN, MET, or NGLN 3 & 4) (Deth et al. 2008) as shown in Fig. 3, all of which are potential candidate genes of autism, it might be presumed that oxidative stress initiates this complex genetic cascade in a variety of interrelated adaptive and developmental mechanisms in autism.

The precise links between oxidative stress and genetic mechanisms other than the DNA methylation process have yet to be explored, especially with regard to inducible neuromodulators, such as Cox-2 or NOS IIA.

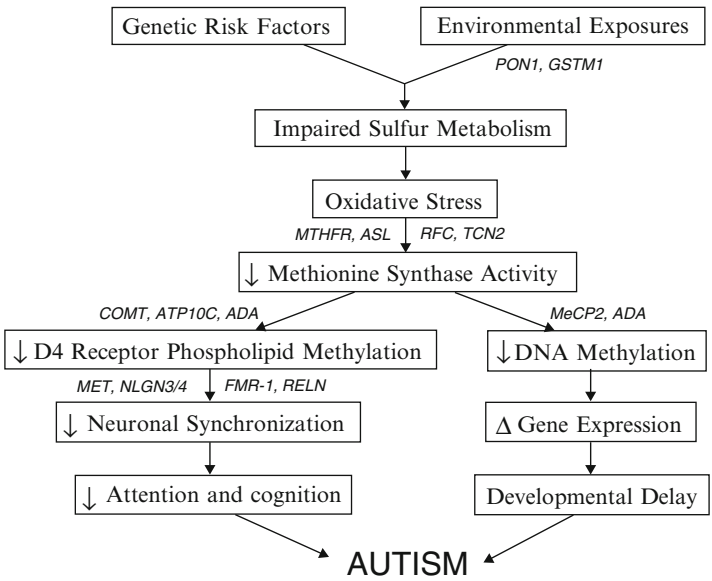

Fig. 3 A redox/methylation hypothesis of autism. Environmental factors (e.g., heavy metals and xenobiotics) can precipitate oxidative stress in a vulnerable subpopulation possessing risk genes (shown in italics), initiating multiple adaptive responses involving sulfur metabolism. Inhibition of methionine synthase broadly reduces methylation activity, with DNA methylation and dopamine-stimulated phospholipid methylation being important examples. Reduced DNA methylation interferes with epigenetic events that are fundamental to normal development. Impairment of dopamine-stimulated phospholipid methylation limits frequency-dependent synchronization of neuronal networks, reflected as deficits in attention and cognition. While all cell types are subject to similar effects, which may be manifested as autism-associated symptoms, neuronal cells exhibit higher sensitivity to oxidative stress (Reprinted from Deth et al. 2008, with permission from Elsevier)

6 Clinical Implications

Many different genes and genetic polymorphisms regarding oxidative stress and human adaptability have been associated with the development of autism. However, in the clinical realm, it is not simple to understand the complex interplay of specific events that cause oxidative stress and genetic susceptibility in the child and the mother. For example, fetal hypoxia, one of the possible causes of oxidative stress, is hardly measurable due to the retrospective nature of perinatal data collection in the proband once diagnosed with autism. Recently, few prospective large cohort studies were conducted to examine the perinatal and prenatal risk factors in autism, but it is difficult to include genetic susceptibility in the analysis (Williams and Marshall 2001).

The second limitation in clinical understanding is that the genes involved in the oxidative pathway are not only associated with autism and similar neurodevelopmental disorders but also with a variety of systemic illnesses, such as cancer, hepatotoxicity, and autoimmune and neurodegenerative disorders. The causative genes implicated in these diseases have similar characteristics as other candidate genes of autism; therefore, the elucidation of the exact roles of these genes in causing autistic psychopathology and differences in the pathophysiological mechanisms of other systemic disorders needs further study.

The treatment of autism is mainly focused on early behavioral intervention. Biological therapies directly involving genetic polymorphisms are not yet available for the treatment of autism. Based on research evidence concerning the abnormalities in methionine and folate metabolism, nutritional interventions, such as supplementation of folic or folinic acid, betaine, and vitamin B6 or B12, have been attempted, but the behavioral outcome after treatment was not fully satisfactory (Main et al. 2010). Even before the direct therapeutic modification of genetic function, the selective supplementation of a certain nutritional element might be attempted; however, the specific function of a gene, the interactions of multiple genes involved, and the specific relationship between environmental factors and genetic polymorphisms have to be further explored to determine the major target of therapeutic intervention.

Another future task is to explore the relationship between specific *endophenotypes* as quantitative traits of autism and genes involved in the oxidative pathway. More stringent and careful phenotyping, including neurocognitive tests and/or brain imaging modalities, and insight from animal knockout studies will help to uncover more powerful and specific phenotypes regarding oxidative stress and related genetic susceptibility.

References

Abrahams BS, Geschwind DH (2008) Advances in autism genetics: on the threshold of a new neurobiology. Nat Rev Genet 9(5):341–355

Adams M, Lucock M, Stuart J, Fardell S, Baker K, Ng X (2007) Preliminary evidence for involvement of the folate gene polymorphism 19bp deletion-DHFR in occurrence of autism. Neurosci Lett 422(1):24–29

Bill BR, Geschwind DH (2009) Genetic advances in autism: heterogeneity and convergence on shared pathways. Curr Opin Genet Dev 19(3):271–278
Bowers K, Li Q, Bressler J, Avramopoulos D, Newschaffer C, Fallin MD (2011) Glutathione pathway gene variation and risk of autism spectrum disorders. J Neurodev Disord 3(2):132–143
Buyske S et al (2006) Analysis of case-parent trios at a locus with a deletion allele: association of GSTM1 with autism. BMC Genet 7:8
Cohen-Kerem R, Koren G (2003) Antioxidants and fetal protection against ethanol teratogenicity. I. Review of the experimental data and implications to humans. Neurotoxicol Teratol 25(1):1–9
Constantino JN (2011) The quantitative nature of autistic social impairment. Pediatr Res 69(5 Pt 2):55R–62R
D'Amelio M et al (2005) Paraoxonase gene variants are associated with autism in North America, but not in Italy: possible regional specificity in gene-environment interactions. Mol Psychiatry 10(11):1006–1016
Deplancke B, Gaskins HR (2002) Redox control of the transsulfuration and glutathione biosynthesis pathways. Curr Opin Clin Nutr Metab Care 5(1):85–92
Deth R, Muratore C, Benzecry J, Power-Charnitsky VA, Waly M (2008) How environmental and genetic factors combine to cause autism: a redox/methylation hypothesis. Neurotoxicology 29(1):190–201
Do KQ, Cabungcal JH, Frank A, Steullet P, Cuenod M (2009) Redox dysregulation, neurodevelopment, and schizophrenia. Curr Opin Neurobiol 19(2):220–230
dos Santos PA, Longo D, Brandalize AP, Schuler-Faccini L (2010) MTHFR C677T is not a risk factor for autism spectrum disorders in South Brazil. Psychiatr Genet 20(4):187–189
Economopoulos KP, Sergentanis TN (2010) GSTM1, GSTT1, GSTP1, GSTA1 and colorectal cancer risk: a comprehensive meta-analysis. Eur J Cancer 46(9):1617–1631
Folstein SE, Rosen-Sheidley B (2001) Genetics of autism: complex aetiology for a heterogeneous disorder. Nat Rev Genet 2(12):943–955
Fombonne E (2009) Epidemiology of pervasive developmental disorders. Pediatr Res 65(6):591–598
Frosst P et al (1995) A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 10(1):111–113
Geier DA, Geier MR (2006) A clinical and laboratory evaluation of methionine cycle-transsulfuration and androgen pathway markers in children with autistic disorders. Horm Res 66(4):182–188
Geschwind DH (2011) Genetics of autism spectrum disorders. Trends Cogn Sci 15(9):409–416
Ghanizadeh A (2011) Malondialdehyde, Bcl-2, superoxide dismutase and glutathione peroxidase may mediate the association of sonic hedgehog protein and oxidative stress in autism. Neurochem Res 37:899–901
Goin-Kochel RP, Porter AE, Peters SU, Shinawi M, Sahoo T, Beaudet AL (2009) The MTHFR 677C→T polymorphism and behaviors in children with autism: exploratory genotype-phenotype correlations. Autism Res 2(2):98–108
Grafodatskaya D, Chung B, Szatmari P, Weksberg R (2010) Autism spectrum disorders and epigenetics. J Am Acad Child Adolesc Psychiatry 49(8):794–809
Gronau S, Koenig-Greger D, Jerg M, Riechelmann H (2003) GSTM1 enzyme concentration and enzyme activity in correlation to the genotype of detoxification enzymes in squamous cell carcinoma of the oral cavity. Oral Dis 9(2):62–67
Hamanishi T et al (2004) Functional variants in the glutathione peroxidase-1 (GPx-1) gene are associated with increased intima-media thickness of carotid arteries and risk of macrovascular diseases in Japanese type 2 diabetic patients. Diabetes 53(9):2455–2460
Hayes JD, Pulford DJ (1995) The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Biochem Mol Biol 30(6):445–600
Herbert MR (2010) Contributions of the environment and environmentally vulnerable physiology to autism spectrum disorders. Curr Opin Neurol 23(2):103–110
James SJ et al (2006) Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. Am J Med Genet B Neuropsychiatr Genet 141B(8):947–956

James SJ, Melnyk S, Jernigan S, Hubanks A, Rose S, Gaylor DW (2008) Abnormal transmethylation/transsulfuration metabolism and DNA hypomethylation among parents of children with autism. J Autism Dev Disord 38(10):1976

James SJ et al (2010) A functional polymorphism in the reduced folate carrier gene and DNA hypomethylation in mothers of children with autism. Am J Med Genet B Neuropsychiatr Genet 153B(6):1209–1220

Kim HW et al (2009) Family-based association study between NOS-I and -IIA polymorphisms and autism spectrum disorders in Korean trios. Am J Med Genet B Neuropsychiatr Genet 150B(2):300–306

LaSalle JM (2011) A genomic point-of-view on environmental factors influencing the human brain methylome. Epigenetics 6(7):862–869

Leonard H et al (2008) Relation between intrauterine growth and subsequent intellectual disability in a ten-year population cohort of children in Western Australia. Am J Epidemiol 167(1):103–111

Leoncini S et al (2011) Oxidative stress in Rett syndrome: natural history, genotype, and variants. Redox Rep 16(4):145–153

Lev-Ram V, Makings LR, Keitz PF, Kao JP, Tsien RY (1995) Long-term depression in cerebellar Purkinje neurons results from coincidence of nitric oxide and depolarization-induced Ca^{2+} transients. Neuron 15(2):407–415

Lev-Ram V, Nebyelul Z, Ellisman MH, Huang PL, Tsien RY (1997) Absence of cerebellar long-term depression in mice lacking neuronal nitric oxide synthase. Learn Mem 4(1):169–177

Liu X et al (2011) Population- and family-based studies associate the MTHFR gene with idiopathic autism in simplex families. J Autism Dev Disord 41(7):938–944

Losh M, Sullivan PF, Trembath D, Piven J (2008) Current developments in the genetics of autism: from phenome to genome. J Neuropathol Exp Neurol 67(9):829–837

Maher P (2006) Redox control of neural function: background, mechanisms, and significance. Antioxid Redox Signal 8(11–12):1941–1970

Main PA, Angley MT, Thomas P, O'Doherty CE, Fenech M (2010) Folate and methionine metabolism in autism: a systematic review. Am J Clin Nutr 91(6):1598–1620

Ming X, Johnson WG, Stenroos ES, Mars A, Lambert GH, Buyske S (2010) Genetic variant of glutathione peroxidase 1 in autism. Brain Dev 32(2):105–109

Mohammad NS, Jain JM, Chintakindi KP, Singh RP, Naik U, Akella RR (2009) Aberrations in folate metabolic pathway and altered susceptibility to autism. Psychiatr Genet 19(4):171–176

Na L, Wartenberg M, Nau H, Hescheler J, Sauer H (2003) Anticonvulsant valproic acid inhibits cardiomyocyte differentiation of embryonic stem cells by increasing intracellular levels of reactive oxygen species. Birth Defects Res A Clin Mol Teratol 67(3):174–180

Pasca SP, Dronca E, Kaucsar T, Craciun EC, Endreffy E, Ferencz BK, Iftene F, Benga I, Cornean R, Banerjee R, Dronca M (2009) One carbon metabolism disturbances and the C677T MTHFR gene polymorphism in children with autism spectrum disorders. J Cell Mol Med 13(10):4229–4238

Pasca SP, Dronca E, Nemes B, Kaucsar T, Endreffy E, Iftene F, Benga I, Cornean R, Dronca M et al (2010) Paraoxonase 1 activities and polymorphisms in autism spectrum disorders. J Cell Mol Med 14(3):600–607

Percy AK (2011) Rett syndrome: exploring the autism link. Arch Neurol 68(8):985–989

Perry SW, Norman JP, Litzburg A, Gelbard HA (2004) Antioxidants are required during the early critical period, but not later, for neuronal survival. J Neurosci Res 78(4):485–492

Phillis JW, Horrocks LA, Farooqui AA (2006) Cyclooxygenases, lipoxygenases, and epoxygenases in CNS: their role and involvement in neurological disorders. Brain Res Rev 52(2): 201–243

Robertson RP, Harmon JS (2007) Pancreatic islet beta-cell and oxidative stress: the importance of glutathione peroxidase. FEBS Lett 581(19):3743–3748

Robertson HR, Feng G (2011) Annual Research Review: transgenic mouse models of childhood-onset psychiatric disorders. J Child Psychol Psychiatry 52(4):442–475

Rommelse NN, Geurts HM, Franke B, Buitelaar JK, Hartman CA (2011) A review on cognitive and brain endophenotypes that may be common in autism spectrum disorder and attention-deficit/

hyperactivity disorder and facilitate the search for pleiotropic genes. Neurosci Biobehav Rev 35(6):1363–1396
Schilter B, Turesky RJ, Juillerat M, Honegger P, Guigoz Y (1993) Phase I and phase II xenobiotic reactions and metabolism of the food-borne carcinogen 2-amino-3,8-dimethylimidazo[4,5-f] quinoxaline in aggregating liver cell cultures. Biochem Pharmacol 45(5):1087–1096
Schmidt RJ et al (2011) Prenatal vitamins, one-carbon metabolism gene variants, and risk for autism. Epidemiology 22(4):476–485
Serajee FJ, Nabi R, Zhong H, Huq M (2004) Polymorphisms in xenobiotic metabolism genes and autism. J Child Neurol 19(6):413–417
Shen Y, Yu HM, Yuan TM, Gu WZ, Wu YD (2007) Intrauterine infection induced oligodendrocyte injury and inducible nitric oxide synthase expression in the developing rat brain. J Perinat Med 35(3):203–209. doi:10.1515/JPM.2007.058
Simic T, Savic-Radojevic A, Pljesa-Ercegovac M, Matic M, Mimic-Oka J (2009) Glutathione S-transferases in kidney and urinary bladder tumors. Nat Rev Urol 6(5):281–289
Sogut S et al (2003) Changes in nitric oxide levels and antioxidant enzyme activities may have a role in the pathophysiological mechanisms involved in autism. Clin Chim Acta 331(1–2): 111–117
Sweeten TL, Posey DJ, Shankar S, McDougle CJ (2004) High nitric oxide production in autistic disorder: a possible role for interferon-gamma. Biol Psychiatry 55(4):434–437
Ulrey CL, Liu L, Andrews LG, Tollefsbol TO (2005) The impact of metabolism on DNA methylation. Hum Mol Genet 14(Spec No 1):R139–R147. doi:10.1093/hmg/ddi100
Verrotti A, Scardapane A, Franzoni E, Manco R, Chiarelli F (2008) Increased oxidative stress in epileptic children treated with valproic acid. Epilepsy Res 78(2–3):171–177
Williams KM, Marshall T (2001) Protein concentration of cerebrospinal fluid by precipitation with Pyrogallol Red prior to sodium dodecyl sulphate-polyacrylamide gel electrophoresis. J Biochem Biophys Methods 47(3):197–207
Williams G, King J, Cunningham M, Stephan M, Kerr B, Hersh JH (2001) Fetal valproate syndrome and autism: additional evidence of an association. Dev Med Child Neurol 43(3): 202–206
Williams TA et al (2007) Risk of autistic disorder in affected offspring of mothers with a glutathione S-transferase P1 haplotype. Arch Pediatr Adolesc Med 161(4):356–361
Yochum CL, Bhattacharya P, Patti L, Mirochnitchenko O, Wagner GC (2010) Animal model of autism using GSTM1 knockout mice and early post-natal sodium valproate treatment. Behav Brain Res 210(2):202–210
Yoo HJ et al (2008) Association between PTGS2 polymorphism and autism spectrum disorders in Korean trios. Neurosci Res 62(1):66–69
Zahir FR, Brown CJ (2011) Epigenetic impacts on neurodevelopment: pathophysiological mechanisms and genetic modes of action. Pediatr Res 69(5 Pt 2):92R–100R
Zawia NH, Lahiri DK, Cardozo-Pelaez F (2009) Epigenetics, oxidative stress, and Alzheimer disease. Free Radic Biol Med 46(9):1241–1249
Zhang J et al (2010) Association of GSTT1, GSTM1 and CYP1A1 polymorphisms with susceptibility to systemic lupus erythematosus in the Chinese population. Clin Chim Acta 411(11–12):878–881
Zhang ZJ et al (2011) Glutathione S-transferase M1 (GSTM1) and glutathione S-transferase T1 (GSTT1) null polymorphisms, smoking, and their interaction in oral cancer: a HuGE review and meta-analysis. Am J Epidemiol 173(8):847–857