Peer Reviewed, Open Access, Free
Published Quarterly
Mangalore, South India
ISSN 0972-5997
Volume 8, Issue 1; Jan-Mar 2009

Review

Assessment of Metabolic Parameters For Autism Spectrum Disorders

Authors
Ananth N Rao, Minakshi Koch, Suresh Kumar V, Sabyasachi Ghosh, Shobha G,
Metabolism Laboratory, Narayana Hrudayalaya, Bangalore

Address For Correspondence
Dr. Ananth N. Rao,
Consultant & Head,
Metabolism Laboratory,
Narayana Hrudayalaya, Bangalore 560099
E-mail: ananthnrao@yahoo.co.in

Citation
Rao AN, Koch M, Suresh Kumar V, Ghosh S, Shobha G. Assessment of Metabolic Parameters For Autism Spectrum Disorders. *Online J Health Allied Scs.* 2009;8(1):1

URL
http://www.ojhas.org/issue29/2009-1-1.htm

Submitted: Jan 20, 2009; Suggested revision Apr 10, 2009; Revised: Apr 17, 2009 Accepted: Apr 20, 2009 Published: May 5, 2009

Abstract:
Autism is a brain development disorder that first appears during infancy or childhood, and generally follows a steady course without remission. Impairments result from maturation-related changes in various systems of the brain. Autism is one of the five pervasive developmental disorders (PDD), which are characterized by widespread abnormalities of social interactions and communication, and severely restricted interests and highly repetitive behavior. The reported incidence of autism spectrum disorders (ASDs) has increased markedly over the past decade. The Centre for Disease Control and Prevention has recently estimated the prevalence of ASDs in the United States at approximately 5.6 per 1000 (1 of 155 to 1 of 160) children. Several metabolic defects, such as phenylketonuria, are associated with autistic symptoms. In deciding upon the appropriate evaluation scheme a clinician must consider a host of different factors. The guidelines in this article have been developed to assist the clinician in the consideration of these factors.
Key Words: Autism, Biomarkers, Immunology, Metabolic profile

Introduction:

The Autism spectrum disorders are more common in the pediatric population than are some better known disorders such as diabetes, spinal bifida, or Down syndrome.(1) Prevalence studies have been done in several states and also in the United Kingdom, Europe, and Asia. A recent study of a U.S metropolitan area estimated that 3.4 of every 1,000 children 3-10 years old had autism.(2)

Many ASD children are highly attuned or even painfully sensitive to certain sounds, textures, tastes and smells. Many children with ASD have some degree of mental impairment. When tested, some areas of ability may be normal, while others may be especially weak. One in four children with ASD develops seizures, either starting in early childhood or adolescence.(3) Seizures, caused by abnormal electrical activity in the brain, can produce a temporary loss of consciousness (a "blackout"), a body convulsion, unusual movements, or staring spells. Metabolic errors related to autism include errors of carbohydrate metabolism (4), errors of peptide metabolism (5), purine and pyrimidine disorders (6), and malabsobtion.(7) Autisms generally have abnormalities in related and overlapping areas such as oxidative stress (8), decreased methylation capacity and limited transsulfation (9,10), increased toxic burden-primarily of heavy metals and especially of mercury (11,12), immunological dysregulation with a unique inflammatory bowel disease and immune activation of glial cells in the brain (13-16) and central nervous system hypo perfusion or abnormal regulation of blood supply to the brain.(17)

Most recent data from the U.S and the U.K suggest that 1-2% of males under 12 may have autism.(18, 19)

Autism Metabolic profile and polymorphisms:

An endophenotype may be a biochemical, neurologic, hormonal, or immunologic biomarker associated with the disease state. Thus, the abnormal metabolic profile that was discovered in autistic children is an endophenotype that may reflect subtle changes in gene products that regulate flux through methionine transmethylation and transsulfuration pathways. Even small variations in gene expression and enzyme activity, if expressed chronically, could have a significant impact on downstream metabolic dynamics.(20)

Relative to controls, autistic children had a significant increase in the frequency of the reduced folate carrier RFC-1 homozygous 80GG (33% vs. 26%) and heterozygous 80GA (52% vs. 41%). Children with either the RFC-1 GA or GG genotypes were approximately two times more likely to be autistic (OR: 2.26 and 1.96, respectively). Importantly, a significant interaction between heterozygous RFC-1 80GA genotype and both the MTHFR 677CT and TT genotypes was observed among in the autistic children with odds ratios of 3.24 and 4.4, respectively. In addition, an interaction between the homozygous RFC-180GG and the MTHFR 677CT genotypes conferred a three fold increase autism susceptibility. Finally, an interaction between 3 and 4 loci was found for the compound heterozygous MTHFR677CT/1298AC and the RFC 80AG and GG genotypes. TheRFC-1 80 G allele is associated with decreased intracellular folate transport and the MTHFR 677 T allele reduces the synthesis of metabolically active folate. Together, common variants in the RFC and MTHFR genes conferred greater susceptibility to autism than either alone and suggest a potential etiologic role for impaired folate-dependent one-carbon metabolism in the susceptibility to autism. Consistent with low intracellular folate availability, methionine levels were decreased among most autistic children. Thus, the metabolic and genetic data support the possibility that the observed alterations in methionine metabolism may be due, in part, to a genetic predisposition for a functional folate deficiency.(20)

TCN2 is the major transport protein required for the cellular uptake of vitamin B12 by receptor-mediated endocytosis.(21) Previous studies indicate that a common 776C>G transition in the TCN2 gene (proline>arginine) decreases the binding affinity of TCN2 for vitamin B12 and reduces the transport of B12 into cells.(22,23) Vitamin B12 is an essential cofactor for the MS reaction and accepts the methyl group from 5-methylfolate to generate methionine from homocysteine in the initial step of the methionine transmethylation pathway. The frequency of the homozygous TCN2 776GG variant was significantly increased among the autistic children compared to controls (26% vs. 16%) and the GG variant was associated with a 1.7-fold increased risk of autism. Although speculative, the low methionine levels found in many autistic children support the possible contribution of all three variant alleles, independently or combined, to impaired methionine synthesis. In addition, children with a genetic predisposition to impaired methionine synthesis would be especially vulnerable to further reduction in enzyme activity with exposure to endogenous or exogenous oxidative stress.(24)

The third genetic variant found to be significantly more frequent among autistic children was the COMT 472 G allele. The methylation of dopamine by COMT is an important mechanism for dopamine inactivation and dopaminergic tone in the CNS.(25) The G>A transition at position 472 (valine>methionine) has been shown to influence protein expression and enzyme activity in an allelic dose/response manner.(26) The val allele is associated with thermostability and high activity whereas the met allele is associated with low activity and thermolability.(26) Compared with met carriers, individuals homozygous for the val allele showed poorer attentional control and performance on tests of executive cognition associated within efficient precortical activity.(27) In other studies, the met allele, which encodes the low activity variant, was associated with better performance on tests of prefrontally mediated

cognition.(28,29)The high activity homozygous GG (val/val) genotype was present in 29% of autistic cases and 20% of unaffected controls and was associated with a 1.74-fold increased susceptibility to autism.

Children with combined RFC-1 heterozygous 80GA and GST M1 null genotypes had a 3.78-fold increased susceptibility to autism and children with both the RFC homozygous GG and GST M1null genotypes had a 2.67-fold increase in risk. In contrast, a decrease in MTRR homozygous GG genotype among autistic children was suggestive of a protective effect.(20)

Given the relatively small number of cases and controls in the present study, it encourages one to note that several susceptibility alleles that perturb a common metabolic pathway were increased among the autistic children. This supports the possibility that some forms of autism could be a manifestation of a genetic predisposition to abnormal methionine/glutathione metabolism and oxidative stress. Further, the abnormal metabolic profile observed in a significant proportion of autistic children suggests the provocative possibility that some autistic behaviors could be a neurologic manifestation of a genetically based systemic metabolic derangement.

An area on chromosome 16p near the telomere was the next most significant, with an MLS of 1.97 in the UK families, and 1.51 in all families.(30)

Porphyrinuria and Autism:

Porphyrins are involved in the production of heme which is a necessary ingredient of the P450 enzymes which are critical for detoxification of chemicals and toxins including pesticides. Heme is also necessary to remove beta-amyloid plaques from the brain.(31) In a recent study, children with severe autism secreted 2-3 times more beta-amyloid precursor protein than children without autism.(32) Certain metals, particularly toxic metals such as mercury, lead, and arsenic, will inhibit different enzymes of the heme porphyrin pathway and will thus cause different and specific porphyrin patterns (or "profiles") in the urine, the analysis of which can help us determine which metal is involved, and to what degree.(33)
One recent prospective study of 115 children with autism demonstrated porphyrinuria when compared to 119 control children.(34) In this study, 53% of the autistic children had elevated urinary porphyrin levels. This study enrolled 115 children with autism, 63 with PDD-NOS, and 88 children with other neurological disorders such as Asperger disorder, attention deficit, hyperactivity, epilepsy, and cerebral palsy. When compared to the control group, children with autism had a mean increase of 2.6-fold ($p < 0.001$) in coproporphyrin (which I refer to as "copro"). This elevation persisted when normalized to urinary levels of creatinine. The elevation in coproporphyrin also correlated with the severity of autism.

Another prospective study on 37 autistic patients confirmed that the severity of autism was directly correlated to the degree of porphyrinuria. Non-chelated autistic children had more porphyrins present in the urine compared to children with PDD, Asperger syndrome, or control children.

Immunology and Autism

Although autism is likely a multifactorial disorder with diverse etiologies, evidence is accumulating that a combination of genetic predisposition, an environmental insult, and resulting alterations in immunity lead to the development of autoimmunity in many of the children who have a period of normal development and then develop autistic symptoms.
Several investigators have reported significant changes in various immune responses in children with autism. These changes demonstrate dysregulation of the immune system (deficiency in some components of the immune system and excesses in others). In addition, certain genes in the major histocompatibility complex (that regulates immune responses) appear to be involved in autism. Based upon immunological abnormalities, various treatment modalities have been applied to children with autism. In this brief review, these immunological changes and various biological therapies are analyzed and summarized.(35)

Innate and adaptive immune responses in children with developmental regression and autism spectrum disorders (ASD, N=71), developmentally normal siblings (N=23), and controls (N=17) were determined. With lipopolysaccharide (LPS), a stimulant for innate immunity, peripheral blood mononuclear cells (PBMCs) from 59/71 (83.1%) ASD patients produced >2 SD above the control mean (CM) values of TNF-alpha, IL-1beta,and/or IL-6 produced by control PBMCs. ASD PBMCs produced higher levels of proinflammatory/counter-regulatory cytokines without stimuli than controls. With

stimulants of phytohemagglutinin (PHA), tetanus, IL-12p70, and IL-18, PBMCs from47.9% to 60% of ASD patients produced >2 SD above the CM values of TNF-alpha depending on stimulants. Results indicate excessive innate immune responses in a number of ASD children that may be most evident in TNF-alpha production.(36)

Reported approaches and yields:

Several screening instruments have been developed to quickly gather information about a child's social and communicative development within medical settings. Among them are the Checklist of Autism in Toddlers (CHAT) (37), the modified checklist for Autism in Toddlers (M-CHAT), (38), the screening tool for autism in Two-Year-Olds (STAT) (39), and the Social Communication Questionnaire (SCQ) (40).Two other tests that should be used to assess any child with a developmental delay are a formal audiologic hearing

evaluation and a lead screening. Children with an autistic disorder usually have elevated blood lead levels.(41)
The current estimates for autism are now are reported to be on the order of 10-60 per 10,000 individuals, if all forms of ASD's are considered. In fact the Centre for Disease Control and Prevention has recently estimated the prevalence of ASD'S in the United States at approximately 5.6 per 1000 (1 of 155 to 1 of 160) children (42,43) This wide range of prevalence points to a need for earlier and more accurate screening for the symptoms of ASD.

The occurrence of autism and autistic-like findings simultaneously with certain genetic diseases (44) has led to the intensification of studies of the cause of autism. Whiteley and Shattock (45) discussed the opioid-excess theory in autism spectrum disorders. This theory suggests that autism is the result of metabolic disorder whereby peptides with opioid activity derived from dietary sources, in particular foods that contain gluten and casein, pass through an abnormally permeable intestinal membrane and enter the central nervous system.

A 4-year old girl presented with only psychiatric findings rather than any physical or neurologic pathologic findings, and the diagnosis was reached when physical findings emerged.(46) She had multi-axial diagnoses. First, her condition was diagnosed as pervasive developmental disorder not otherwise specified (axis I disorder). She also received a diagnosis of borderline intelligence functioning (axis II disorder) and metabolic disorder placed in the order of general medical conditions (axis III disorder), according to the criteria of the Diagnostic and Statistical manual of mental disorders, fourth edition (DSM-IV).(47) This fact underlines the importance of keeping in mind the "metabolic disorder" that causes psychiatric findings such as behavioral problems, hyperactivity and autistic-like findings during infancy and early childhood and is accompanied by mild or severe mental retardation and cognitive impairment. Central nervous system functions are oversensitive to the metabolic changes that emerge during fetal or early postnatal life. Spade et al (48) described a 4-year old boy who had normal development but bizarre behavior, such as laughing and crying for no reason. Normal results were obtained in this case from general physical and neurologic examinations. This case was diagnosed as OTC when the patient was aged 8 years because of the absence of physical findings.(48) Behavioral problems and autistic-like findings similarly emerge with mental retardation in some other metabolic disorders caused by innate metabolic damage. Another metabolic disease is Phenylketonuria, which is similarly often accompanied by autistic findings.

The metabolic profile of children diagnosed with autistic disorder with regressive onset was found to be severely abnormal. The autistic children were found to have significant decreases in methionine levels and in the ratio of plasma S- adenosylmethionine (SAM) to S-adenosylhomocysteine (SAM/SAH ratio), an index of methylation capacity. Total glutathione levels (GSH, the major intracellular antioxidant) were decreased and oxidized glutathione disulfide (GSSG) was increased, resulting in a threefold reduction in the redox ratio of reduced (active) GSH to oxidized (inactive) glutathione (GSH/GSSG).(20)

Using the abnormal metabolic phenotype in autistic childrenas a guide for the selection of functional candidate genes, common SNPs in genes encoding methylenetetrahydrofolate (MTHFR 677C>T, MTHFR 1298A>C), methioninesynthase reductase (MTRR 66A>G), transcobalamin II (TCN2 776C>G), catechol-O methyltransferase (COMT472G>A), glutathione-S-transferase (GST M1 null, GST T1 null), reduced folate carrier (RFC 80A>G), glutamate carboxypeptidase(GCPII1561C>T) were evaluated. These are among several high frequency low penetrance polymorphisms that have been previously shown to modulate metabolite levels in the methionine transmethylation and transsulfuration pathways.(49-52) Significant increases in odds ratios, allele frequencies and genotype distributions among autistic children were found for RFC-1 80A>G, TCN2 776C>G, and COMT 472G>A genes. An increase in the frequencies of MTHFR 677CT and GST M1 null genotypes among autistic cases achieved borderline significance. A decrease in the MTRR homozygous GG genotype and G allele frequency also achieved borderline significance among cases.(53)

Internationally Preferred biomarkers and Lab Tests:
A list that may be intended to target core issues in autism may include the following:

A. Immune blood markers:

1. Autoantibodies to endovasculature (54) performed at Washington University in St. Louis predicts immune involvement in speech.
2. Neopterin/ Biopterin:(55) Neopterin predicts the degree of cell mediated immune activation and Biopterin is a measure of attempts to down-regulate immune activation. In this urine is preferred over blood.
3. ASO and Anti-DNase B: These if significantly elevated indicate recent strep infection. (56)
4. Vaccine titers: Can be used to show immunocompetency and may reflect a response to IVIG.
5. Immunoglobulin subsets IgG (1-4), IgM, IgA and IgE: They are most helpful in sickly or autoimmune children where they can predict response to IVIG therapy.

B. Immune Urine markers:

1. Urinary N-Methylhistamine: It is a useful marker of significant inflammatory bowel disease.(57)

C. Oxidative stress Blood markers:

1. Reduced Glutathione: It is the opposite of Glutathione so it is inversely related. The higher the number the better.
2. GSSG, which is oxidized glutathione.
3. Levels of major antioxidant proteins in the serum i.e., transferrin (iron-binding protein) and ceruloplasmin (copper-binding protein) are significantly reduced in autistic children as compared to their developmentally normal non-autistic siblings (58)
4. Blood ammonia and lactate: Reflect mitochondrial function and as such reflect the state of mitochondrial function in the presence of likely oxidative stress.(59)

D. Oxidative stress urine markers:

1. 8 Hydroxyguanine (8-OHG): Is a marker for RNA oxidation in the mitochondria and cytoplasm of cells and easy to obtain for intracellular oxidative stress (60).
2. Isoprostane looks at fatty acid oxidation and reflects cell membrane stress (61)

E. Heavy Metal Markers Blood:

1. Packed erythrocyte levels of toxic metals (mercury, lead, and arsenic in particular) reflect ongoing exposure or rapid turnover from tissue. Lead in particular is trapped in bone and can be released during growth spurts without renewed exposure.

F. Heavy Metal Markers Urine:

1. Urinary Fractionated porphyrins are an ideal way to assess the metals that are left behind. It can tell if mercury is present but is a little hard to distinguish how much is mercury versus lead.

G. Decreased methylation capacity and limited transsulfation- blood:

1. Plasma Cysteine or cystine (the double form of cysteine) and plasma Methionine are frequently deficient in Autism. Decrease in either or both of these may help to determine Methyl B12 responder status. Cysteine is the sulfur containing amino acid that will act as the rate limiting step in production of glutathione- the key intracellular defence against oxidative stress. Methionine is the main donor of methyl via an intermediate S-adenosylmethionine (SAM or SAMe)

H. Intestinal permeability : Abnormal absorbtion of lactulose and mannitol can be used to determine altered permeability in the gastrointestinal tract.(62)

I. Oxalates: Can be measured in the urine and if high, support reduction in oral oxalates and perhaps reduce Vitamin C intake.

J. Urine Mucopolysaccharides and Organic acids: Trifiletti and Packard (63) emphasized that any organic condition that produces cortical dysfunction could present with psychiatric symptoms. At present it is not possible to associate a specific metabolic disease, but it is known that the genetic basis and consequently the fundamental biochemical defect are important in the pathogenesis.

K. Serum lactate, amino acids, ammonia, and Acyl Carnitines profile.

L. Serum and Urine Uric acid:

M. If elevated, Hypoxanthine Guanine Phosphoribosyl Transferase (HGPRT) and Phosphoribosylpyrophosphate (PRPP) synthetase testing.

N. If low, Purine/ Pyrimidine panel (Uracil excretion, Xanthine, Hypoxanthine).

Discussion:

Although there are many concerns about labeling a young child with an ASD, the earlier the diagnosis of ASD is made, the earlier needed interventions can begin. Proven biomarkers are essential for diagnosis. Given the fact that ASD is a multifactorial disease, choices of a biomarker are limited, since one cannot perform an array of investigations on every child, which in most cases may amount to over-investigating. Accumulated evidence indicates a few markers and an approach to the diagnosis. Fragile X syndrome has been suggested to be ruled in all cases of suspected ASD. Other investigations, mentioned in the article are relevant at this point of time, but a more aggressive approach in the research of autism will definitely lead to a better package of biomarkers for suspected ASD. This, we hope will pave way for prenatal diagnosis too.

Evidence over the last 15 years indicates that intensive early intervention in optimal educational settings for at least 2 years during the preschool year's results in improved outcomes in most young children with ASD.(1) Autism treatment, particularly in the areas of language and social skills, is extremely important and should be started early on to help increase the chances of developing language and social skills. In recent years, experts have made remarkable strides in better understanding autism, including developing more effective methods of diagnosis in treatment. In the department of metabolic diseases and research, Narayana Hrudayalaya we are continuing to investigate this complex disorder in the hopes of not only improving diagnoses and interventions, but also discovering autism's causes and possible means of prevention.

References:

1. Fillipek PA, Accardo PJ, Baranek GT, Cook Jr. EH, Dawson G, Gordon B, Gravel JS, Johnson CP, Kellen RJ, Levy SE, Tuchman RF, Volkmar FR. The screening and diagnosis of autism spectrum disorders. *Journal of Autism and Developmental Disorders* 1999;29(2):439-484.
2. Yeargin-Allsopp M, Rice C, Karapurkar T, Doernberg N, Boyle C, Murphy C. Prevalence of Autism in U.S metropolitan area. *Journal of the American Medical Association.* 2003 Jan 1;289(1):49-55
3. Volkmar FR. Medical Problems, Treatments, and Professionals. In: Powers MD, ed. Children with Autism: A Parent's Guide, Second Edition. Bethesda, MD: Woodbine House, 2000;73-74.
4. Coleman M, Brass JP. Autism and lactic acidosis. *Journal of autism and developmental disorders* 1985;15:1-8
5. Reichelt KL, Saelid G, Lindback T. Childhood autism: A complex disorder. *Biological Psychiatry* 1986;21:1279-1290
6. Simmonds HA, Duley JA, Fairbanks LD, McBride MB. When to investigate for pure and pyrimidine disorders. Introduction and review of clinical and laboratory indications 1997;20(2):214-226
7. Goodwin MS, Cowen MA, Goodwin TC. Malabsobtion and cerebral dysfunction. A multivariate and comparative study of autistic children *J Autism Child Schizophr* 1971;1(1):48-62
8. Yao Y, Walsh WJ, McGinnis WR, Pratico D. Altered vascular phenotype in Autism: correlation with oxidative stress. *Arch Neurol.* 2006 Aug;63(8):1161-64.
9. James SJ, Melnyk S, Jernigan S, Cleves MA, Halsted CH, Wong DH, Cutler P, Bock K, Bradstreet JJ, Baker SM, Gaylor DW. Metabolic endophenotype and related genotypes are associated with oxidative stress in children with Autism. *Am J Med Genet B Neuropsychiatr Genet.* 2006;141:947-956.
10. James SJ, Cutler P, Melnyk S, Jernigan S, Janak L, Gaylor DW, Neubrander JA. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. *Am J Clin Nutr.* 2004 Dec;80(6):1611-7
11. Bradstreet, JJ et al. A Case Control study of Mercury Burden in Children with Autistic Spectrum Disorders. *Journal of American Physicians and Surgeons.* 2003;8(3):76—79
12. Nataf R, Skorupka C, Amet L, Lam A, Springbett A, Lathe R. Porphyrinuria in childhood autistic disorder: implications for environmental toxicity. *Toxicol Appl Pharmacol.* 2006 Jul 15;214(2):99-108. Epub 2006 Jun16.
13. Jyonouchi H, Geng L, Ruby A,Zimmerman -Bier B. Dysregulated innate immune responses in young children with autism spectrum disorders : their relationship to gastrointestinal symptoms and dietary intervention. *Neourophyschobiology.* 2005;51(2):77-85
14. Balzola L, Barbon V, Repici A, Rizzetto M, Clauser D, Gandione M, Sapino A. Panenteri IBD-like diseasein a patient with regressive autism shown for the first time by the wireless capsule enteroscopy: another piece in the jigsaw of this gut-brain syndrome? *Am J Gastroenterol.* 2005 Apr;100(4);979-81
15. Ashwood P, Anthony A, Torrente F, Wakefield AJ. Spontaneous mucosal leucocyte cytokine profiles in children with autism and gastrointestinal functions: mucosal immune activation and reduced counter regulatory interleukin-10. *J Clin Immunol.* 2004 Nov;24(6):664-73
16. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and meuroinflammation in the brain of patients with autism. *Ann Neurol.* 2005 Jan;57 (1):67-81. Erratum in: Ann Neurol.2005 Feb;57(2):304.
17. Ito H, Mori K, Hashimoto T, Miyazaki M, Hori A, Kagami S, Kuroda Y. Findings of brain 99m-Tc-ECD SPECT in high functioning autism-3-dimensional stereotactic ROI template analysis of brain SPECT. *J Med Invest.* 2005;52(1-2):49-56.
18. Baird G, Simonoff E, Pickles A, Chandler S, Loucas T, Meldrum D, Chairman T. Prevence of the disorders of the autism spectrum in a population cohort of children in south Thames: the Special Needs and Autism Project (SNAP). *Lancet* 2006 Jul 15;368:210-5.
19. Morbidity and mortality weekly. Mental health in the United States: Parental report of Diagnosed Autism in children aged 4-17 years. United States, 2003-2004. May 5, 2006;55(17);482-486.
20. James SJ, Melnyk S, Jernigan S, Cleves MA et al. Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. *Am. J. of Genetics Part B (Neuropsychiatric Genetics).* 2006;141:1-10.
21. Seetharam B. Receptor mediated endocytosis of cobalamin. *Annu. Rev Nutr.* 1999;19:173-195.
22. Afman LA, Lievens KJ, Van der Put NM, Trijbels FJ, Blom HJ.Single nucleotide polymorphisms in the transcobalamin gene: Relationship with transcobalamin concentrations and risk for neural tube defects. *Eur J Hum Genet.* 2002;10:433-438.
23. Miller JW, Ramos MI, Garrod MG, Flynn MA, Green R. Transcobalamin II 775G>C polymorphism and indices of vitamin B12 status in healthy older adults. *Blood* 2002;100:718-720.
24. Mosharov E,Cranford MR, Banerjeee R. The quantitatively important relationship between homocysteine metabolism and glutathione synthesis by the transulfuration pathway and its regulation by redox changes. *Biochemistry* 2000;39:13005-13011.
25. Nieoullon A.Dopamine and the regulation of cognition and attention. *Prog Neurobiol.* 2002;67:53-83.
26. Chen JS, Lipsika BK, Halim N,Ma QD, Matsumoto M,Melhem S, Kolachana BS, Hyde TM, Herman MM. Apud J, Egan MF, Kleiman JE, Weinberger DR. Functional analysis of genetic variation in catechol-o-methyltransferase (COMT). Effects on

MRNA, protein and enzyme activity in postmortem human brain. *Am J Hum Genet.* 2004;75:807-821.

27. Blasi G, Mattay VS, Bertolino A, Elvevag B, Calliott JH, Das S, Kolachana BS,Egan MF, Goldberg TE, Weinberg DR. Effect of Catechl-o-158 methyltransferase val met genotype on attention control. *J Neurosci.* 2005;25:5038-5045.
28. Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, Golman D, Weinberger DR. Effect of COMT Val 108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci USA.* 2001;98:6917-6922.
29. Diamond A, Briand L, Fossella J, Gehlbach L. Genetic and neurochemical modulation of prefrontal cognitive functions in children. *Am. J Psychiatry* 2004;161:125-132.
30. International Molecular Genetic Study of Autism Consortium. A full genome screen for autism with evidence for linkage to a region on chromosome 7q. *Hum Mol Genet.* 1998;7(3):571 –578
31. Atamna H, Boyle K. Amyloid-beta peptide binds with heme to form a peroxidase: Relationship to the cytopathologies of Alzheimer's disease. *PNAS* 2006;103;3381–6.
32. Sokol DK, Chen D, Farlow MR, Dunn DW, Maloney B, Zimmer JA, Lahiri DK. High levels of Alzheimer beta-amyloid precursor protein (APP) in children with severely autistic behavior and aggression. *J Child Neurol* 2006 Jun;21(6):444–9.
33. Woods JS. Altered porphyrin metabolism as a biomarker of mercury exposure and toxicity. *Can J Physiol Pharmacol* 1996;74 (2):210–5.
34. Nataf R, Skorupka C, Amet L, Lam A, Springbett A, Lathe R. Porphyrinuria in childhood autistic disorder: implications for environmental toxicity. *Toxicol Appl Pharmacol* 2006;214(2):99–108.
35. Gupta S. Immunological treatments for autism. *J Autism Dev Disord.* 2000;30(5):475-9.
36. Jyonouchi H, Sun S, Le H. Pro inflammatory and regulatory cytokine production associated with innate and adaptive immune responses in children with autism spectrum disorders and developmental regression. *J Neuroimmunol.* 2001;120(1-2):170-9.
37. Baird G, Charman T, Baron-Cohen S, Cox A, Sweetenham J, Wheelwright S, Drew A. A screening instrument for autism at 18 months of age: A 6-Year follow up study. *Journal of the American Academy of Children and Adolescent Psychiatry.* 2000;39:694-702.
38. Robbins DL, Flein D, Barton ML, Green JA. The modified checklist for autism in toddlers: an initial study investigating the early detection of autism and pervasive developmental disorders. *Journal of Autism and Developmental Disorders* 2001;31(2):149-151.
39. Stone WL, Coonrod EE, Ousley OY. Brief report: screening tool for autism in two-year-olds (STAT): development and preliminary data. *Journal of autism and Developmental Disorders* 2000;30(6):607-612.
40. Berument SK, Rutter M, Lord C, Pickles A, Bailey A. Autism screening questionnaire: diagnostic validity. *British Journal of Psychiatry* 1999;175:444-451.
41. Fillipek PA, Accardo PJ, Ashwal S, Baranek GT, Cook Jr. EH, Dawson G, Gordon B, Gravel JS, Johnson CP, Kellen RJ, Levy SE, Minshew NJ, Ozonoff S, Prizant BM, Rapin I, Rogers SJ, Stone WL, Teplin SW,Tuchman RF, Volkmar FR. The screening and diagnosis of autism spectrum disorders. *Journal of Autism and Developmental Disorders* 1999;29(2):439-484.
42. Fombonne E. The prevalence of autism. *JAMA* 2003;289:87-89.
43. Mental health in the United States; parental report of diagnosed autism in children aged 4-17 years- United States, 2003-2004. *MMWR. Morbid Mortal Wkly Rep* 2006;55:481-486.
44. Lotspeich LJ, Ciaranello RD. The neurobiology and genetics of infantile autism. *Int Rev Neurobiol* 1993;35:87-129.
45. Whiteley P, Shattock P. Biochemical aspects in autism spectrum disorders: updating the opioid excess theory and presenting new opportunities for biomedical intervention. *Expert Opin Ther Targets* 2002;6(2):175-83.
46. Isik Gorker, Umran Tuzun. Autistic like findings associated with a urea cycle disorder in a 4- year old girl. *J Psychiatry Neurosci* 2005;30(2):133-135
47. American Psychiatric association. Diagnostic and Statistical manual of mental disorders. 4th ed. Washington: The Association; 1994.
48. Spade M, Guardamagna O, Rabier D, Van der Meer SB, Parvy P, Bardet J, et al. Recurrent episodes of bizarre behaviour in a boy with ornithine transcarbamylase deficiency: diagnostic failure of protein loading and allopurinol challenge tests. *J Pediatr* 1994;125(2):249-51.
49. James SJ, Pogribna M, Pogribny IP, Melnyk S, Hine RJ, Gibson JB, Yi P, Tafoya DL, Swenson DH, Wilson VL, Gaylor DW. Abnormal folate metabolism and mutation in the methylenetetrahydrofolate reductase gene may be maternal risk factors for Down syndrome. *Am J Clin Nutr* 1999;70:495–501.
50. Stern LL, Shane B, Bagley PJ, Nadeau M, Shih V, Selhub J. Combinedmarginal folate and riboflavin status affect homocysteine methylation incultured immortalized lymphocytes from persons homozygous for the MTHFR C677T mutation. *J Nutr.* 2003;133:2716–2720.
51. Beagle B, Yang TL, Hung J, Cogger EA, Moriarty DJ, Caudill MA. The glycine N-methyltransferase (GNMT) 1289 C->T variant influences plasma total homocysteine concentrations in young women after restricting folate intake. *J Nutr* 2005;135:2780–2785.
52. Castel-Dunwoody KM, Kauwell GP, Shelnutt KP, Vaughn JD, Griffin ER, Maneval DR, Theriaque DW, Bailey LB. Transcobalamin 776C->G polymorphism negatively affects vitamin B-12 metabolism. *Am J Clin Nutr.* 2005;81:1436–1441.

53. Gottesman II, Gould TD. The endophenotype concept in psychiatry: Etymology and strategic intentions. *Am J Psychiatry.* 2003;160:636–645.
54. Connolly AM, Chez MG, Pestronk A, Arnold ST, Mehta S, Deuel RK. Serum autoantibodies to brain in Landau- Kleffner variant, autism, and other neurological disorders. *J Pediatr.* 1999;134(5):607-13
55. Sweeten TL, Posey DJ, McDougle CJ. Blood monocyte count and neopterin levels in children with autistic disorder. *Am J Psychiatry.* 2003;160(9):1691-3
56. Messahel S, Pheasant AE, Pall H, Ahmed Choudhury J, Sungum- Paliwal RS, Vostanis P. Urinary levels of neopterin and biopterin in autism. *Neurosci Lett.* 1998;241(1):17-20.
57. Kim JW, Grant JE, Kim SI, Swanson TA, Bernstein GA, Jaszcz WB, Williams KA, Schlievert PM. A possible association of recurrent streptococcal infections and acute onset f obsessive-compulsive disorder. *J Neuropsychiatry Clin Neurosci.* 2004;16(3):252-60.
58. Chauhan A, Chauhan V. Oxidative stress in autism. *Pathophysiology.* 2006 Aug;13(3):171-81.
59. Filipek PA, Juranek J, Nguyen MT, Cummings C, Gargus JJ. Relative carnitine deficiency in autism. J Autism Dev Discord. 2004 Dec;34(6):5615-23
60. Svoboda P, Maekawa M, Kawai K, Tominaga T, Savela K, Kasai H. Urinary 8-hydroxyguanine may be a better marker of oxidative stress than 8-hydroxydeoxyguanosine in relation to the life spans of various species. *Antioxid Redox signal.* 2006 May-Jun;8(5-6):985-92.
61. Ming X, Stein TP, Brimacombe M, Johnson WG, Lambert GH, Wagner GC. Increased excretion of a lipid peroxidation biomarker in autism. *Prostaglandins Leukot Essent Fatty Acids.* 2005;73(5):379-84
62. D'Eufemia P, Celli M, Finocchiaro R, Pacifico L, Viozzi L, Zaccagnini M, Cardi E, Giardini O. Abnormal intestinal permeability in children with autism. *Acta Paediatr.*1996;85(9):1076-9.
63. Trifiletti RR, Packard AM. Metabolic disorders presenting with behavioral symptoms in the school aged child. *Child Adolesc Psychiatr Clin N Am* 1999;8(4):791-806