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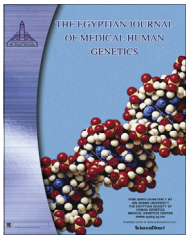

ORIGINAL ARTICLE

Study of genotype–phenotype correlation of methylene tetrahydrofolate reductase (MTHFR) gene polymorphisms in a sample of Egyptian autistic children

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KEYWORDS

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Abstract *Background:* Classical autism belongs to a group of heterogeneous neurobehavioral disorders known as autism spectrum disorders (ASDs) characterized by abnormalities in social interaction, impaired communication, and repetitive stereotypic behaviors. Overall, there is an increased risk of ASDs associated with common mutations affecting the folate/methylation cycle. This study aimed at identification of the C677T polymorphic genotypes of MTHFR gene among the Egyptian children with autism and to correlate them with different phenotypes.

Subjects and methods: This case-control study included 20 children with autism (4.57 ± 1.36 years) (13 males and 7 females) and a normal control group. Assessments by DSM-IV-TR criteria, Stanford-Binet intelligence scale and childhood autism rating scale (CARS) were done. Assay for MTHFR gene mutation C677T was performed on amplified DNA by PCR and subsequent reverse hybridization to immobilized allele-specific biotinylated oligonucleotides probes.

Results: The relation between low birth weight and occurrence of autism is highly significant ($P < 0.01$). The delayed motor and social milestones showed a statistically highly significant difference in cases of autism compared to controls ($P < 0.01$); 50% of autistic patients were heterozygous (CT) for the MTHFR gene, and 15% were homozygotes for the mutant genotype (TT). For the homozygous wild type genotype, 35% of patients were CC ($P < 0.05$). The segregation of T allele in the homozygous 677TT genotype occurred in 30% of autistic children. Frequency of the T-allele in autistic children is 0.4 compared to an allele frequency of 0.3 among controls ($P < 0.01$). According to the CARS classification, 70% were severely affected of whom 42.8% were carrying the CT

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genotype. There was no significant difference between CARS (degree of severity of autism) and C677T polymorphism. There was no significant difference between various genotypes as regards the mean for CARS. There was no statistically significant difference as regards mean gestational age, birth weight and mean age at sitting down among the patient group with different genotypes ($P > 0.05$).

Conclusion: Although the 677CT variant alleles significantly increased in patients with autism, it is unlikely that this association alone is sufficient to produce the complex array of symptoms associated with autism. Therefore, a search for additional genomic, metabolic, epigenetic and environmental risk factors should be undertaken.

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1. Introduction

Autism is a complex neuro developmental disorder that is increasingly being recognized as a public health issue [1]. It is currently estimated that 3–6 children out of every 1000 worldwide have autism spectrum disorders (ASDs) [2], with three to four fold higher incidence in males than in females [3]. In the year 2014, the Centers for Disease Control and Prevention (CDC) released new data on the prevalence of autism in the United States. This surveillance study identified 1 in 68 children (1 in 42 boys and 1 in 189 girls) as having autism spectrum disorder (ASD) [4]. It is classified under the pervasive developmental disorders (PDDs) that involve a combination of impairments in communication, reciprocal social interactions, and stereotyped patterns of interest/behavior. PDDs include autism (classical autism which is considered the most severe form of ASD); Asperger's syndrome (which generally preserves an individual's linguistic development); childhood disintegrative disorder, and PDD – not otherwise specified (PDD–NOS) [5,6]. Autism spectrum disorders are characterized by language impairment, restricted interests, stereotypic motor behaviors, hyperactivity, sensory disturbances and self injury [7]. It is also associated with seizure disorder [8], gastrointestinal disturbances [9] and autoimmune disorders in some patients [10].

The methylene tetrahydrofolate reductase (MTHFR) gene codes for an essential enzyme in folate metabolism. There is an evidence of increased risk of autism spectrum disorders (ASDs) 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 ASDs. Increased folinic acid during pregnancy and early development may offset the genomic risk factors. 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 phenotypes of ASDs [11].

The present study aimed at identification of the C677T gene mutation in the MTHFR gene among the Egyptian sample of patients with autism and to correlate the detected polymorphic genotypes with different phenotypes among the children involved in the study.

2. Patients and methods

2.1. Patients

This case control study enrolled 20 child patients with autism diagnosed by DSM-IV-TR criteria [12]. Patients were recruited from the Psychiatric Clinic, Pediatric Hospital, Ain Shams University. They were 13 males (65%) and 7 females (35%). Their ages ranged from 3 to 7 years (mean age 4.57 ± 1.36 years). The control group included 20 healthy children [13 males (65%) and 7 females (35%)]. Their ages ranged from 3 to 8 years (mean 4.9 ± 1.58 years). The control children were referred to the psychiatric clinic to exclude the presence of autism spectrum disorders (ASDs). All of the patients were subjected to the following:

Detailed history taking was done with special emphasis on the onset, course and duration of the disease, consanguinity of the parents; antenatal history with special emphasis on history of threatened abortion, any fetal loss, parity and chronic illness. Natal and postnatal histories including gestational age, complications during labor, history of prematurity or intrauterine growth retardation, birth weight; perinatal problems and postnatal course especially the occurrence of neonatal hypoxia, resuscitation, pallor and jaundice. The developmental history included age of sitting without support, walking unassisted, first spoken word, combining words, accurate details of cognitive abilities, gross and fine motor functions, feeding disorders, abnormal sleep patterns and history of vaccination. In addition, family history was taken for any similar conditions, genetic diseases and other psychological or mental disorders in the family. Thorough clinical examination with laying stress on neurological examination was done.

2.2. Methods

- Confirmation of diagnosis was performed using a DSM-IV-TR criteria questionnaire of autism, i.e. impairments of language, social skills, and restricted stereotyped interest or activity [12].
- Assessment of mental age using the Stanford-Binet intelligence scale (2003) [13] to calculate the intelligence quotient (IQ). Subnormal intellectual function is diagnosed when IQ is below 70.

- Assessment of severity of autistic symptoms using the childhood autism rating scale (CARS) [14] which rates the child on a scale from one to four in each of fifteen areas (relating to people, emotional response, imitation, body use, object use, listening response, fear or nervousness, verbal communication, non-verbal communication, activity level, and consistency of intellectual response, adaptation to change, visual response, taste, smell, touch response and general impression) was done.
- Assay for the detection of methylene tetrahydrofolate reductase (MTHFR) gene mutation C677T was performed based on polymerase chain reaction (PCR) amplification of the DNA and subsequent reverse hybridization to immobilized allele-specific biotinylated oligonucleotide probes (Vienna lab diagnostics® GmbH, Vienna, Austria). DNA isolation was obtained from peripheral blood samples using the spin column method and GEN^X TRACT Resin of Vienna lab diagnostics®. In Vitro amplification (PCR) was performed on 5–40 μg/ml DNA template. The PCR protocol was performed on a thermal cycler HVD™ as follows: Pre-PCR 94 °C/2 min. Thermocycling for 30 cycles each consists of 94 °C/15 s, 58 °C/30 s and 72 °C/30 s. Final extension at 72 °C/3 min. Analysis of the amplified products on 3% agarose gel electrophoresis to detect a 202 bp fragment was done. Hybridization of the amplified PCR products was conjugated using the streptavidin–alkaline phosphatase solution with subsequent serial washing using 0.05% NaN_3-containing solution. Detection of the hybridized PCR products was done using nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) solution.
- MTHFR gene C677T polymorphism was interpreted as a homozygote mutant, heterozygote or normal wild type genotypes.
- **Statistical analysis:** The results were analyzed using the Statistical Package of Social Sciences (SPSS) computer software program, version 11.0 (Chicago, IL, USA). Quantitative data were presented as mean ± SD for normally distributed data and as medians and percentiles for skewed data. Qualitative data were presented in the form of frequencies and percentages. For normally distributed parameters, differences among groups were tested by the t test and a one-way analysis of variance (ANOVA). For Qualitative data, differences among groups were tested using Pearson's chi-square test (X^2). A P value less than 0.05 was considered statistically significant.

Informed consent was taken from parents of the infants.

3. Results

Among the patient group, consanguinity was detected in 20% of patients compared to 15% in the control group. In the patient group, 10% have similar autistic patients in the family; 15% have a history of epilepsy in the family, 10% of the patients have a history of prematurity .Thirteen (65%) were breast fed and seven (35%) were artificially fed. The mean for gestational age in our patients was 39 ± 25 weeks while in the control the mean gestational age was 39.1 ± 0.5 weeks .The mean for birth weight in our patients was 2.67 ± 0.389 kg compared to 3.03 ± 0.309 kg in the control group .The relation between gestational age and occurrence of autism was not significant statistically ($P > 0.05$) while the relation of low birth weight to autism was highly significant ($P < 0.01$). In our patients, the mean age for sitting was 8.5 ± 1.1 months while in the control group it was 6.65 ± 0.87 months. Mean age for the first spoken word in our patients was 2.6 ± 0.69 years while in the control group it was 1.13 ± 0.18 years. These delayed motor and developmental milestones showed statistically highly significant difference in patients of autism compared to controls. Among our patient group, 18 had IQ scores ranging from 35 to 65 with a mean of 54 ± 14.7 while the control group had normal IQ ranging from 95 to 115 with a mean of 96 ± 8.9. The IQ was significantly lower in autistic patients than in controls ($P < 0.01$) (Table 1).

In the present study, 10 patients (50%) were heterozygous (CT) for the MTHFR gene compared to only 6 in the control group (30%). However this difference was not significant ($P > 0.05$). The homozygote mutant genotype TT was detected in 3 patients (15%) compared to 0% in the control group ($P > 0.05$). For the homozygous wild genotype, 7 patients (35%) had the CC genotype compared to 14 (70%) of the control group. This was statistically significant ($P < 0.05$) (Table 2).

The segregation of the T allele in the homozygous 677TT genotype occurred in 3 patients (15%) among the autistic children, while in the heterozygous 677CT genotype it occurred in 10 (50%) of the autistic group children. Frequency of the T-allele in autistic children is 0.4 compared to an allele frequency of 0.3 among controls ($P < 0.\ 0\ 1$) (Tables 2 and 3).

Two of the autistic patients of the study (10%) had normal IQ, while ten of them (50%) had mild mental retardation and six patients (30%) had moderate mental retardation and two patients (10%) had severe retardation with no statistically significant difference among different genotypes. In this study, according to the CARS classification there were no mild cases, while there were 6 patients (30%) with moderate affection; 4 (66.7%) of whom were heterozygous for C667T, and the majority (14) (70%) of patients were severely affected of whom 6 patients (42.8%) were carrying the CT genotype and also 6 patients (42.8%) were CC in genotype. This indicates that there was no significant difference between CARS (degree of severity of autism) and C677T polymorphism ($P > 0.05$). In the patients of the study, mean IQ score of the heterozygous group (CT) was 50.8 ± 13.67, mean IQ score of the group with mutant genotype (TT) was 58.66 ± 5.5, and mean IQ score of normal genotype (CC) was 57 ± 18.94. Mean CARS of the heterozygous group was 36.3 ± 5.01, mean CARS of the group with mutant genotypes was 37.3 ± 4.61 and mean CARS of the group with normal genotypes was 37.1 ± 3.98 with no significant difference (Table 4).

There was no statistically significant difference as regards mean gestational age, birth weight and mean age at sitting among the patient groups with different genotypes ($P > 0.05$) (Table 5).

4. Discussion

There is an agreement that autism is one of the most puzzling diseases. It is a neuro developmental disorder characterized by impaired social interaction [15], which is usually diagnosed

Table 1 Characteristics of the patient and control groups included in the study.

Parameters	Patients	Controls	Chi-square test	*P*-value
Consanguinity (no., %)				
Yes	4 (20%)	3 (15%)	0.173	> 0.05**
No	16 (80%)	17 (85%)		
Family history of autism (no., %)				
Yes	2 (10%)	0 (0%)	0.529	> 0.05**
No	18 (90%)	20 (100%)		
Family history of epilepsy (no., %)				
Yes	3 (15%)	0 (0%)	1.44	> 0.05**
No	17 (85%)	20 (100%)		
History of prematurity:				
Yes	2 (10%)	0 (0%)	0.526	> 0.05**
No	18 (90%)	20(100%)		
Feeding				
Breast	13 (65%)	16 (80%)	1.129	> 0.05**
Artificial	7 (35%)	4 (20%)		
Mean gestational age (weeks)	39 ± 1.25	39.1 ± 0.5	1.48	> 0.05**
Mean birth weight (kg)	2.67 ± 0.389	3.03 ± 0.309	3.2	< 0.01*
Mean age for sitting (month)	8.5 ± 1.1	6.65 ± 0.87	5.88	< 0.01*
Mean age at weaning (year)	1.59 ± 0.3	1.61 ± 0.31	0.206	> 0.05**
Mean age at 1st spoken word (year)	2.6 ± 0.69	1.13 ± 0.18	6.63	< 0.01*
Mean level of IQ score	54 ± 14.7	96 ± 8.8	11.003	< 0.01*

* Highly significant difference.
** Non-significant difference.

Table 2 Genotypes of MTHFR among the patient and control groups.

Genotype	Patients		Control		Chi square test	*P*-value
	No.	%	No.	%		
Heterozygous(CT)	10	50	6	30	0.33	> 0.05**
Mutant(TT)	3	15	0	0	1.87	> 0.05**
Wild type (CC)	7	35	14	70	2.3	< 0.05*

* Significant difference.
** Non-significant difference.

Table 3 Segregation and frequency of T-allele versus C-allele in the Genotypes of Autistic and Control groups.

Group	C-allele		T-allele	
	No.	Frequency	No.	Frequency
Autistic	24	0.6	16	0.4
Control	34	0.85	6	0.3

$P < 0.01$(HS).

before the age of three years, and is characterized by deficits in social reciprocity and in language skills that are associated with repetitive behaviors and restricted interests [16].

In our study, 80% of autistic children were diagnosed before the age of 3 years. This is in agreement with Gray and Tonge [17] who found that parents become concerned about autistic behavior at the age of 12–30 months of age, however, there is often a wide variation in the age at which autistic children present for diagnosis to a specialized developmental center [18].

In our study 65% of patients were males and 35% were females. McKusick [19] found that autism affects males more than females and occurring at a ratio of 4:1. Also Shu et al. [20], Allsopp et al. [21] and Newschaffer et al. [22] reported that autism is more than twice as common in boys as in girls, and this ratio increases to 5:1 at the high-ability end of the autism spectrum. This may indicate that males are predominantly affected with autism than females.

In the present study, 80% of parents of patients are non consanguineous compared to 85% in the control group. This is in agreement with El-Baz et al. [23] who reported that the rate of consanguinity is lower among parents of patients with autism than in normal controls which means that consanguinity has no role in autism. Similar findings were reported by many authors [24-26].

In our study, family history of autism was present in 10% among patients compared to 0% in the control group. Families of individuals with autism tend to demonstrate a set of cognitive disorders that are not seen in other family groups [27]. A family history of autism was reported in 16% of autistic patients versus 1% among the control [23].

Table 4 Correlation between C667T polymorphic genotypes with CARS and IQ.

IQ and CARS C677T	CT genotype (mean ± SD)	TT genotype (mean ± SD)	CC genotype (mean ± SD)	*P*-value
(i) **I Q**				
1- Normal IQ	1 (5%)	0 (0%)	1 (5%)	
2-Mild MR	4 (20%)	3 (15%)	3 (15%)	
3-Moderate MR	4 (20%)	0 (0%)	2 (15%)	
4-Severe MR	1 (5%)	0 (0%)	1 (5%)	
IQ (mean ± SD) * a	50.8 ± 13.67	58.66 ± 5.5	57 ± 18.94	>0.05***
(ii) **CARS**** c				
1-Moderate(=6)	4 (66.7%)	1 (16.6%)	1 (16.6%)	>0.05***
2-Severe (=14)	6 (42.8%)	2 (14.2%)	6 (42.8%)	>0.05***
(Mean ± SD)* b	36.3 ± 5.01	37.3 ± 4.61	37.1 ± 3.98	>0.05***

* Anova F test: a = 0.502, b = 0.096.
** Chi-square: 1.2, I.Q. mean of whole group, MR = mental retardation.
*** Non-significant difference.

Table 5 Mean age for developmental milestones among different genotypes in the study.

Milestone	Heterozygous CT (mean ± SD)	Mutant TT (mean ± SD)	Normal CC (mean ± SD)	F (anova test)	*P*-value
Gestational age (month)	38 ± 1.3	39 ± 0.00	39.1 ± 1.4	0.069	>0.05*
Birth weight (kg)	2.65 ± 0.44	2.63 ± 0.057	2.72 ± 0.423	0.094	>0.05*
Age of sitting (month)	8.5 ± 1.26	8.33 ± 0.57	8.57 ± 1.13	0.044	>0.05*

* Non-significant difference.

In our study, all developmental and social milestones were significantly delayed among autistic children compared to the control group. About 96% of autistic children had motor developmental delay. Qualitative impairment in social interaction and communication was also more commonly observed than restricted interests and activities [28]. Children with autism may be delayed in acquiring motor activity, such as bicycle riding. They may be poorly coordinated or have an abnormal gait or posture and poor hand writing [29]. Some of the noted behaviors in autism include delayed speech and language skills, and not to point or wave ''bye-bye'' [30].

In this study, history of low birth weight was found to be highly significant among autistic children than controls. Kolevzon et al. [31] suggested the presence of non heritable prenatal and perinatal risk factors for autism. An association is suggested between autism and obstetric complications, prenatal or intrapartum use of medications [32]. A significantly higher incidence of low birth weight and using instrumental tools during delivery was reported among cases with autism than controls [23]. Perinatal risk factors such as breech presentation, low Apgar score, low birth weight (2500 g), gestational age at birth of less than 35–37 weeks, and being small for gestational age were associated with a statistically significant increased risk of autism [33]. In 2007, a review of risk factors demonstrated associated obstetric conditions that included low birth weight, gestation duration and hypoxia during childbirth with autism. Studies focusing on single perinatal risk factor have reported a positive association for low birth weight (<2500 g), and gestational age at birth of less than 37 weeks [34].

In our study, 90% of autistic patients presented with mild to severe mental retardation and 10% with normal mentality. According to CARS, severe cases were 14 (70%), moderate cases were six (30%), while no mild cases were found. Autistic children have spectrum of IQ ranging from 0 to 60 [35]. It is clear that autism is a biological brain disorder [15]. Genetic factors are the most significant causes for Autism Spectrum Disorders [36]. Early studies of twins estimated heritability to be over 90% [37]. Many of the non-autistic twins had learning or social disabilities [38].

In the present study the homozygote mutant 677TT genotype was present in 15% of the autistic children while the heterozygous 677CT genotype occurred in 50% of the autistic children group. This was statistically highly significant as compared to the control group (<0.01). The T allele frequency was 0.8 in autistic patients compared to an allele frequency of 0.3 among the control group (<0.01). The 677T allele leads to valine substitution at amino acid 222 encoding a thermo labile enzyme with reduced activity in folate metabolism. About ten percent of the North American Population is T-homozygous for this polymorphism. There is an ethnic variability in the frequency of the T allele. The frequency in Mediterranean/Hispanics is greater than the frequency in Caucasians which, in turn, is greater than in Africans/African-Americans [39].

Our results are in agreement with Boris et al. [11] who reported that homozygous 677TT genotype was present in 23% of the ASD children and the heterozygous 677CT genotype in 56% of children in the ASD group. The 677CT polymorphisms, whether homozygous or heterozygous, are significantly associated with ASD. The homozygous (TT) individuals are reported to have an approximately 50% decrease in MTHFR thermo labile enzyme activity, and the heterozygous (CT) a 30% decrease in enzyme activity as measured in their lymphocytes. The homozygosity of 677T allele of the MTHFR gene is more prevalent in the typical autism group [11,40,41]. MTHFR C677T polymorphism contributes to increased

ASD risk, and periconceptional folic acid may reduce ASD risk in those with MTHFR 677C > T polymorphism [42].

To conclude, although the 677CT variant alleles significantly increased in patients with autism, it is unlikely that this association alone is sufficient to produce the complex array of symptoms associated with autism. Therefore, a search for additional genomic, metabolic, epigenetic and environmental risk factors should be undertaken.

Conflict of interest

The authors declare no conflict of interest. There is no financial and personal relationship with other people or organizations that could inappropriately influence this work.

References

[1] Main AE, Angley MT, Thomas P, Fenech M. Folate and methionine metabolism in autism. Am J Clin Nutr 2010;91:1598–620.

[2] Mendoza RL. The economics of autism in Egypt. Am J Econ Bus Adm 2010;2(1):12–9.

[3] Butler MG, Dasouki MJ, Zhou X-P, Talebizadeh Z, Brown M, Takahashi TN, et al. Subset of individuals with autism spectrum disorders and extreme macrocephaly associated with germline PTEN tumour suppressor gene mutations. J Med Genet 2005;42:318–21.

[4] Davis KJ. SIG 16 perspectives on school-based issues. ASHA 2014;15(1):1–6. http://dx.doi.org/10.1044/sbi15.1.1.

[5] Abha C, Ved C. Review oxidative stress in autism. Pathophysiology 2006;13:171–81.

[6] Kuehn BM. Studies probe autism anatomy, genetics. J Am Med Assoc (JAMA) 2006;295:19–20.

[7] MacFabe DF, Cain NE, Boon F, et al. Effects of the enteric bacterial metabolic product propionic acid on object-directed behavior, social behavior, cognition, and neuroinflammation in adolescent rats: Relevance to autism spectrum disorder. Behav Brain Res 2008;217:47–54.

[8] Besag FMC. Behavioral aspects of pediatric epilepsy syndromes. Epilepsy Behav 2004;5:S3–S13.

[9] White JF. Intestinal pathophysiology in autism. Exp Biol Med (Maywood) 2003;228(6):639–49.

[10] Ashwood P, Van de Water J. Is autism an autoimmune disease? Autoimmun Rev 2004;3(7–8):557–62.

[11] Boris M, Goldblatt APA, Galanko J, James SJ. Association of MTHFR gene variants with autism. J Am Phys Surg 2004;9(4):106–8.

[12] American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders. Fourth ed., text revision (DSM-IV Text Revision). 2000, ISBN 10 0890420254 and ISBN 13: 9780890420256. American Psychiatric Publishing, Inc., Arlington, Virginia, U.S.A. < http://www.psychiatryonline.com/DSMPDF/dsm-iv.pdf > < http://www.psych.org/MainMenu/Research/DSMIV.aspx > .

[13] Stanford-Binet intelligence scales (SB-5), two years to adult 2003; fifth ed. Available online at: < http://www.riverpub.com/products/clinical/sbis5/home.html > .

[14] Schopler E, Reichler RJ, Renner BR. The childhood autism rating scale (CARS) for diagnostic screening and classification of autism. New York: Irvington; 1986.

[15] Kidd PM. Autism, an extreme challenge to integrative medicine. Part I: The knowledge base. Alternat Med Rev 2002;7:292–316.

[16] Eigsti IM, Shapiro T. A system's neuroscience approach to autism: biological, cognitive and clinical perspectives. Ment Retard Dev Disabil Res Rev 2003;9:205–15.

[17] Gray KM, Tonge BJ. Are there early features of autism in infants and preschool children ? J Pediatr child health 2001;37:221–6.

[18] Mandell DS, Listerud J, Levy SE, Pioto-Martin JA. Race differences in the age at diagnosis among Medicaid-eligible children with autism. J Am Acad Child Adolesc Psychiatry 2008;41:1447–56.

[19] McKusick VA, editor. Mendelian inheritance in man. A catalogue of human genes and genetic disorders. 12th ed. Baltimore: John Hopkins University Press; 1998. OMIM available from the National Centre for Biotechnology information: < http://www/ncbi/nim/nih/gov/omim > .

[20] Shu B, Lung F, Change Y. The mental health in mothers with autistic children: a case-control study in southern Taiwan. Kaohsiung J Med Sci 2000;16(6):308–14.

[21] Allsopp MY, Rice C, Karapurkar T, Doernberg N, Boyle C, Murphy C. Prevalence of autism in a US metropolitan area. JAMA 2003;289:49–55.

[22] Newschaffer CJ, Croen LA, Daniels J, et al. The epidemiology of autism spectrum disorders. Ann Rev Public Health 2007;28:235–58.

[23] El-Baz F, Ismael NA, Nour El-Din SM. Risk factors for autism: an Egyptian study. Egyptian J Med Hum Genet 2011;12:31–8.

[24] Salem AM, Ismail S, Zarouk WA, Abdul Baky O, Sayed AA, Abd El-Hamid S, et al. Genetic variants of neurotransmitter-related genes and miRNAs in Egyptian autistic patients. Sci World J 2013;2013:1–7.

[25] Elshahawi H, Shaker N, Essawy H, Eissaa A, Sadek H, Haroon A. Risk factors and clinical presentations of autism in an Egyptian sample. Egypt J Neurol Psychiat Neuro surg 2007;44(1):69–77.

[26] Bilder D, Judith PZ, Judith M, William M. Prenatal, perinatal and neonatal factors associated with autism spectrum disorders. Pediatrics 2009;123:1293.

[27] Muhle R, Trentacoste SV, Rapin I. The genetics of autism. Pediatrics 2004;113:472–86 [Retrieved, June 30, 2007].

[28] Juneja M, Mukherjee SB, Sharma S. A descriptive hospital based study of children with autism. Indian Pediatr 2005;42(5):453–8.

[29] McPartland PJ, Klin A. Asperger's syndrome. Adolesc Med Clin 2006;17(3):771–88.

[30] Filipek PA, Accordo PJ, Baranek GT. The screening and diagnosis of autistic spectrum disorders. J Autism Dev Disord 1999;29(6):439–84.

[31] Kolevzon A, Gross R, Reichenberg A. Prenatal and perinatal risk factors for autism: a review and integration of findings. Arch Pediatr Adolesc Med 2007;161(4):326–33.

[32] Bolton P, Pickles A, Harrington R, et al. Season of birth: issues, approaches and findings for autism. J Child Psychol Psychiatry 1992;33:509–30.

[33] Burd L, Severud R, Kerbeshian J, Klug MG. Prenatal and perinatal risk factors for autism. J Perinat Med 1999;27(6):441–50.

[34] Limperopoulos C, Bassan H, Gauvreau K, Robertson RL Jr, Sullivan NR, Benson CB, et al. Does cerebellar injury in premature infants contribute to the high prevalence of long-term cognitive, learning, and behavioral disability in survivors? Pediatrics 2007;120:584. http://dx.doi.org/10.1542/peds.2007-1041.

[35] Baron-Cohen S, Hoekstra RA, Knickmeyer R, et al. The autism spectrum quotient (AQ): adolescent version. J Autism Dev Disord 2006;36:343–50.

[36] Geschwind K. Association of fragile X with autism. Am J Psychiatry 2005;192(142):9.

[37] Freitag CM. The genetics of autistic disorders and its clinical relevance: a review of the literature. Mol Psychiatry 2007;12(1):2–22.

[38] Persico AM, Bourgeron T. Searching for ways out of the autism maze: genetic, epigenetic and environmental clues. Trends Neurosci 2006;29(7):349–58.

[39] Schneider JA, Rees DC, Liu YT, Clegg JB. Worldwide distribution of a common methylenetetrahydrofolate reductase mutation. Am J Hum Genet 1998;62(5):1258–60.
[40] Rozen R. Genetic predisposition to hyperhomocysteinemia: deficiency of methylenetetrahydrofolate reductase (MTHFR). Thromb Haemost 1997;78(1):523–6.
[41] Guo T1, Chen H, Liu B, Ji W, Yang C. Methylenetetrahydrofolate reductase polymorphisms C677T and risk of autism in the Chinese Han population. Genet Test Mol Biomarkers 2012;16(8):968–73.
[42] Pu D, Shen Y, Wu J. Association between MTHFR gene polymorphisms and the risk of autism spectrum disorders: a meta-analysis. Autism Res 2013;6(5):384–92.