The Redox/Methylation Hypothesis of Autism

Richard C Deth, PhD

Department of Pharmaceutical Sciences, Northeastern University, Boston

Abstract

The alarming increase in autism rates has brought attention to possible adverse effects of environmentally encountered toxic substances on neurodevelopment. Recent studies of autistic children reveal evidence of oxidative stress and neuroinflammation, consistent with the metabolic consequences of a toxic insult. Sulfur metabolism provides detoxification of heavy metals and xenobiotics, maintains cellular redox status, and supports a multitude of methylation reactions, including DNA methylation. When toxic exposures cause oxidative stress, it leads to impaired DNA methylation and can disrupt epigenetic regulation of gene expression, which is critical for normal development. Dopamine stimulates a unique form of methylation involving the D4 receptor subtype, known as phospholipid methylation, which appears to play a role in synchronization of neural networks during attention. The supply of methyl groups for this process depends on the folate- and vitamin B_{12}-dependent enzyme methionine synthase, whose activity is inhibited during oxidative stress. Based on these metabolic relationships, a redox/methylation hypothesis of autism has been formulated, providing a molecular framework for understanding how environmental toxins can disrupt cognitive development. Preliminary studies suggest that metabolic interventions that normalize redox and methylation status may offer benefit in autism, and the underlying mechanisms may also have importance for other neurological and neuropsychiatric disorders.

Keywords

Autism, methylation, attention-deficit–hyperactivity disorder (ADHD), D4 dopamine receptor, epigenetic, glutathione, methionine synthase, methylcobalamin, neuroinflammation, neurodevelopmental disorder, oxidative stress, phospholipid methylation

Disclosure: The author has no conflicts of interest to declare.
Received: January 29, 2009 **Accepted:** September 7, 2009 **Citation:** *US Psychiatry*, 2010;3:48–52
Correspondence: Richard C Deth, PhD, Department of Pharmaceutical Sciences, Northeastern University, Boston, MA 02115. E: r.deth@neu.edu

The prevalence of autism has increased more than 10-fold in the US during the past two decades,[1] raising public concern and increasing research efforts to identify factors that might be responsible. Earlier work established the importance of genetic factors,[2] but it is highly unlikely that such a dramatic increase reflects purely genetic factors. Consequently, there has been increasing attention on the role of one or more 'environmental factors' whose exposure might lead to impaired development.[3,4] Not surprisingly, many theories have been put forth, in part reflecting the vast number of xenobiotic substances encountered in contemporary society. Most controversial among these is the proposal that mercury, derived from the vaccine preservative thimerosal, might play an important role.[5] However, removal of mercury from most childhood vaccines has not been associated with a decrease in autism.[6] Nonetheless, the mercury debate continues as other potential toxins receive attention, including heavy metals (e.g. lead and aluminum),[7,8] drugs (e.g. pre-natal terbutaline, antibiotics),[9,10] and chemicals (e.g. bisphenol A, pesticides).[11,12] Emerging awareness of the role of neuroinflammation and oxidative stress in autism not only illuminates the origins of this neurodevelopmental disorder,[13–21] but also sheds light on other neurological, neuropsychiatric, and neurodegenerative disorders. This review focuses on those metabolic pathways regulating the redox status of cells (i.e. the balance between reduced and oxidized states), because these pathways also support the process of methylation, in which a carbon atom (methyl group) is added to a molecule. The importance of methylation reactions is increasingly appreciated, especially for its central role in the epigenetic regulation of gene expression.

Oxidative Stress and Methylation

Many xenobiotics adversely affect metabolic pathways concerned with maintaining cellular redox status, which may represent a shared mechanism for contributing to autism. This possibility is strongly supported by recent metabolic studies that have found a pattern of significant oxidative stress in autistic children, highlighted by a decrease in glutathione (GSH), the body's principal antioxidant.[13,14,19–21] GSH, a tripeptide containing the sulfur amino acid cysteine, binds heavy metals and xenobiotics, restricting their toxicity and promoting their excretion. Reciprocally, heavy metals and xenobiotics inhibit the metabolic pathways that synthesize GSH and serve to maintain sufficient levels of its reduced form. Notably, proteins containing selenium are critical for sustaining reduced GSH, and these proteins are inhibited by mercury with remarkable affinity, promoting a condition of oxidative stress.[22,23]

Adequate levels of GSH are essential for normal function of all cells, and aerobic metabolism (i.e. mitochondrial utilization of oxygen) places a high demand on GSH status, especially in the brain, which consumes a disproportionately higher amount of oxygen than other tissues. Numerous metabolic mechanisms have evolved to monitor redox status and respond as needed to maintain GSH levels. Key among these is the folate- and vitamin B_{12}-dependent enzyme methionine synthase, which is inhibited during oxidative stress, resulting in diversion of its homocysteine substrate toward synthesis of GSH rather than converting it to methionine, as illustrated in *Figure 1*. Under normal circumstances, increased synthesis of GSH restores redox balance, allowing methionine synthase to resume conversion of homocysteine to methionine. However, if oxidative stress is not resolved, methionine synthase activity remains inhibited.[24,25]

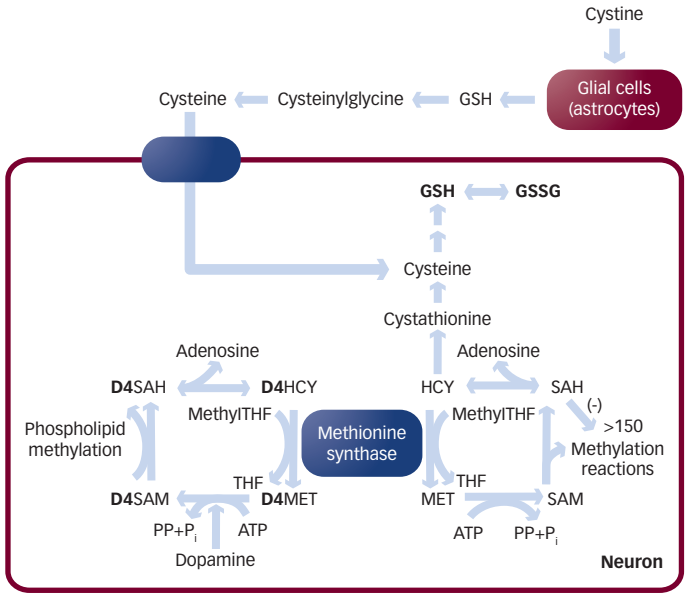
The influence of redox status on methionine synthase is the gateway to regulation of more than 150 different methylation reactions in which a carbon atom is transferred from the donor S-adenosylmethionine (SAM). Perhaps most important for autism is the methylation of sites in DNA and histones that results in inhibition of gene expression as a fundamental step in epigenetic regulation.[26] Development can be described as a program of orchestrated epigenetic transitions resulting in progression of pluripotent stem cells into differentiated cell types with distinctive functional capabilities. Altered patterns of DNA and/or histone methylation can interfere with normal development, resulting in developmental disorders, as has been well-documented in Rett, fragile X, Angelman, and Prader-Willi syndromes.[27–30] Accordingly, it is reasonable to propose that impaired methylation occurring in response to xenobiotic-induced oxidative stress would also result in adverse developmental consequences. This is the essence of the redox/methylation hypothesis of autism.

Inhibition of methionine synthase during oxidative stress results in lower levels of methionine and SAM, which is observed in plasma of autistic children. In addition, a portion of the accumulated homocysteine is converted to S-adenosylhomocysteine (SAH), a potent inhibitor of methylation reactions (see *Figure 1*). Together, the combination of low SAM and high SAH exerts a powerful negative effect on methylation reactions, including methylation of DNA and histone. Other important methylation reactions that are also inhibited during oxidative stress include the synthesis of melatonin and creatine and methylation of catecholamine neurotransmitters. Recent studies have linked changes in DNA methylation with learning and memory creation.[31–33] This fascinating concept implies that perceptual and emotional experiences are able to stably modulate gene expression, resulting in altered neuronal architecture and synaptic connectivity, and can facilitate formation of associative networks. Within this framework, impaired methylation during early years could disrupt processes that are essential for normal cognitive development.

Dopamine-stimulated Phospholipid Methylation

Of particular importance to neuropsychiatry is the ability of dopamine to stimulate methylation of membrane phospholipids, an exclusive activity of the D4 dopamine receptor subtype (see *Figure 1*), first reported by our laboratory in 1999.[34] Phosphatidylethanolamine (PE), the particular phospholipid methylated by the D4 receptor, is localized at the inner surface of the plasma membrane, where it is converted to phosphatidylcholine (PC). The newly synthesized PC then 'flips' to the outer membrane surface, where it is the predominant phospholipid. Dopamine-stimulated phospholipid methylation therefore affects the asymmetrical distribution of PE versus PC, which can have a very important impact on the function of other neurotransmitter receptors, ion channels, and other membrane proteins located near the D4 receptor. Studies have linked deficits in PC formation with a loss of cognitive abilities, while PC supplementation improves cognition.[35,36]

Figure 1: Redox and Methylation Pathways in Human Neuronal Cells



Sulfur metabolism supports synthesis of the antioxidant glutathione (GSH) and the methionine cycle of methylation (lower right). Methionine synthase regulates the flow of homocysteine (HCY) to either transsulfuration, via cystathionine and cysteine, or to methionine (MET), for synthesis of the methyl donor S-adenosylmethionine (SAM). S-adenosylhomocysteine (SAH) is a powerful inhibitor of methylation reactions. Lower activity of methionine synthase during oxidative stress increases GSH synthesis at the expense of methylation. Methionine synthase also supports the cycle of dopamine-stimulated phospholipid methylation (lower left), carried out by a methionine residue found only in the D4 dopamine receptor, which is impaired during oxidative stress.

D4 dopamine receptor activity plays an important role in attention, and a specific variant of the D4 receptor gene is widely recognized as an important risk factor for attention-deficit–hyperactivity disorder (ADHD).[37] The dramatic rise in ADHD prevalence during the past several decades and its 4:1 predominance in males versus females are similar to the pattern seen in autism, suggesting a shared etiology. The D4 receptor gene displays remarkable genetic variability among humans. In a worldwide sample, the overall frequency of the seven-repeat form was about 25%, although in native South Americans it is 80%, while it is less than 3% in native Asians.[38] Most, but not all, studies have found a three- to five-fold higher risk of ADHD associated with the presence of at least one seven-repeat allele.[39] The seven-repeat allele shows evidence of positive selection since its initial appearance 40,000–50,000 years ago, suggesting a beneficial function for an extended period of time, although now it is associated with risk for impaired attention.[40]

D4 receptor involvement in attention involves modulation of the frequency at which neural networks fire in synchrony. During

attention, involved brain regions exhibit a synchronized frequency in the 30–80Hz range, designated as gamma frequency, meaning that their information content is 'on or off' in a co-ordinated manner. As a consequence, the combined synchronized information is able to interact and selectively contribute to attention. Synchronized activity in other frequency ranges (e.g. theta, alpha, and beta) also contributes to consciousness and awareness, but gamma frequency information appears to be particularly salient, giving rise to the quality of attention. Moreover, attended information is preferentially committed to memory. We recently proposed a molecular mechanism by which dopamine-induced changes in membrane properties could tune neural networks to gamma frequency.[41]

Measurement of synchronized brain activity in medical students during attention, using magnetoencephalography (MEG), revealed that individuals carrying the seven-repeat D4 dopamine receptor exhibited significantly higher power in the gamma activity frequency range compared with individuals with either two or four repeats.[42] This observation strongly suggests that the seven-repeat form of the D4 receptor has exhibited positive selection during evolution because it increases the capacity for gamma synchronization, but is now a source of ADHD risk for some individuals.

Dopamine-stimulated phospholipid methylation is absolutely dependent on methionine synthase for its supply of methyl groups, which is not a trivial matter, since it is estimated that each D4 dopamine receptor can transfer up to 50 methyl groups/second when fully activated.[43] Methylfolate provides the methyl groups to methionine synthase, and vitamin B_{12} (cobalamin) directly participates in their transfer to homocysteine. The efficiency of dopamine-stimulated phospholipid methylation, and potentially the effectiveness of D4-receptor-mediated gamma synchronization, could therefore be adversely affected by oxidative stress, since it inactivates methionine synthase. While this provides a potential explanation for the recent rise in ADHD, MEG studies of ADHD subjects have not found a decrease, but rather an increase in gamma synchrony compared with control subjects.[44] However, despite stronger gamma synchrony, ADHD subjects failed to encode memories from the attention episode as efficiently as controls. These findings suggest that inhibition of methionine synthase may disrupt the link between attention and memory in ADHD. Impaired neuronal synchrony, including decreased gamma synchrony, is a well-documented deficit in autism,[45–47] consistent with an inability of methionine synthase activity to adequately support D4-receptor-mediated phospholipid methylation.

Neuroinflammation in Autism

Inflammation is the response of an organism to injury or insult. The classic signs of inflammation, redness, heat, swelling, pain, and loss of function primarily reflect vascular responses to local injury. However, the term inflammation also encompasses the altered metabolic state that all cells can display as part of an innate response to threatening changes in their environment. The metabolic shift recruits cellular resources geared toward cell survival, and, as a consequence, the inflammatory response limits resource availability for metabolic activities that are unique to the particular differentiated cell type. In other words, inflammation is associated with a loss of function while cells focus on staying alive. Clearly, inflammation is a useful response when needed, but once the threat is resolved, inflammation is an unwelcome guest that causes chronic diseases such as arthritis, colitis, etc. In the case of the brain, neuroinflammation causes a loss of function, whose consequences can vary depending on the age at which it occurs. Neuroinflammation during early years can interfere with normal development, contributing to disorders such as autism, whereas neuroinflammation in later years contributes to Alzheimer's and Parkinson's diseases, as well as other neurodegenerative disorders.[48,49] Intriguingly, recent studies link schizophrenia to lower levels of brain glutathione.[50]

Microglial cells serve as sentinels and responders to threats in the extracellular environment of the brain, similar to the role of macrophages in the periphery. They are activated during neuroinflammation, for example, in response to foreign materials or pathogens, to produce and release an array of pro-inflammatory cytokines that then act on neurons and astrocytes to elicit inflammatory responses. Studies of *post mortem* brain samples from autistic subjects showed clear evidence of neuroinflammation, including activated microglia and elevated levels of cytokines.[51,52] Elevated levels of pro-inflammatory cytokines in plasma have also been reported,[53] along with increased plasma and urinary levels of other biomarkers of oxidative stress, such as oxidized forms of DNA, RNA, lipids, and metabolites.[54,55] In conjunction with the lower ratio of reduced glutathione to oxidized glutathione (GSH/GSS), these findings reveal that autism is a systemic metabolic disorder in which neuroinflammation contributes to developmental delay, learning disabilities, epilepsy, and other behavioral manifestations.

While neuroinflammation plays a central role in autism, no infectious agent has been identified and it does not appear that the inflammatory response is directed toward a foreign insult. Alternatively, inflammation can result from the direct effects of xenobiotics on metabolic pathways that participate in the inflammatory response. Thus, in a sense, chemical and heavy metal exposures induce a state of inflammation by mimicking the innate response that cells depend on to respond to threatening insults. A shift of cellular redox status to a more oxidized state is a key component of the inflammatory response, and toxic substances that promote sustained oxidative stress are prime candidates for causing neuroinflammation. Shielded by the blood–brain barrier, the central nervous system (CNS) represents a unique redox environment, and the brain is especially vulnerable when this barrier is breached, particularly early in its development.

Implications for Treatment of Autism

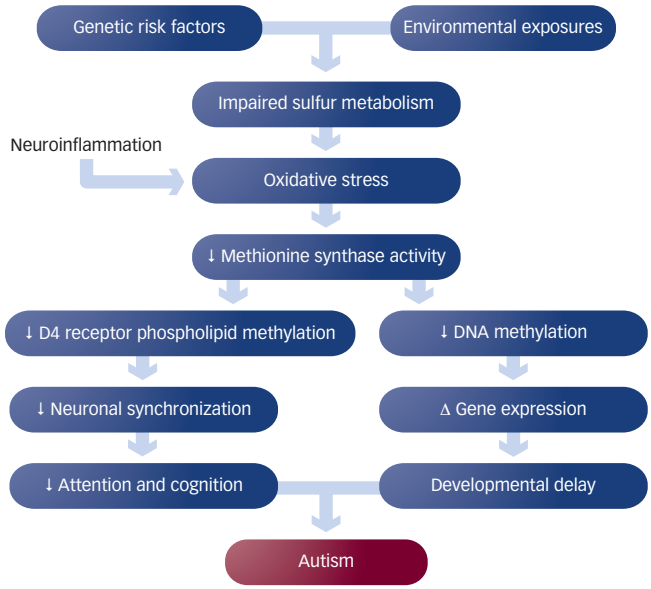
The increased prevalence of autism, along with its developmental nature, creates an imperative for treatment, but the lack of options places both clinicians and parents in a difficult, almost untenable, position. With the exception of risperidol, which provides largely behavioral control, there are currently no US Food and Drug Administration (FDA)-approved treatments for autism, reflecting, at least in part, a lack of consensus about the underlying cause. The controversy over possible involvement of neurotoxic substances, such as mercury, further compounds the problem, as clinical researchers and pharmaceutical companies assume the role of stakeholders in the debate, and distance themselves from findings that might provide

valuable clues and guidance. Moreover, awareness of the metabolic pathways of oxidative stress and redox regulation is limited within the medical community, and practitioners are not familiar with non-prescription, so-called 'biomedical,' treatments that could potentially address oxidative stress and impaired methylation. The result, for autistic children, is an unfortunately narrow set of treatment options within the traditional medical system. Intense behavioral approaches, such as applied behavioral analysis (ABA), can be effective, but the underlying metabolic condition goes untreated.

'Biomedical' treatment for autism encompasses a wide range of interventions, including nutritional supplements, antioxidants, dietary restrictions, heavy metal chelation, hyperbaric oxygen, and more. Only a very limited number of peer-reviewed clinical studies have been published to evaluate the effectiveness of these treatments. Most pertinent to the redox/methylation hypothesis are those carried out by Dr S Jill James at the University of Arkansas Children's Hospital. In a preliminary study, significantly abnormal plasma levels of redox and methylation metabolites were documented in 20 autistic subjects, some of whom were then treated under open-label conditions with an initial regimen of folinic acid (leucovorin) and betaine (trimethylglycine), followed by the further addition of methylcobalamin (Methyl-B12).[13] Plasma metabolite levels partially normalized in response to each stage of this methylation support regimen. No data were presented for neurocognitive changes, although a brief comment claimed improvement. In a more recent study, 40 autistic subjects were treated with a combination of folinic acid and methylcobalamin for a period of three months.[56] Their plasma levels of GSH and cysteine were significantly increased, as was the ratio of GSH to GSSG, indicative of improved redox status. However, thiol metabolite levels still remained below those in the control group. A parent rating scale indicated improvement in neurocognitive status in association with improved redox status, but the results were not reported due to the potential for bias.

While these preliminary results are encouraging, more robust blinded and placebo-controlled studies are needed, including neurocognitive testing measures. Validation of the importance of oxidative stress and impaired methylation in autism hinges on documentation of clinical improvement when these metabolic conditions are corrected. At the same time, clinicians dealing with autism need to learn more about the relevant metabolic pathways, including which laboratory tests can be ordered to individually assess redox and methylation status, and what supportive treatments are available to correct abnormalities.

Figure 2: The Redox/Methylation Hypothesis of Autism



Exposure to environmental toxins promotes oxidative stress, particularly in individuals carrying genetic risk factors related to sulfur metabolism. The resultant decrease in methionine synthase activity can impair dopamine-stimulated phospholipid methylation and its role in attention and attention-based learning. Lower methionine synthase activity also decreases DNA methylation and its critical role in epigenetic regulation of gene expression during development. Autism reflects the impact of neuroinflammation, oxidative stress, and impaired methylation on the developing brain.

Summary

Increasing rates of autism suggest a causative environmental factor, which is strongly supported by the documented presence of neuroinflammation in the brain, as well as by systemic metabolic disturbances that are commonly caused by heavy metals and xenobiotics. As summarized in *Figure 2*, environmental exposures can interact with genetic factors in vulnerable individuals to cause oxidative stress and decrease methylation. Adverse effects of impaired methylation on gene expression in the developing brain, and on the role of dopamine-stimulated phospholipid methylation in attention, can combine to provide a molecular framework for understanding the origins of autism. This redox/methylation framework has led to novel metabolic treatment approaches, which are currently being evaluated in clinical studies. Despite ongoing controversy over the cause of the 'autism epidemic,' researchers and clinicians must find common ground to collaborate in an effort to bring meaningful benefit to those who are affected, as soon as possible. ■

1. Yeargin-Allsopp M, Rice C, Karapurkar T, et al., Prevalence of autism in a US metropolitan area, *JAMA*, 2003;289:49–55.
2. Smalley SL, Asarnow RF, Spence MA, Autism and genetics. A decade of research, *Arch Gen Psychiatry*, 1988;45:953–61.
3. Herbert MR, Russo JP, Yang S, et al., Autism and environmental genomics, *Neurotoxicology*, 2006;27:671–84.
4. Hertz-Picciotto I, Croen LA, Hansen R, et al., The CHARGE study: an epidemiologic investigation of genetic and environmental factors contributing to autism, *Environ Health Perspect*, 2006;114:1119–25.
5. Bernard S, Enayati A, Roger H, et al., The role of mercury in the pathogenesis of autism, *Mol Psychiatry*, 2002;7:S42–3.
6. Schechter R, Grether GK, Continuing increases in autism reported to California's developmental services system: mercury in retrograde, *Arch Gen Psychiatry*, 2008;65:19–24.
7. Shannon M, Graef JW, Lead intoxication in children with pervasive developmental disorders, *J Toxicol Clin Toxicol*, 1996;34:177–81.
8. Blaylock RL, Strunecka A, Immune-glutamatergic dysfunction as a central mechanism of the autism spectrum disorders, *Curr Med Chem*, 2009,16:157–70.
9. Manev R, Manev H, Aminoglycoside antibiotics and autism: a speculative hypothesis, *BMC Psychiatry* 2001;1:5.
10. Connors SL, Crowell DE, Eberhart CG, Copeland J, et al., beta2-Adrenergic receptor activation and genetic polymorphisms in autism: data from dizygotic twins, *J Child Neurol*, 2005;20:876–84.
11. Masuo Y, Morita M, Oka S, Ishido M, Motor hyperactivity caused by a deficit in dopaminergic neurons and the effects of endocrine disruptors: a study inspired by the physiological roles of PACAP in the brain, *Regul Pept*, 2004;123:225–34.
12. D'Amelio M, Ricci I, Sacco R, Liu X, et al., Paraoxonase gene variants are associated with autism in North America, but not in Italy: possible regional specificity in gene–environment interactions, *Mol Psychiatry*, 2005;10:1006–16.
13. James SJ, Cutler P, Melnyk S, et al., Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism, *Am J Clin Nutr*, 2004;80:1611–17.
14. James SJ, Melnyk S, Jernigan S, et al., Metabolic endophenotype and related genotypes are associated with

oxidative stress in children with autism, *Am J Med Genet B Neuropsychiatr Genet*, 2006;141:947–56.
15. McGinnis WR, Oxidative stress in autism, *Altern Ther Health Med*, 2004;10:22–36.
16. Kern JK, Jones AM, Evidence of toxicity, oxidative stress, and neuronal insult in autism, *J Toxicol Environ Health B Crit Rev*, 2006;9:485–99.
17. Chauhan A, Chauhan V, Oxidative stress in autism, *Pathophysiology*, 2006;13:171–81.
18. Deth R, Muratore C, Benzecry J, et al., How environmental and genetic factors combine to cause autism: A redox/methylation hypothesis, *Neurotoxicology*, 2008;29:190–201.
19. Suh JH, Walsh WJ, McGinnis WR, Lewis A, et al., Altered sulfur amino acid metabolism in immune cells of children diagnosed with autism, *Am J Biochem Biotech*, 2008;4:105–13.
20. Zimmerman AW, Jyonouchi H, Comi AM, et al., Cerebrospinal fluid and serum markers of inflammation in autism, *Pediatr Neurol*, 2005;33:195–201.
21. Geier DA, Kern JK, Garver CR,et al., A prospective study of transsulfuration biomarkers in autistic disorders, *Neurochem Res*, 200934:386–93.
22. Carvalho CM, Chew EH, Hashemy SI, et al., Inhibition of the human thioredoxin system. A molecular mechanism of mercury toxicity, *J Biol Chem*, 2008;283:11913–23.
23. Wataha JC, Lewis JB, McCloud VV, Shaw M, Effect of mercury(II) on Nrf2, thioredoxin reductase-1, and thioredoxin-1 in human monocytes, *Dent Mater*, 2008;24:765–72.
24. Tchantchou F, Graves M, Shea TB, Expression and activity of methionine cycle genes are altered following folate and vitamin E deficiency under oxidative challenge: modulation by apolipoprotein E-deficiency, *Nutr Neurosci*, 2006;9:17–24.
25. Persa C, Pierce A, Ma Z, Kabil O, The presence of a transsulfuration pathway in the lens: a new oxidative stress defense system, *Exp Eye Res*, 2004;79:875–86.
26. Jiang Y, Langley B, Lubin FD, Renthal W, Epigenetics in the nervous system, *J Neurosci*, 2008;28:11753–9.
27. Shahbazian MD, Zoghbi HY, Rett syndrome and MeCP2: linking epigenetics and neuronal function, *Am J Hum Genet*, 2002;71:1259–72.
28. LaSalle JM, Ritchie RJ, Glatt H, Lalande M, Clonal heterogeneity at allelic methylation sites diagnostic for Prader-Willi and Angelman syndromes, *Proc Natl Acad Sci U S A*, 1998;95:1675–80.
29. Jiang YH, Sahoo T, Michaelis RC, et al., A mixed epigenetic/genetic model for oligogenic inheritance of autism with a limited role for UBE3A, *Am J Med Genet A*, 2004;131:1–10.
30. Zhao X, Pak C, Smrt RD, Jin P, Epigenetics and Neural developmental disorders: Washington DC, September 18 and 19, 2006, *Epigenetics*, 2007;2:126–34.
31. Sweatt JD, Experience-dependent epigenetic modifications in the central nervous system, *Biol Psychiatry*, 2009;65:191–7.
32. Levenson JM, Sweatt JD, Epigenetic mechanisms in memory formation, *Nat Rev Neurosci*, 2005;6:108–18.
33. Si K, Lindquist S, Kandel E, A possible epigenetic mechanism for the persistence of memory, *Cold Spring Harb Symp Quant Biol*, 2004;69:497–8.
34. Sharma A, Kramer ML, Wick PF, Liu D, D4 dopamine receptor-mediated phospholipid methylation and its implications for mental illnesses such as schizophrenia, *Mol Psychiatry*, 1999;4:235–46.
35. Troen AM, Chao WH, Crivello NA, D'Anci KE, Cognitive impairment in folate-deficient rats corresponds to depleted brain phosphatidylcholine and is prevented by dietary methionine without lowering plasma homocysteine, *J Nutr*, 2008;138:2502–9.
36. Wurtman RJ, Synapse formation and cognitive brain development: effect of docosahexaenoic acid and other dietary constituents, *Metabolism*, 2008;57:S6–10.
37. Swanson JM, Kinsbourne M, Nigg J, et al., Etiologic subtypes of attention-deficit/hyperactivity disorder: brain imaging, molecular genetic and environmental factors and the dopamine hypothesis, *Neuropsychol Rev*, 2007;17:39–59.
38. Chang FM, Kidd JR, Livak KJ, et al., The world-wide distribution of allele frequencies at the human dopamine D4 receptor locus, *Hum Genet*, 1996;98:91–101.
39. Gornick MC, Addington A, Shaw P, et al., Association of the dopamine receptor D4 (DRD4) gene 7-repeat allele with children with attention-deficit/hyperactivity disorder (ADHD): an update, *Am J Med Genet B Neuropsychiatr Genet*, 2007;144:379–82.
40. Ding YC, Chi HC, Grady DL, et al., Evidence of positive selection acting at the human dopamine receptor D4 gene locus, *Proc Natl Acad Sci U S A*, 2002;99:309–14.
41. Kuznetsova AY, Deth RC, A model for modulation of neuronal synchronization by D4 dopamine receptor-mediated phospholipid methylation, *J Comput Neurosci*, 2008;24:314–29.
42. Demiralp T, Herrmann CS, Erdal ME, et al., DRD4 and DAT1 polymorphisms modulate human gamma band responses, *Cereb Cortex*, 2007;17:1007–19.
43. RC Deth, A Kuznetsova, M Waly, Attention-related signaling activities of the D4 dopamine receptor. In: Posner M (ed.), *Cognitive Neuroscience of Attention*, New York: Guilford Publications Inc., 2004;269–82.
44. Lenz D, Krauel K, Schadow J, et al., Enhanced gamma-band activity in ADHD patients lacks correlation with memory performance found in healthy children, *Brain Res*, 2008;1235:117–32.
45. Rojas DC, Maharajh K, Teale P, Rogers SJ, Reduced neural synchronization of gamma-band MEG oscillations in first-degree relatives of children with autism, *BMC Psychiatry*, 2008;8:66.
46. Orekhova EV, Stroganova TA, Prokofyev AO, et al., Sensory gating in young children with autism: relation to age, IQ, and EEG gamma oscillations, *Neurosci Lett*, 2008;434:218–23.
47. Just MA, Cherkassky VL, Keller TA, Minshew NJ, Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity, *Brain*, 2004;127:1811–21.
48. Zhou C, Huang Y, Przedborski S, Oxidative stress in Parkinson's disease: a mechanism of pathogenic and therapeutic significance, *Ann NY Acad Sci*, 2008;1147:93–104.
49. Rojo LE, Fernández JA, Maccioni AA, et al., Neuroinflammation: implications for the pathogenesis and molecular diagnosis of Alzheimer's disease, *Arch Med Res*, 2008;39:1–16.
50. Gysin R, Kraftsik R, Sandell J, Bovet P, et al., Impaired glutathione synthesis in schizophrenia: convergent genetic and functional evidence, *Proc Natl Acad Sci U S A*, 2007104:16621–6.
51. Vargas DL, Nascimbene C, Krishnan C, et al., Neuroglial activation and neuroinflammation in the brain of patients with autism, *Ann Neurol*, 2005;57:67–81.
52. Li X, Chauhan A, Sheikh AM, Patil S, et al., Elevated immune response in the brain of autistic patients, *J Neuroimmunol*, 2009 Jan 19 (Epub ahead of print).
53. Grigorenko EL, Han SS, Yrigollen CM, et al., Macrophage migration inhibitory factor and autism spectrum disorders, *Pediatrics*, 2008;122:e438–45.
54. Ming X, Stein TP, Brimacombe M, et al., Increased excretion of a lipid peroxidation biomarker in autism, *Prostaglandins Leukot Essent Fatty Acids*, 2005;73:379–84.
55. Yao Y, Walsh WJ, McGinnis WR, Praticò D, Altered vascular phenotype in autism: correlation with oxidative stress, *Arch Neurol*, 2006;63:1161–4.
56. James SJ, Melnyk S, Fuchs G, Reid T, et al., Efficacy of methylcobalamin and folinic acid treatment on glutathione redox status in children with autism, *Am J Clin Nutr*, 2009;89:425–30.