Research Article

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Evaluation of MTHFR Genetic Polymorphism as a Risk Factor in Egyptian Autistic Children and Mothers

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Abstract

Autistic spectrum disorders (ASD) is now considered a multifactorial neurodevelopmental disorder, with increasing prevalence worldwide. Many evidences had showed a role of disturbed folate metabolism in increasing risk for autism, with methylenetetrahydrofolate reductase (MTHFR) being a pivotal enzyme controlling it.

This study aims at examining two polymorphisms in MTHFR gene as they are linked to decreased enzyme activity. MTHFR C677T and A1298C polymorphisms are studied in Egyptian ASD children and their mothers in addition to control group. We examined MTHFR 677 C/T and 1298 A/C in 24 autistic children and their mothers and 30 control children and 42 control mothers.

Genotype frequency of MTHFR 1298 AC/CC is significantly higher in autistic children; compared to controls. It was also significantly higher in autism mothers compared to control mothers with 3.2, and 2.1 increased fold risk for MTHFR 1298 AC, and AC+CC genotypes, respectively. No significant changes in C677T genotyping was found in neither autism children nor their mothers.

Conclusion: These data supports an increased risk for ASD in association with MTHFR 1298 AC/CC polymorphism and hence a role of folate/methylation cycle disturbances is suspected in autism.

Keywords: Autism; MTHFR; Folate; Polymorphism

Introduction

Autism spectrum disorder (ASD) is complex neurodevelopment disorders characterized mainly by impaired social interaction, disrupted communication, and restricted repetitive behavior [1]. The latest autism prevalence studies were 1 in 110 children with a male to female ratio of 4:1 [2]. The Middle East ranged from 1.4 per 10000 in Oman [3], to 29 per 10,000 in the United Arab Emirates [4]. ASD disorders are multifactorial and environmental factors play an important role [5], however genetic elements have been extensively studied [6].

Folate plays an important role in neurological development because it act as a methyl group transporter [7]. Folate level is determined by interactions of nutritional, metabolic, and genetic factors [8]. Methylenetetrahydrofolate reductase (MTHFR) is located on chromosome 1 (1p36.3) and involved in DNA methylation process.

Several MTHFR mutations influence its enzyme activity with C677T and A1298C being the most important. [9].The T677T mutation showed evidence of only 30% of normal activity in invitro studies, while the heterozygous C677T is corresponding to 65% of enzyme activity [10].

Most of previous studies have focused on MTHFR C677T polymorphism in ASD patients [11,-13], with fewer studies have examined the role of MTHFR A1298C in this disorder [14-16]. The present study assesses both MTHFR C677T and the A1298C polymorphisms in Egyptian families with autistic children and their mothers for the first time in comparison to control children and mothers.

Materials and Methods

Subjects

Twenty-four autistic children ranging from 3-11 years and 20 of their mothers were included, while 4 mothers were excluded due to medical conditions and taking supplements. The control group included 30 normal healthy children matched for age and sex and 42 healthy control mothers. We recruited this number of normal mothers to have data on prevalence of studied polymorphisms in Egyptian female population.

All the families were recruited from the Autism Disorders Clinic, Medical Research Center of Excellence, National Research Center. Exclusion criteria included; Children with visual, hearing, motor impairment identified metabolic, and neurological disorders. Autistic children were diagnosed using Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV-R [1], Childhood Autism Rating Scale (CARS) [17] and the Autism Diagnostic Interview Revised (ADI-R) [18]. Caregivers consent was obtained for all the studied cases.

All cases were subjected to detailed history taking including three generation pedigrees construction, with detailed peri-natal history. Similarly affected cases and other family findings. Pre, peri and postnatal history were also included in our questionnaire.

Genomic DNA extraction

Blood samples were collected on EDTA and genomic DNA was extracted from whole blood using Axygene DNA Extraction Kit.

Mutational analysis

The C677T and A1298C polymorphisms located in MTHFR gene exons 4 and 7, respectively were examined by genomic DNA amplification followed by restriction endonuclease digestion (PCR-RFLP analysis) [19]. Exon 4 amplification was performed in a reaction mixture of 50 μl containing approximately 100 ng genomic DNA, 10 mM dNTPs, 50 pico mole from each primer (forward 5' TCC CTG TGG TCT CTT CAT CC 3' and reverse 5' ACT CAG CAC TCC ACC CAG AG 3') and 2 units of Taq DNA polymerase (Qiagene, USA) in 1X buffer containing 1.5 mM MgCl2 (supplied by the manufacturer).

PCR products were digested with Hinf I. The C allele is showing a single band of 360 bp, while T allele shows 2 bands at 210 and 150 bp.

Exon 7 amplification using three primers that were designed to amplify the domain surrounding nucleotide1298 in a semi-nested PCR. First round PCR contained the primer pair, forward (F1) '5 TCA GGG GCA GAA TTT ACA GG 3' and reverse (R1) 5' ACA GGA TGG GGA AGT CAC AG 3'. Using this pair, a band of 377 bp representing the full length of exon (7) was amplified. In the second round PCR a forward primer (F2) '5 GAA GAG CAA GTC CCC CAA G 3' and the reverse primer (R1) were utilized to produce a 221bp band. MBOII is used to determination of genotype; a the A allele is determined by 3 band; 151, 30 and 28 bp and the C allele shows 2 band 179 and 30 bp. The digestion products were run on 2.5% agarose gel andvisualized on a UV transilluminator.

Statistical analysis

Data were statistically described in terms of frequencies (number of cases) and percentages. Comparison between case and control groups regarding each haplotype as well as each allele was done using Chi square (2) test. Yates continuity correction equation was used instead when the expected frequency was less than 5. Odds ratio (OR) with 95% confidence intervals (95% CI) was calculated for each mutant haplotype against the wild type. Hardy Weinberg equilibrium was also tested. P values less than 0.05 was considered statistically significant. All statistical calculations were done using SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

Results

Risk factors for autism were obtained through self-answered questionnaires completed by parents with trained study staff present to provide clarifications. Among the studied cases, Positive Consanguinity was present in 16% of cases. Prenatal risk factors are antiepileptic drugs in only 2 cases while neonatal jaundice was present in 16% of cases.

MTHFR A1298C Genotype

The genotype frequency of MTHFR 1298 AC and CC genotypes were significantly higher in autistic children (p=0.001) compared to normal children, with detailed genotypes frequencies shown in Table 1. In addition, the genotypes are deviated from Hardy Weinberg Equilibrium (p=0.0001) (Table 2).

Autistic mothers showed 1.33 and 3.2, increased fold risk for MTHFR 1298 A/C and AC+CC respectively. In this group, MTHFR 1298 AC was significantly higher in autism children mothers (80%) compared to control mothers (47.6 %) with a deviation from Hardy Weinberg Equilibrium (P=0.007) (Table 3).

MTHFR C677T Genotype

The frequencies of CC, CT and TT genotypes in autism and control children are shown in Table 1 with no significant changes (p=0.2) with no deviation from Hardy Weinberg Equilibrium. No significant allele frequencies distribution between mothers of both groups was seen (Table 3).

Exons	Genotype	Control Children (N=30)	Autistic children (N=24)	X2 test	P value
C677T	CC	66.6%	45.8 %	1.59	0.207
	CT	26.6%	45.8%	1.39	0.238
	TT	6.6%	8.3%	0.08	0.771
	CT+TT	33.3%	54.1%	1.69	0.207
A1298C	AA	40%	0%	10.14	0.001
	AC	53.3%	95.8 %	9.98	0.002
	CC	6.6%	4.16%	0.04	0.842
	AC+CC	60%	100%	10.14	0.001

Table 1: Genotype Frequencies of MTHFR C677T & A1298C Polymorphisms in Normal and Autistic children

C677T	Observed CC	Observed CT	Observed TT	Expected CC	Expected CT	Expected TT	X2	P HWE
Control Children	20	8	2	19.20	9.60	1.20	0.833	0.361
Autistic Children	11	11	2	11.34	10.31	2.34	0.107	0.744
A1298C	Observed AA	Observed AC	Observed CC	Expected AA	Expected AC	Expected CC	X2	P HWE
Control Children	12	16	2	13.33	13.33	3.33	1.200	0.273
Autistic Children	0	23	1	5.51	11.98	6.51	20.314	0.000

Table 2: Hardy Weinberg Equilibrium of MTHFR C677T and A1298C in Autistic and Normal Children

C677T	Observed CC	Observed CT	Observed TT	Expected CC	Expected CT	Expected TT	X2	P HWE
Control mothers	20	17	5	19.34	18.32	4.34	0.218	0.640
Case Mothers	10	8	2	9.80	8.40	1.80	0.045	0.831
A1298C	Observed AA	Observed AC	Observed CC	Expected AA	Expected AC	Expected CC	X2	P HWE
Control mothers	8	20	14	7.71	20.57	13.71	0.032	0.857
Case Mothers	2	16	2	5.00	10.00	5.00	7.200	0.007

Table 3: Hardy Weinberg Equilibrium of C677Tand A1298C in mothers of autism and control children

Discussion

ASD is a multifactorial disorder and are influenced by environmental risk factors [20]. Epigenetic mechanisms play a vital role in the expression of autism phenotypes and are affected by nutritional status and medications. MTHFR gene polymorphisms have been implicated in birth defects diseases, neurological disorders, and cancers. They have been also studied in several neuropsychiatric disorders, such as Alzheimer's Disease [21] , Schizophrenia [22], and attention deficit hyperactivity disorders [23], . The role of folate metabolism is suspected to be more eminent in birth defects and neurodevelopment disorders as vascular degenerative changes related polymorphisms are prevelant in neurodegenerative disorders [24,25].

Several studies have investigated the association of genetic polymorphisms in various pathways and the risk of autism. In our group, we studied previously a possible genetic association between autism and iron metabolism genes' polymorphisms [26,27]. To our knowledge there is no study investigated the polymorphism of the MTHFR gene and autism in our population. Disturbances of the folate metabolic pathway and polymorphism at position C677T and A1298C of MTHFR gene in autism have been reported in many populations. However, results were not consistent and are still in debate. This can be attributed by regional and ethnic variations.

The mechanisms of MTHFR C677T polymorphisms as a risk factor of autism are still unclear. Several studies have suggested that autism may be associated with metabolic abnormalities in the folate pathway, which plays an important role in DNA methylation, thus altering gene expression. Few studies have evaluated the second common MTHFR Polymorphism -- A1298C, which encodes glutamine 429 alanine substitutions and it affects the regulatory domains of the enzyme.

High frequency of C677T genotype in Mexican women (18.1%) compared to white women (7.9%) was found. The frequency of compound heterozygosity (677 CT+1298 AC) was higher in Mexican white women compared to Asian and African American women.

In our pilot study, we evaluated the most 2 common MTHFR polymorphisms; C677 T and A1298C in autistic children and their mothers compared to normal healthy children and their mothers. We found a possible genetic association between autism and MTHFR 1298A/C genotypes among Egyptians. The frequency of the 1298C allele in the Egyptian control group was (20%) which is relatively lower than those reported in the Japanese and Africans who had 79% and 91% frequencies respectively. Moreover, our results showed that the frequency of MTHFR 1298AC genotypes was significantly higher among autism mothers (80%) compared to controls (20%).

This supports previous positive studies regarding this polymorphisms in autism among other populations e.g Korean and North american as they showed MTHFR A1298C genotype distribution deviation from Hardy Weinberg Equilibrium in autism, although the last study showed combined effect of MTHFR A1298C and C677T genotypes [16,28].

There was a trend of increased 677 C/T allele among our autism children, although this did not reach significance. Higher MTHFR 677 T allele frequency was found in Indian autistic children with a 2.79 fold increased risk, and the risk was dose dependent with high significance [14]. However, no significant difference in MTHFR allele was found in Caucasian Romanian ASD patients compared to controls [11] and in Brazilian autism patients [12]. Another case control study revealed a significantly higher frequency of 677T allele genotype in autistic compared to normal population in simplex families [16].

To conclude, the present study indicates that MTHFR exon 7 A1298C polymorphism represents a risk factor in association with autism and this highlightes the role of folate in prevention of autism in accordance with other populational studies. Folate fortification of food would be recommended among our country and similar populations for prevention of autism although further clinical trials studies are warranted.

Author Disclosures

There is no conflict of interest in association with this work.

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