Curr Pediatr Res 2018; 22 (4): 277-284 *ISSN 0971-9032*
www.currentpediatrics.com

A contribution of Methylenetetrahydrofolate Reductase (MTHFR) gene polymorphisms in children with attention deficit hyperactivity disorder.

Khaled Ismail AbdElShakoor[1]*, Howida Hosny El-Gebaly[2], Tarek Mostafa Kamal[3], Eman Ahmed Zaky[4]

[1]Faculty of Postgraduate Childhood Studies, Medical Studies Department, Ministry of Health, Ain Shams University, Al-Khalifa Al-Ma'moun street, Cairo, Egypt
[2]Faculty of Postgraduate Childhood Studies, Department of Pediatrics, Ain Shams University, Egypt
[3]Faculty of Medicine, Department of Human Genetics, Ain Shams University, Egypt
[4]Faculty of Medicine, Department of Pediatrics, Ain Shams University, Egypt

Abstract

Background: Attention Deficit Hyper-Activity Disorder (ADHD) is a neuro-behavioral, complex disorder influenced by many genes. The MTHFR gene C677T and A1298C polymorphisms affect both nucleotide synthesis and DNA methylation. This study aimed to assess the relationship between Methylenetetrahydrofolate Reductase (MTHFR) gene polymorphisms and ADHD in a sample of Egyptian children.

Methods: MTHFR gene polymorphisms were evaluated in 60 participants, 30 ADHD patients and 30 controls of healthy children with normal developmental and psychiatric evaluation with comparable age and sex. The patients were recruited from Psychiatric clinic, Faculty of Postgraduate Studies for Childhood-Ain Shams University, Cairo, Egypt during the period from January to August 2015 with age ranged from 6 to 12 years. MTHFR C677T and A1298C alleles distribution was investigated via polymerase chain reaction (PCR) and reverse hybridization.

Results: The recorded genetic results showed heterozygous advantage (Heterosis) regarding studied C677T allele genotype with statistically significant association reported in controls compared to ADHD cases (p=0.0159). Genotype distributions of A1298C allele showed statistically high significant association with ADHD cases compared to controls (p=0.0002). A significant association was found between males of ADHD cases and hetero- homozygous A1298C allele compared to controls (p=0.0079). Meanwhile, ADHD females showed statistically significant higher distribution of the hetero- homozygous genotypes compared to controls (p=0.0072).

Conclusions: There was an evident association between ADHD phenotype and MTHFR A1298C gene polymorphism, and there was a heterozygous advantage (Heterosis) regarding C677T allele genotype and ADHD cases leading to absence of association between MTHFR C677T gene polymorphism and ADHD.

Keywords: MTHFR gene, A1298C allele, C677T allele, Genotype, Phenotype, Attention Deficit Hyperactivity Disorder (ADHD).

Accepted November 06th, 2018

Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is a psychiatric disorder of the neurodevelopmental type [1]. Defined by symptoms of developmentally inappropriate inattention, hyperactivity, or acting impulsively that are not appropriate for a person's age [2]. The symptoms must begin by the age of six to twelve years and continue to be present for more than six months [3]. A recent meta-analysis estimated the world-wide prevalence of ADHD to be about 7.2% and diagnosed in about 2 to 16% in school aged children [4]. It is a chronic condition that affects millions of children and often persists into adulthood; the social and economic costs of childhood ADHD are considerable [5].

Family twin and adoption studies suggested that 70% heritability rate for the disorder [6]. Evidence from

animal and human studies implicated degranulation of the frontostriatal and fronto-cerebellar catecholaminergic circuits in children and adolescents with ADHD [7]. Hypoactivity of the dorsolateral prefrontal cortex, caudate, and thalamus was also demonstrated in ADHD probands [8]. Although the exact cause is unknown, heredity prenatal or perinatal factors, exposure to toxins and heavy metals, socio-psychological stress, diet, structural and functional abnormalities of the brain contributed to ADHD etiology [9].

Maternal folate deficiency during gestation was reported to affect childhood hyperactivity [10]. Folate plays an important role in homocysteine and S-Adenosyl-Methionine (SAM) biosynthesis, low folate level affects neural stem cell proliferation, decreases apoptosis, and alters DNA biosynthesis [11]. Deficiency of various maternal nutrients including folate has been associated with neurodevelopmental disorder like ADHD and autism [10]. Abnormal folate transport into the fetal central nervous system has been related to cerebral folate deficiency associated with developmental delay, cognitive impairment and reduced memory function, while higher plasma folate level showed a correlation with better cognitive performance [12-14].

Methylenetetrahydrofolate Reductase (MTHFR) is important for folate chemical reactions. MTHFR gene is located at the end of the short arm of the chromosome 1p 36.3 [15]. The enzyme methylenetetrahydrofolate Reductase (MTHFR) helps converting folate in folate metabolic cycle to other metabolites used for cellular processes including methylation of the gene promoter enhancers, proteins, RNA, DNA, amino acids and phospholipids synthesis. MTHFR enzyme converts 5, 10-methylentetrahydrofolate to 5-methyltetrahydrofolate, which is the predominant circulating form of folate, and this reaction is required for a multistep process which converts homocysteine to other amino acid methionine. Methyl tetrahydrofolate donates its methyl group to convert homocysteine into methionine in the presence of methionine synthetase and vitamin B12 during the generation of S-adenosyl methionine, a major source of methyl group in the brain [9]. MTHFR polymorphisms C677T and A1298C affect nucleotide synthesis and DNA methylation [16]. Several studies link folate/homocysteine levels with cognitive functions, as patients with folate deficiencies in the CNS exhibited cognitive deficits [12].

Decreased MTHFR activity leads to increased plasma homocysteine and decreased methionine concentration. Methionine can be used to produce SAM, Homocysteine and S-Adenosyl-Homocysteine (SAH). The SAM regulates methylation of DNA, RNA, protein, and phospholipids. In the brain, SAM-dependent methylations generate neurotransmitters (Catecholamines and indoleamines), phospholipids, and myelin [17]. MTHFR gene is a key regulator for folate versus homocysteine levels [18]. The MTHFR C677T and A1298C polymorphisms affect both nucleotide synthesis and DNA methylation [16]. The MTHFR C677T polymorphism is associated with reduced folate bioavailability and folate metabolites that "mimics" low dietary folate intake [19]. The MTHFR A1298C polymorphism is less severe, and homozygous carries of this allele have a moderate 30-40% reduction of the enzyme activity, yet its function remains controversial [20]. MTHFR polymorphisms A1298C allele, especially in its homozygous expression, can result in a disturbance in the biochemical tetra-hydro biopterin (BH4) and methylation pathways. BH4 is a key factor in the synthesis of serotonin, dopamine, epinephrine, and norepinephrine. Optimal functioning of these neurotransmitters is integrally involved in behavioral symptoms that define ADHD [21].

Scanty studies investigated the association between ADHD and MTHFR gene polymorphism. Accordingly, the aim of this study was to determine the relationship between common MTHFR gene mutations (C677T and A1298C) and ADHD as a phenotype in a sample of Egyptian children.

Materials and Methods

Study design and research

Out of 402 reviewed patients regularly attending the Psychiatric clinic, Faculty of Postgraduate Childhood Studies. Ain Shams University, Cairo, Egypt, and according to criteria of inclusion and exclusion, a sample of 30 ADHD patients were enrolled in this study. The patients were chosen by convenient simple random method of sampling [22].

Outpatient Clinic Hospital based, case control study was conducted during the period from January to August 2015, where data was collected from the participants' parents or legal guardians. After explaining the purpose of the study, parents were informed that participation in this study was voluntary, and they could exit from the study at any time without providing an explanation. The subjects would remain anonymous, and the results of the study would only be used for scientific purposes. Verbal and written consent were obtained from parents, confidentiality of all data and test results of all studied children were protected. This study was approved by Ethical Committee of the Scientific Research, Faculty of Postgraduate Childhood Studies, Ain Shams University, this work was carried out in concordance with Ethics Code of the World Medical Association (Declaration of Helsinki).

All studied cases were subjected to full history taking, physical and neurological examinations, and were further evaluated using the following:

A) **Diagnostic criteria of the DSM-IV TR criteria (2000):** To settle ADHD diagnosis.

B) **The IQ was assessed by using Stanford-Binet Intelligence Scale, Fifth Edition (SB5):** The Arabic version, Abu El-Neil [23], conducted by a trained licensed psychologist; it is used to

assess intellectual ability, it contains 10 subtests, and three areas to be assessed: general cognitive functioning, verbal and non-verbal intelligence. Five factors were formed into groups along verbal/nonverbal measures: Fluid Reasoning, Knowledge, Quantitative Reasoning, Visual-Spatial Processing, and Working Memory. Together, the ten subtests yield an overall estimate of cognitive functioning, which is the Full-Scale Intelligence Quotient [24].

C) **Pediatric Symptom Checklist (PSCL):** The Pediatric Symptom Checklist is a psychosocial screen designed to facilitate the recognition of cognitive, emotional, and behavioral problems. We used the Arabic validated version of Pediatric Symptom Checklist (PSC). The PSCL consists of 35 items that are rated as "Never," "Sometimes," or "Often" present and scored 0, 1, and 2, respectively. The total score is calculated by adding together the score for each of the 35 items. For children and adolescents ages 6 through 16, a cutoff score of 28 or higher indicates psychological impairment. For children ages 4 and 5, the PSCL cutoff score is 24 or higher [25].

D) **Conner's rating scale Scales-Revised (CRS-R):** The scales used are the Arabic versions of the Conner´s parent rating scale revised (CRS-R) version, items are scored on 14 subscales, excellent specificity for ADHD, multidimensional scales that assess ADHD and comorbid conditions [26].

E) **ADHD rating scaling-IV:** It includes 18 items; 9 for inattention symptoms and 9 items for symptoms of hyperactivity and impulsivity [27].

Genetic analysis

Genomic DNA of the patients and controls was isolated from peripheral blood samples and the extracted genomic DNA sample underwent assay for methylene tetrahydrofolate reductase (MTHFR) gene polymorphic loci (C677T and A1298C) detection. This was performed by polymerase chain reaction (PCR) and reverse hybridization to screen and amplifying both MTHFR gene mutations, one involving base pair 677 and other one involving base pair 1298. MTHFR C677T allele was assayed for the detection of methylene tetrahydrofolate reductase (MTHFR) gene polymorphism, it was performed based on polymerase chain reaction (PCR) and reverse hybridization. The procedures were performed according to instructions of MTHFR Stripassay™ kits obtained from Vienna lab diagnostics® GmbH, Vienna, Austria.

MTHFR A1298C allele specific amplification was done for A1298C polymorphism as follows:

A) *In vitro* amplification (PCR) according to the published techniques [28,29].

1. Selective amplification of 256-bp fragment of the MTHFR gene Primers for the A1298C polymorphism in MTHFR gene using the following primers:

R-5'-CAC TTT GTG ACC ATT CCG GTT TG-3'

F-5'-CTT TGG GGA GCT GAA GGA CTA CTA C-3'

2. Genotyping was performed following amplification of template genomic DNA in PCR.
3. The restriction fragment length polymorphism (RFLP) was done by using a restriction enzyme MboII according to manufacturer's instructions (Sibzyme™ - Russia).
4. The genotypes of the PCR products were determined by electrophoresis.
5. The PCR products were all documented by Gel Documentation System and Software for DNA analysis (InGenius Syngene™–UK).

B) The distribution of genotypes and allele frequencies were all statistically compared in all patients versus healthy controls.

The amplified DNA extracted from homozygous patient with A1298C allele appeared at a band length of 176 bp in length compared to the size marker, with three smaller fragments of 30, 28, and 22 bp. The presence of the A1298C polymorphism abolishes an MboI cut site; thus, DNA from a patient homozygous for the A1298C allele appeared at a band length of 204 bp with smaller fragments of 30 and 22 bp.

Ethical consideration

Written informed consent was obtained from parents after explanation of the aim of the study and its benefits.

Statistical analysis

The collected data was tabulated and statistically analyzed using the statistical package for the social science (SPSS) on the computer (version 17.3, SPSS Inc., Chicago IL, USA). The t-student test was used to compare the mean age values of the ADHD cases and the control group. The Chi-square test was used to compare countable variables between the ADHD group and the controls. The significant level was tested at 0.05.

Results

Cases and controls were comparable regarding age and sex distribution at p=0.073 and p=0.21 respectively (Table 1). Both ADHD cases and controls showed statistically in-significant difference regarding their parent's consanguinity. Studied ADHD cases revealed statistically significant family history of medical illness (10%) and psychiatric illness (8.33%) (p=0.0248) compared to controls. Bladder control mean age showed very high significant difference among studied ADHD cases and controls p=0.0005 (Table 1). Speech disorders were significantly high among ADHD cases (25%) compared to controls p=0.0019 (Table 1). Associated

Table 1. *Statistical comparison among enrolled ADHD cases and controls regarding different variables.*

Variable/Group	ADHD (N=30)	Control (N=30)	Test	p-value
Age	8.147 ± 1.75	9.04 ± 2.02	1.826	0.073
Gender				
Male	26 (43%)	21 (35%)	1.571	0.21
Female	4 (7%)	9 (15%)		
Family History				
Normal	19 (31.67%)	27 (45%)	6.480	0.0248
Medical illness	6 (10.00%)	3 (5%)		
Psychiatric illness	5 (8.33%)	0 (0.00%)		
Consanguinity Rate				
Non-cognate	25 (41.67%)	26 (43.33%)	0.353	0.838
First degree	2 (3.33%)	1 (1.67%)		
Second degree	3 (5%)	3 (5%)		
Bladder control age	2.95 ± 1.05	2.15 ± 0.56	3.67	0.001
Speech Disorder				
Positive	15 (25%)	3 (5%)	9.603	0.0019
Negative	15 (25%)	27 (45%)		
Aggressive Behavior				
Positive	19(32%)	4 (7%)	13.819	0.0002
Negative	11 (18%)	26 (43%)		
Attention				
Normal	1 (2%)	30 (50%)	52.325	0.0001
Decreased	2 (48%)	0 (0%)		
Academic performance				
Normal	0 (0%)	28 (47%)	48.817	0.0001
Poor	30 (50%)	2 (3%)		

t-student test was used in statistical comparison regarding the mean age and the age of bladder control.
Chi-square test (χ^2) was used for statistical comparison regarding gender, family history, consanguinity rate, Speech Disorder, Aggressive Behavior, Attention and Academic performance.
Statistical insignificant at $p>0.05$, $p<0.01$=statistical highly significant.

Table 2. *MTHFR gene alleles frequency among enrolled ADHD cases and controls.*

Gene	Allele	ADHD (N=30)	Control (N=30)	χ^2	p-value
C677T	CC	16 (27%)	6 (10%)	5.823	0.0159
	CT	14 (23%)	24 (40%)		
	TT	0 (0%)	0 (0%)		
A1298C	AA	7 (11.67%)	21 (35%)	16.922	0.0002
	AC	13 (21.67%)	8 (13.33%)		
	CC	10 (16.66%)	1 (1.66%)		

Chi-square test (χ^2) was used for statistical comparison.
Statistical insignificant at $p>0.05$, $p<0.01$=statistical highly significant.

aggressive behavior was significantly very high among enrolled ADHD cases (32%) compared to controls p=0.0002 (Table 1). Cognitive functions assessment showed significantly higher prevalence of inattention (96.67%), and poor academic performance (100%) among studied ADHD cases compared to controls p=0.0001, and p=0.0001 respectively (Table 1). The IQ score mean value of enrolled ADHD cases was equal to 94.1 (SD ± 10.192) with minimum value of 80 and maximum value of 115. MTHFR gene alleles of enrolled ADHD cases and controls are presented in Table 2. There was heterozygous advantage (Heterosis) regarding C677T allele genotype, statistically significant association was found in control group compared to ADHD cases (p=0.0159). The genotype frequency of A1298C allele showed statistically high significant association with ADHD cases compared to controls (p=0.0002). MTHFR A1298C allele frequency with respect to gender was shown in Table 3. The ADHD male cases presented statistically significant association of A1298C AC (23.44%) and CC (17%) genotypes compared to controls (p= 0.02). Similarly, the ADHD females showed statistically significant higher frequency of A1298C AC

Table 3. *MTHFR A1298C alleles frequency in respect to gender of enrolled ADHD cases.*

Gender	Group	*AA*	*AC*	*CC*	χ^2	p value
Male	ADHD (N=26)	7 (14.9%)	11 (23.4%)	8 (17%)	7.69	0.02
	Control (N=21)	13 (27.6%)	7 (14.9%)	1 (2.1%)		
Female	ADHD (N=4)	0 (0%)	2 (15.38%)	2 (15.38%)	9.87	0.0072
	Control (N=9)	8 (61.53%)	1 (7.69%)	0 (0%)		

Chi-square test (χ^2) was used for statistical comparison.
Statistical insignificant at p>0.05, p<0.01=statistical highly significant.

Table 4. *Frequency distribution of the A1298C polymorphism regarding Conner's rating scale (inattentive subtype) of enrolled ADHD cases.*

Genetic	Conner's inattention						Chi-square test	
	Mild (N=2)		Moderate (N=2)		Severe (N=26)			
	No.	%	No.	%	No.	%	χ^2	p-value
AA	1	3.33%	0	0%	6	20%	2.396	0.663
AC	0	0%	1	3.33%	12	40%		
CC	1	3.33%	1	3.33%	8	26.7%		

Chi-square test (χ^2) was used for statistical comparison.
Statistical insignificant at p>0.05, p<0.01=statistical highly significant.

Table 5. *Frequency distribution of the A1298C polymorphism regarding Conner's rating scale (hyperactive subtype) of enrolled ADHD cases.*

Genetic	Conner's hyperactive								Chi-square test	
	No hyperactivity (N=2)		Mild (N=1)		Moderate (N=3)		Severe (N=24)			
	No.	%	No.	%	No.	%	No.	%	χ^2	p-value
AA	0	0%	0	0.0%	0	0.0%	7	23.3%	3.577	0.734
AC	1	3.33%	1	3.33%	2	6.67%	9	30%		
CC	1	3.33%	0	0.0%	1	3.33%	8	26.67%		

Chi-square test (χ^2) was used for statistical comparison.
Statistical insignificant at p>0.05, p<0.01=statistical highly significant.

(15.38%), and CC (15.38%) genotypes compared to controls (p=0.0072). There were no statistically significant association between MTHFR A1298C different genotypes (AA, AC, CC) and ADHD subtypes, but they were more encountered among severe inattentive cases according to Conner's rating scale at (20%, 40% and 26.7%) respectively as shown in Tables 4 and 5.

Discussion

ADHD is a very complex disorder. The number of factors contributing to its symptomatology is vast- and growing. MTHFR is a genetic abnormality that somewhat found common in individuals with ADHD, its presence could explain a lot and offer additional treatment [30].

Genetic inheritance and many environmental factors have been proven to increase child's risk of being born with ADHD, which comprises a spectrum of symptoms ranging in severity, its causes are likely to mimic a similar pattern [31]. The genetic instructions for a child comprise a mash up of instructions from each parent. The language of these instructions is our individual genetic code. Some genetic factors pass to the offspring just as they appeared in one parent, other genetic traits are created by a new combination of different parent genes [31].

Parents with ADHD are more likely to have kids with ADHD. MTHFR gene controls a biological process in the body that affects neurological functions, MTHFR gene is a name of both a gene and an enzyme in the human body, methylenetetrahydrofolate reductase, the gene tells the body how to make the enzyme, this enzyme is important to process folate or folic acid (B9) properly, it turns folate into its bioavailable form methyl folate through a process called methylation, the methyl folate then converts amino acids to different forms for different body functions, including dopamine and serotonin manufacturing. Accordingly, if the MTHFR gene is mutated, the enzyme cannot be produced correctly, which in-turn disrupts dopamine and serotonin production, the key players in ADHD. MTHFR gene malformation reduces the change capacity of folate into methyl folate (as low as 10% for homozygous and

50% for heterozygous) [32].

The present study revealed that male predominance in ADHD cases was 86.7% close to 84.3% reported by Novik et al. [33] who studied the influence of gender on 1478 ADHD child in Europe. The female to male ratio in the current study was 1:6.5, this ratio is in concordance with a ratio range of 1:3 to 1:16 formerly mentioned by Novik et al. [33], and in agreement with a study conducted in Fayoum city, Egypt among ADHD school aged children by Abouel-ata et al. [34]; revealing a ratio of 1:5. However, another study in Menoufia Governate, Egypt among ADHD primary school children by Farahat et al. [35] reported a ratio of 1:3.5, such ratio difference could be attributed to the hospital-based sample in the current study versus the community-based sample of Farahat's study.

The present study revealed that ADHD cases' age ranged from 6 to 12 years with mean age of 8.147 ± 1.75 which agrees with Cheon et al. [36] who reported a mean age of 8.4 ± 1.77. Current study showed positive family history of medical and psychiatric illness in ADHD cases of 20% and 16.67% respectively, however Farahat et al. [35] reported 62.8% and 66% respectively in Menoufia Governate. The consanguinity rate recorded in the current study was 16.67% compared to 34.8% in Jordan [37], consanguinity is a long-standing habit in Egypt that increases the risk of multi-factorial disorders as ADHD [38]. ADHD and incontinence are common childhood disorders which usually co-occur at much higher rates than expected by chance. The current study demonstrated a statistically significant higher mean age of bladder control among studied ADHD cases compared to controls (p=0.005), which is in concordance with findings of von Gontard et al. [39]. The present study revealed that mean IQ score of ADHD cases was 94.1 ± SD 10.19 compared to a study by Cheon et al. [36] which showed a value of 111.8 ± SD 17.4.

As is the norm for most psychiatric phenotypes, many etiological studies have primarily focused on the interplay of genetic and environmental factors. Family, twin and adoption studies provided an overwhelming evidence for an inherited contribution to the ADHD pathogenesis [6]. The current study aimed at comparing children with ADHD and children without regarding the presence of MTHFR mutant allele and it revealed a significant presence of MTHFR C677T allele wild-, hetero-type with the absence of any mutation in the control group (p=0.016), denying an association between C677T genotype and ADHD phenotype expression. This result is in concordance to those of Gokcen et al., who showed no difference concerning MTHFR C677T allele in ADHD cases (p=0.678) [9]. Similarly, another study by Krull et al. [40] showed absence of an association between C677T genotype and inattentive symptoms of ADHD. However, the studied ADHD cases had a high significant mutant MTHFR A1298C allele compared to controls (p=0.0002). A previous study by Gokcen et al. [9] showed that ADHD group had a higher prevalence of the mutant A1298C allele than the control group (p=0.033). Additionally, another study by Krull et al. [40] highlights a possible link between A1298C polymorphism and inattentive symptoms in ADHD group. Inattentive symptoms are usually prominent in ADHD female children [41]. In the present study, all females (4/4) of the ADHD group were found to have MTHFR A1298C polymorphism (p=0.0015). This result corresponds to that of Gokcen et al. [9] findings showed that 8 of 9 females in the ADHD group had the A1298C mutation. In the current study, the enrolled male ADHD cases had significant higher prevalence of A1298C mutation compared to the controls (p=0.02).

The findings of this study revealed an association between ADHD in both genders and MTHFR A1298C gene mutation. On the other hand, there was no association between ADHD and MTHFR C677T gene mutation. Results of the present study encourage to detect MTHFR A1298C gene mutation early in suspected ADHD cases, assist in prompt intervention, understand MTHFR gene mutation mapping in ADHD, guide future researches regarding additional treatment and gene therapy.

Conclusion

It can be concluded that there was an evident association between ADHD phenotype and MTHFR A1298C gene polymorphism, and there was a heterozygous advantage (Heterosis) regarding C677T allele genotype and ADHD cases leading to absence of association between MTHFR C677T gene polymorphism and ADHD. There was a positive correlation between ADHD and family history of medical and psychiatric illness. Bladder control mean age was significantly higher among studied ADHD cases than controls, speech disorders, associated aggressive behavior, and poor academic performance were significantly higher among ADHD compared to controls.

Study Limitations and Future Scope

Thereby, the present study included 30 ADHD cases and 30 matched controls which could be accounted as a limited sample size. Thus, we recommend carrying nationwide community-based studies to investigate the prevalence of A1298C allele in ADHD phenotypes.

References

1. Caroline S, Clauss-Ehlers. Topics in cross-cultural school psychology: In: Encyclopedia of cross-cultural school psychology (1st ed.). Springer 2010;pp:64-90.
2. Childress AC, Berry SA. Pharmacotherapy of attention-deficit hyperactivity disorder in adolescents. Drugs. 2012;72:309-25.
3. Dulcan MK, Ballard RR, Jha P, et al. Neuro-developmental disorders: In: Concise guide to child and adolescent psychiatry (5th ed.). American Psychiatric Association Publications, Washington, DC. 2017;pp:25-76.

4. Thomas R, Sanders S, Doust J, et al. Prevalence of attention deficit/hyperactivity disorder: A systemic review and meta-analysis. Pediatrics. 2015;135:1-8.

5. Leibson CL, Katusic SK, Barbaresi WJ, et al. Use and costs of medical care for children and adolescents with and without attention-deficit/hyperactivity disorder. JAMA. 2001;285:60-6.

6. Faraone SV, Perlis RH, Doyle AE, et al. Molecular genetics of attention deficit/hyperactivity disorder. Biological Psychiatry. 2005;57:1313-23.

7. Biederman J, Faraone SV. Attention deficient hyperactivity disorder. Lancet. 2005;366:237-48.

8. Dickstein SG, Bannon K, Castellanos FX, et al. The neural correlates of attention deficient hyperactivity disorder (ADHD): An ALE meta-analysis. J Child Psychology and Psychiatrics. 2006;47:1051-62.

9. Gokcon C, Kocak N, Pekgor A. Methylene tetrahydrofolate reductase gene polymorphism in children with attention deficient hyperactivity disorder. Int J Med Sci. 2011;8:523-8.

10. Schlotz W, Jones A, Phillips DI, et al. Lower maternal folate status in early pregnancy is associated with childhood hyperactivity and peer problems in offspring. J Child Psychol Psychiatr. 2010;51:594-602.

11. Zeisel SH. Importance of methyl donors during reproduction. Am J Clin Nutr. 2009;89:673S-7S.

12. Moretti P, Peters SU, Gaudio DD,, et al. Brief report: Autistic symptoms, developmental regression, mental retardation, epilepsy, and dyskinesias in CNS folate deficiency. J Autism Dev Disord. 2008;38:1170-7.

13. Pavarino EC, Zampier BL, Biselli JM, et al. Abnormal folate metabolism and maternal risk for Down syndrome: In: Genetics and etiology of down's syndrome (1st ed.). Intechopen Brazil. 2011;pp:97-120.

14. De Lau ML, Refsum H, Smith A, et al. Plasma folate concentration and cognitive performance: Rotterdam scans study. Am J Clin Nutr. 2007;86:728-34.

15. Goyette P, Sumner JS, Milos R, et al. Human methylenetetrahydrofolate reductase: Isolation of CDNA, mapping and mutation identification. Nat Genet. 1994;7:195-200.

16. Lucock M. Folic acid: Nutritional biochemistry, molecular biology and role in disease processes. Mol Genet Metab. 2000;71:121-38.

17. Miller AL. The methylation, neurotransmitter, and antioxidant connections between folate and depression. Altern Med. 2008;13:216-26.

18. Tangney C, Tang Y, Evans D, et al. Biochemical indicators of vitamin B12 and folate insufficiency and cognitive decline. Neural. 2009;72:361-7.

19. Molloy AM, Daly S, Mills JL. Thermolabile variant of 5, 10-Methylene tetrahydrofolate reductase associated with low red-cell folates: Implications for folate intake recommendations. Lancet. 1997;349:1591-93.

20. Weisberg IS, Jacques PF, Selhub J. The A1298C polymorphism in methylenetetrahydrofolate reductase (MTHFR): *In vitro* expression and association with homocysteine. Atherosclerosis. 2001;156:409-15.

21. Peter LR, Stephen KT, David H, et al. Methylene Tetrahydrofolate Reductase (MTHFR): The incidence of mutations C677T and A1298C in the Ashkenazi Jewish population. Am J Med Genet A. 1999;86:380-4.

22. Langholzl B, Clayton D. Sampling strategies in nested case-control studies. Environ Health Perspectives. 1994;102:47-51.

23. Abu El-Neil M. Stanford binet scale of intelligence (5th ed.), The Arab Foundation for the Preparation, Standardization and Publication of Psychological Tests, Cairo. 2011.

24. Roid GH. Stanford-binet intelligence scales fifth edition (SB:V). Itasca. 2004;19:235-44.

25. Pagano ME, Cassidy LJ, Little M, et al. Identifying psychosocial dysfunction in school-age children: The pediatric symptom checklist as a self-report measure. Psychology in the Schools. 2000;37:91-106.

26. Conners CK. Conners' parent rating scales-revised technical manual, multi-health systems inc. North Tonawanda NY. 1997.

27. Collett BR, Ohan JL, Myers KM. Ten-year review of rating scales-V: Scales assessing attention-deficit/hyperactivity disorder. J Am Academy of Child and Adolescent Psychiatry. 2003;42:1015-37.

28. Hanson NQ, Aras O, Yang F, et al. C677T and A1298C polymorphisms of the methylenetetrahydrofolate reductase gene: Incidence and effect of combined genotypes on plasma fasting and post-methionine load homocysteine in vascular disease. Clin Chem. 2001;47:661-6.

29. Boris M, Goldblatt A, Galanko J, et al. Association of MTHFR gene variants with autism. J Am Physicians and Surgeons. 2004;9:106-8.

30. Gilbody S, Lewis S, Lightfoot T. Methylene Tetrahydrofolate Reductase (MTHFR) genetic polymorphisms and psychiatric disorders: A huge review. Am J Epidemiol. 2007;165:1-13.

31. Zhang L, Chang S, Li Z, et al. ADHD gene: A genetic database for attention deficit hyperactivity disorder. Nucleic Acids. 2012;40:1003-9.

32. Mattson MP, Shea TB. Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders. Trends Neurosci. 2003;26:137-46.

33. Novik TS, Hervas A, Ralston SJ, et al. Influence of gender on attention deficit hyperactivity disorder in Europa-Adore. Eur Child Adolesc Psychiatry. 2006;15:i15-i24.

34. Aboul-ata M, Amin F. The prevalence of ADHD in Fayoum City (Egypt) among school-age children: Depending on a DSM-5-based rating scale. J Atten Disord. 2015;22:127-33.

35. Farahat T, Alkot M, Rajab A, et al. Attention deficit hyperactivity disorder among primary school children in Menoufia governorate, Egypt. International Journal of Family Medicine. 2014;pp:1-7.

36. Cheon KA, Kim BN, Cho SC. Association of 4-repeat allele of the dopamine D4 receptor gene exon III polymorphism and response to methylphenidate treatment in Korean ADHD children. Neuropsychopharmacol. 2007;32:1377-83.

37. Nafi OA, Shaheen AM. Prevalence of Attention Deficit Hyperactive Disorder (ADHD) in school children in Al-Qaser District Jordan. Jordan Med J. 2011;45:37-43.

38. Zaky E. Nurture and human behavior: An endless debate. J Child Adolesc Behav. 2015;3:1-4.

39. Von Gontard A, Equit M. Comorbidity of ADHD and incontinence in children. Eur Child Adoles Psy. 2014;24:127-40.

40. Krull KR, Brouwers P, Jain N, et al. Folate pathway genetic polymorphisms are related to attention disorders in childhood leukemia survivors. J Pediatr. 2008;152:101-5.

41. Wolraich ML, Hannah JN, Pinnock TY, et al. Comparison of diagnostic criteria for attention-deficit hyperactivity disorder in a country-wide sample. J Am Acad Child Adolesc Psychiatry. 1996;35:319-24.

Correspondence to:

Khaled Ismail AbdElShakoor
Faculty of Postgraduate Childhood Studies,
Medical Studies Department,
Ministry of Health, Ain Shams University,
Al-Khalifa Al-Ma'moun street,
Cairo, Egypt
Tel: +202 22594539
E-mail: khaledprime@hotmail.com