Association of MTHFR Gene Variants with Autism

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ABSTRACT

Autism is a complex neurodevelopment disorder with numerous possible genetic and environmental influences.

We retrospectively examined the laboratory data of 168 children sequentially referred to our facility with a confirmed diagnosis of autism or pervasive developmental disabilities (PDD). Since folate and methylation (single carbon metabolism) are vital in neurological development, we routinely screened children for the common mutations of the methylenetetrahydrofolate reductase gene (MTHFR), which regulates this pathway. All children had polymerase chain reaction (PCR) DNA evaluation to determine the frequency of the 677 and 1298 common polymorphisms in the MTHFR gene.

We observed a significantly increased frequency of the homozygous mutation 677CT allele (TT): 23% in the autistic children compared to 11% in the control population ($P<0.0001$). Additionally, the heterozygous 677CT allele (CT) was present in 56% of the autistic children compared to 41% in the control population ($P<0.0001$). Somewhat paradoxically, the normal 1298AA allele was significantly higher in the autistic group, 55%, compared to the controls, 44% ($P<0.05$). Despite the increased frequency of normal 1298AA alleles, the compound 677CT/1298AC heterozygous mutations were more prevalent in the autistic population, 25%, than in controls, 15% ($P=0.01$).

Overall, the data show an increased risk of autism spectrum disorder (ASD) associated with common mutations affecting the folate/methylation cycle. These associations by themselves may provide a partial explanation for a subgroup of children genomically at risk for ASD disorders. Increased folinic acid during pregnancy and early development may offset the genomic risk factors, and this deserves further study. Further, since folate-dependent methylation provides, in part, the methyl group for inactivation of monoamine neurotransmitters via the catecholamine-O-methyltransferase (COMT) system, this observation may help to further differentiate subtypes within the broad phenotype of ASD. A search for additional genomic and environmental risk factors should be undertaken. In particular, the methylation/transsulfation and COMT pathways should be investigated.

Background

It is generally accepted that the prevalence of autism and pervasive developmental disorders (PDD) has risen significantly in the last two decades. These disorders interfere with normal development of language and socialization. Atypical patterns of stereotypic and restricted activities are common features of these syndromes. Multiple theories regarding causality have been generated, and typically these focus on genetic vulnerability and environmental risk factors. As yet, no theory has gained wide acceptance.

Clinically available testing for methylenetetrahydrofolate reductase (MTHFR) gene mutations (polymorphisms) has recently become available and had been incorporated into our evaluation process for developmentally delayed children. The MTHFR gene codes for an essential enzyme in folate metabolism. To further understand this condition, we retrospectively evaluated our findings regarding the genomic variations in the gene. MTHFR enzyme catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Methyltetrahydrofolate is essential in one-carbon-donor metabolism for the remethylation of homocysteine to methionine and the generation of metabolically active tetrahydrofolate in the methionine synthase reaction.[1] Common polymorphisms in the MTHFR gene have been associated with reduced enzyme activity. A detailed review of folate metabolism and MTHFR is available from Schiver et al.[2]

MTHFR is located on chromosome 1 at 1p36.3. Common single nucleotide polymorphisms of the 677C→T and the 1298A→C alleles in the MTHFR gene decrease the activity of the enzyme.[3] The 677C→T allele has been associated with neural tube defects,[4-7] cerebrovascular and cardiovascular disease,[8-15] inflammatory bowel disease,[16] colorectal cancer,[17-18] and psychiatric disorders.[19-20]

The 677C→T and the 1298AC gene variants are prevalent in many populations. The heterozygous 677CT genotype ranges from 13% in Africans to 51% in Italians and 44% in North American Caucasians.[2] [1]The homozygous 677TT rate in the last group is 12%. The prevalence of heterozygous genotype 1298AC among Caucasians in the United States was 47%, and that of the homozygous 1298AA mutant allele was 7.9%.[2] [2]

The 677C→T allele is characterized by a mutation of a cytosine to a thymine giving rise to an amino acid replacement of valine for alanine in the catalytic domain of the enzyme. Homozygosity for the mutant T allele is associated with a 60% reduction in enzyme activity.[2] [3]The 1298A→C mutant allele has a cytosine substitution for adenine, resulting in a glutamate to alanine change within the C-terminal regulatory domain.[2] [4]Compound heterozygosity for both the 677CT and 1298AC is associated with a decrease of approximately 50%-60% in MTHFR activity.[2] [5]

In a study by Ramaekers et al., low 5-methyltetrahydrofolate levels in the spinal fluid of children who had normal neurodevelopment until age 4 to 6 months was associated with subsequent neurological regression.[2] [6]Addition of folinic acid as a dietary supplement corrected the symptoms. The observed favorable response to folinic acid further supports a central role for methylation in at least some developmental disorders.

Materials and Methods: Population

All the 168 Caucasian children, whose charts were retrospectively analyzed, were in the private practice of the principal investigator. A diagnosis of autism (73.8%) or PDD (26.2%) was previously made either by a neurologist, psychiatrist, neuropsychologist, or developmental pediatrician, and was confirmed by the investigator after referral. All the children meet

Table 1. MTHFR C677T and A1298C Genotypes in 168 Children with Autism

MHTFR 1298AC Alleles	MHTFR 677→T Alleles		
	CC	CT	TT
AA	2	52	39
AC	23	42	–
CC	10	–	–

Table 2. Frequency of 677CT Genotypes in Autistic and Control Populations

677C→T	CC	CT	TT	T ALLELE FREQUENCY
Autistic	35 (21%)*	94 (56%)*	39 (23%)*	0.51*
Control	2570 (48%)	2213 (41%)	606 (11%)	0.32

*P<0.0001

Table 3. Distribution of 1298AC Genotype in Autistic and Control Populations

1298AC	AA	AC	CC	C ALLELE FREQUENCY
Autistic	93 (55%)*	65(39%)	10 (6%)	0.25
Controls	70 (44%)	75 (47%)**	14 (9%)	0.32

*P=0.0005
**P=0.04

Table 4. Frequency of Compound Heterozygous Genotypes in Autistic and Control Populations

	677CT and 1298AC	NON 677CT and 1298AC
Autistic	42(25%)*	126 (75%)
Non-Autistic	43(15%)*	236(85%)

*P=0.01

the DSM-IV criteria for their psychiatric diagnosis. In the study, these groups will be referred to as autistic spectrum disorders (ASD). All parents gave informed consent prior to testing, and ethical approval for the retrospective chart review was granted by a private Investigational Review Board (Arizona State University, Tempe, Arizona).

In the study group, there were 142 males (84.5%) and 26 females (15.5%). The distribution by gender was statistically similar for both autism and PDD. Among the 168 children, 149 were diagnosed with regressive autism, and 19 showed no evidence of regression.

The genetic frequencies in the control population for the 677C→T genotypes were derived from Ogino and Wilson's data of MTHFR genotypes in a Caucasian population of 5,389 persons.[27] The 1298A→C polymorphism frequencies in U.S. Caucasians were utilized as reported by Rady et al.[29] The control compound polymorphism (677CT+1298AC) rates were obtained from Weisberg et al.[24]

Laboratory Methods

Blood specimens were previously processed by PCR DNA analysis for MTHFR alleles at the Mayo Clinic (20.2%), North Shore University Hospital-Long Island Jewish Hospital Core Laboratory (58.3%), or Quest Laboratories (21.4%). All laboratories utilize standardized, commercially available PCR primer kits, with accepted internal controls. Laboratory selection was determined by the participants' insurance relationships with the various laboratories utilized. There were no significant differences in the frequencies of reported polymorphism between any of the laboratories.

Statistical Analysis

The Fisher's Exact Test was applied to a two-way frequency table. A null hypothesis of interest was stated, and a *P*-value was calculated. For each of 677C→T and 1298A→C variant alleles the following were compared:
a) Overall distribution of ASD and controls,
b) Proportion of homozygous in ASD and controls,
c) Proportion of variant (i.e., homozygous or heterozygous) in ASD and controls, and
d) Allele frequency in ASD and controls.

Results

The frequency of the MTHFR 677C→T and 1298A→C genotypes in the 168 ASD children are shown in Table 1. The homozygous 677TT allele was present in 39 (23%) of the ASD children and in 606 (11%) of the controls (P<0.0001) (Table 2). The heterozygous 677CT allele occurred in 56% of children in the ASD group. This was significantly greater than the 41% prevalence in the control group (P=0.0001). The 677C→T allele frequency in the 168 ASD children was 0.51 compared to an allele frequency of 0.32 among the 5389 controls (P<0.0001) (Table 2).

The homozygous and heterozygous 1298A→C mutant alleles (1298CC and 1298AC) were similar in the ASD and control groups (Table 3). However, the normal 1298AA allele was significantly higher in the ASD group, being present in 93 (55%) affected children compared to 70 (44%) controls (P=0.0005). The 1298A→C allele frequency was significantly lower in ASD (0.25) than the controls (0.32), with P=0.04.

Heterzygosity for both the 677CT and 1298AC was identified in 25% of the ASD children, but only 15% of the controls (Table 4). This was significant, with P=0.01.

Discussion

The data demonstrate that 677C→T polymorphisms, whether homozygous or heterozygous, are significantly associated with ASD. The homozygous (TT) individuals are reported to have an approximately 50% decrease in MTHFR enzyme activity, and the heterozygous (CT) a 30% decrease in enzyme activity as measured in their lymphocytes.[2,12,2]

The 1298AA normal alleles are more prevalent in the control population than in children with ASD. The compound heterozygous state, 677CT/1298AC, which lowers enzyme activity by 50-60%,[2,2] was found to be significantly more prevalent in the autistic group. Notably, only 2% of children with ASD in our study presented without at least one polymorphism in the MTHFR gene.

It is unlikely that any single polymorphism accounts for the majority of autistic risk factors. The high natural prevalence of MTHFR variants in the absence of autistic symptoms could be interpreted in various ways. Given the rising prevalence of ASD, it may indicate emergence of a new environmental risk factor that exposes this genomic vulnerability commonly present in the folate

pathway. Multiple studies on Down syndrome have shown that polymorphisms in the folate pathway are associated with this syndrome.[2][83-0] Low plasma levels of transcobalamin combined with polymorphisms in methionine synthase reductase interact with MTHFR to increase the risk of neural tube defects.[3][1] This study does not take into account the numerous potential influencing cofactors, which may be additive to the MTHFR observations, e.g. dietary folate, serum folate, dietary B vitamin intakes, amino acid deficiencies, environmental exposures, or heavy-metal exposure. It is likely some combination of these influences the phenotypic expression (ASD symptoms) of the genomic risk factors (MTHFR polymorphisms).

The data support the hypothesis that ASD syndromes are associated with single nucleotide mutations of the MTHFR gene in some cases. Although 677C→T variant alleles (677CT or 677TT) and the heterozygous compound allele (677CT/1298AC) are significantly increased in the ASD group, it is unlikely that this association alone is sufficient to produce the complex array of symptoms associated with ASD. Therefore, a search for additional genomic, metabolic, epigenetic, transposon, and environmental risk factors should be undertaken.

Based on the observed MTHFR-related genetic variations in children with ASD, it is reasonable to evaluate dietary supplementation with folinic acid and its cofactors in the methylation cycle, e.g. B vitamins and trimethylglycine (Betaine), for these children. This would be particularly important in the subgroup shown to carry MTHFR polymorphisms.

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REFERENCES

[1] Rosenblatt D. Methylenetetrahydrofolate reductase. *Clin Invest Med* 2001;24:56-59.

[2] Scriver CR, Beaudet AL, Sly WS, Valle D. *The Metabolic and Molecular Basis of Inherited Disease.* New York, N.Y.: McGraw-Hill; 2000.

[3] Chango A, Boisson F, Barbe F, et al. The effect of 677CT and 1298AC mutations on plasma homocysteine and 5,10-methylene-tetrahydrofolate reductase activity in healthy subjects. *Br J Nutr* 2000;83(6):593-596.

[4] Van der Put NM, Gabreels F, Stevens EM, et al. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? *Am J Hum Genet* 1998;62:1044-1051.

[5] Trembath D, Sherbondy AL, Vandyke DC, et al. Analysis of select folate pathway genes, PAX3, and human T in a Midwestern neural tube defect population. *Teratology* 1999;59:331-341.

[6] Rampersaud E, Melvin EC, Siegel D, et al. NTD Collaborative Group. Updated investigations of the role of methylenetetrahydrofolate reductase in human neural tube defects. *Clin Genet* 2003;63:210-214.

[7] Volcik KA, Shaw GM, Lammer EJ, Zhu H, Finnell RH. Evaluation of infant methylenetetrahydrofolate reductase genotype, maternal vitamin use, and risk of high versus low level spina bifida defects. Birth Defects Res Part. A *Clin Mol Teratol* 2003;67:154-157.

[8] Goyette P, Christensen B, Rosenblatt DS, Rozen R. Severe and mild mutations in cis for the methylenetetrahydrofolate reductase (MTHFR) gene and description of five novel mutations in MTHFR. *Am J Hum Genet* 1996;59:1268-1275.

[9] Kluijtmans LA, Kastelein JJ, Lindemans J, et al. Thermolabile methylenetetrahydrofolate reductase in coronary artery disease. *Circulation* 1997;96:2573-2577.

[10] Reinhardt D, Sigusch HH, Vogt SF, et al. Absence of association between a common mutation in the methylenetetrahydrofolate reductase gene and the risk of coronary artery disease. *Eur J Clin Invest* 1998;28:20-23.

[11] Cattaneo M, Tsai MY, Bucciarelli P, et al. A common mutation in the methylenetetrahydrofolate reductase gene (C677T) increases the risk for deep-vein thrombosis in patients with mutant factor V (factor V:Q506). *Arterioscler Thromb Vasc Biol* 1997;17:1662-1666.

[12] Margaglione M, D'Andrea G, d'Addedda M, et al. The methylenetetrahydrofolate reductase TT677 genotype is associated with venous thrombosis independently of the coexistence of the FV Leiden and the prothrombin A20210 mutation. *Thromb Haemost* 1998;79:907-911.

[13] Kostulas K, Crisby M, Huang WX, et al. A methylenetetrahydrofolate reductase gene polymorphism in ischaemic stroke and in carotid artery stenosis. *Eur J Clin Invest* 1998;28:285-289.

[14] Verhoef P, Rimm EB, Hunter DJ, et al. A common mutation in the methylenetetrahydrofolate reductase gene and risk of coronary heart disease: results among U.S. men. *J Am Coll Cardiol* 1998;32:353-359.

[15] Nakata Y, Katsuya T, Takami S, et al. Methylenetetrahydrofolate reductase gene polymorphism: relation to blood pressure and cerebrovascular disease. *Am J Hypertens* 1998;11:1019-1023.

[16] Mahmud N, Molloy A, McPartin J, et al. *Gut* 1999;45:389-394.

[17] Chen J, Giovannucci E, Kelsey K, et al. A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. *Cancer Res* 1996;56:4862-4864.

[18] Ryan BM, Molloy AM, McManus R, et al. The methylenetetra-hydrofolate reductase (MTHFR) gene in colorectal cancer: role in tumor development and significance of allelic loss in tumor progression. *Int J Gastrointest Cancer* 2001;30:105-111.

[19] Bonig H, Daublin G, Schwahn B, Wendel U. Psychotic symptoms in severe MTHFR deficiency and their successful treatment with betaine. *Eur J Pediatr* 2003;162:200-201.

[20] Bjelland I, Tell GS, Vollset SE, Refsum H, Ueland PM. Folate, vitamin B12, homocysteine, and the MTHFR 677CT polymorphism in anxiety and depression: the Hordaland Homocysteine Study. *Arch Gen Psychiatry* 2003;60:618-626.

[21] Botto LD, Yang Q. 5,10-methylenetetrathydrofolate reductase gene variants and congenital anomalies. *Am J Epidemiol* 2000;151:862-877.

[22] Robien K, Ulrich CM. 5,10-Methylenetetrahydrofolate reductase polymorphisms and leukemia risk: a HuGE minireview *Amer J Epidemiol* 2003;157:571-582.

[23] Weisberg I, Tran P, Christensen B, Sibani S, Rozen R. A second genetic polymorphism in methyltetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. *Mol Genet Metab* 1998;64:159-172.

[24] Weisberg IS, Jacques PF, Selhub J, et al. The 1298AC polymorphism in methylenetetrahydrofolate reductase (MTHFR): in vitro expression and association with homocysteine. *Atherosclerosis* 2001;156:409-415.

[25] Rady PL, Szucs S, Grady J, et al. Genetic polymorphisms of methylenetetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) in ethnic populations in Texas; a report of a novel MTHFR polymorphic site, G1793A. *Am J Med Genet* 2002;107:162-168.

[26] Ramaekers VT, Hausler M, Opladen T Heimann G, Blau N. Psychomotor retardation, spastic paraplegia, cerebellar ataxia, and dyskinesia associated with low 5-methyltetrahydrofolate in cerebrospinal fluid: a novel neurometabolic condition responding to folinic acid substitution. *Neuropediatrics* 2002;33:301-308.

[27] Ogino S, Wilson RB. Genotype and haplotype distributions of MTHFR677C→T and 1298A→C single nucleotide polymorphisms: a meta-analysis. *J Hum Genet* 2003;48:1-7.

[28] Bosco P, Gueant-Rodriguez RM, Anello G, et al. Methionine synthase (MTR) 2756 (AG) polymorphism, double heterozygosity methionine synthase 2756 AG/methionine synthase reductase (MTRR) 66 AG, and elevated homocysteinemia are three risk factors for having a child with Down syndrome. *Am J Med Genet* 2003;121A(3):219-224.

[29] Sheth JJ, Sheth FJ. Gene polymorphism and folate metabolism: a maternal risk factor for Down syndrome. *Indian Pediatr* 2003;40:115-123.

[30] Pogribna M, Melnyk S, Pogribny I, et al. Homocysteine metabolism in children with Down syndrome: in vitro modulation. *Am J Hum Genet* 2001;69:88-95.

[31] Gueant-Rodriguez RM, Rendeli C, Namour B, et al. Transcobalamin and methionine synthase reductase mutated polymorphisms aggravate the risk of neural tube defects in humans. *Neurosci Lett* 2003;344:189-192.