A Study of the Role of Methylenetetrahydrofolate Reductase (MTHFR) Gene Polymorphism in Children With Attention Deficit Hyperactivity Disorder

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A BSTRACT

Background: ADHD is a neuro-behavioral disorder that is typically manifested during childhood and often persists into adulthood, it is a complex disorder influenced by many genes, genetic and environmental risk factors co-occurring in a non-random fashion. The MTHFR gene C677T polymorphism affects both nucleotide synthesis and DNA methylation, and is associated with reduced folate bioavailability and folate metabolites.

Objectives: To assess the relationship between Methylenetetrahydrofolate Reductase (MTHFR) gene polymorphisms (C667T) allele and Attention Deficit Hyperactivity Disorder (ADHD) in a sample of Egyptian children.

Methodology: Out of 402 reviewed patients regularly attending the Psychiatric clinic, Faculty of Postgraduate Studies for Childhood - Ain Shams University, Cairo, Egypt. According to criteria of inclusion and exclusion a sample of 30 ADHD children were enrolled in this study. The patients were selected by simple random sample during the period from January to August, 2015 with age ranged from 6- to 12-year old. A matching number of 30 healthy children with normal developmental and psychiatric evaluation, of compareble age and sex, were randomly recruited as a study control. MTHFR gene polymorphism (C677T) allele was investigated in the selected sample.

Results: There was heterozygous advantage (Heterosis) regarding C677T allele genotype, a statistically significant association was found in control group compared to ADHD cases (p=0.0159). The ADHD group revealed statistically significant family history of medical illness (10%) and psychiatric illness (8.33%) (p=0.0248) compared to control group.

Conclusions: The study found no association between ADHD phenotype and the MTHFR C677T gene polymorphism in Egyptian children with attention deficit hyperactivity disorder. There was positive link between ADHD and family history of medical and psychiatric illness.

Keywords: MTHFR gene, C677T allele, genotype, phenotype, attention deficit hyperactivity disorder (ADHD).

دراسه تأثير تعدد الأشكال الجينية للميثيلين تتراهيدروفولات المختزل

في الأطفال ذوي اضطراب نقص الانتباه و فرط النشاط

مستخلص:

الخلفية العلمية: اضطراب فرط الحركة ونقص الانتباه هو اضطراب سلوكي عصبي يظهر عادة أثناء الطفولة وغالبا ما يستمر إلى مرحلة البلوغ ، وهو اضطراب معقد يتأثر بالعديد من الجينات وكذلك عوامل الخطر الجينية C677Tوالبيئية و التي غالبا تحدث بطريقة غير عشوائية. يؤثر تعدد أشكال الجين ميثيلين تيتراهيدروفولات للأليل على تخليق كل من النوكليوتيدات ومثيلته من الحمض النووي و كذلك يرتبط بتقلص الحيوية في حمض الفوليك ومستقلبات الفولات.

) للميثيلين MTHFRالأهداف: كان الغرض من هذه الدراسه هو تقييم العلاقة بين تعدد الأشكال الجيني ( ) في عينة من الأطفال ADHD و اضطراب فرط الحركه وتشتت الانتباه ((C667T) (MTHFR)تيتراهيدروفولات المصريين.

المنهجيه: تم فحص 402 من المرضى الذين كانوا يحضرون بانتظام عيادة الأمراض النفسية ، كلية الدراسات العليا للطفولة – جامعة عين شمس ، القاهرة ، مصر ، وفقا لمعايير الإدراج والاستبعاد قد تم تسجيل عينة من 30 طفلا من المصابين بحالات اضطراب فرط الحركه و تشتت الانتباه في هذه الدراسة. تم اختيار المرضى بعينة عشوائية بسيطة خلال الفترة من يناير إلى أغسطس 2015 وكانت تتراوح اعمارهم بين 6 و 12 عاما. تم تسجيل عدد مماثل من 30 طفل بصحة جيدة و قد تم تقييم النمو الطبيعي والنفسي لديهم وهم في نفس العمر والجنس مقارنه بالحالات كمجموعه تحكم . تم الفحص و الأختبار للتحقق من تعدد أشكال الجين ميثيلين تيتراهيدروفولات في العينة المحددة و عينه التحكم.C677Tللأليل

النتائج: كانت هناك ميزة متغايرة (Heterosisبخصوص النمط الوراثي للأليل C677T) ، حيث قد وجد ارتباط ذو دلالة إحصائية في مجموعة التحكم مقارنة بحالات اضطراب فرط الحركه و تشتت الانتباه ((P = 0.0159 . كشفت مجموعة حالات فرط الحركه و تشتت الانتباه عن تاريخ عائلي ذو دلالة إحصائية للأمراض الطبيه بنسبه 10% و كذلك الأمراض النفسية بنسبه 8.33 % (P = 0.0248.مقارنة مع مجموعة التحكم)

الاستنتاجات: أظهرت الدراسة أنه لا يوجد ارتباط بين تعدد الأشكال الجيني للميثيلين تيتراهيدروفولات للأليل C677Tحالات فرط الحركه و تشتت الانتباه في الاطفال المصريين محل الدراسه. و

الكلمات الدالة: اضطراب فرط الحركة وتشتت الانتباه، النمط الظاهري، النمط الجيني، الجين ميثيلين تيتراهيدروفولات، الأليل

1. Introduction

Attention deficit hyper-activity disorder (ADHD) is a neuro-behavioral disorder that is typically manifested during childhood and often persists into adulthood. Gene and environmental influences work together in complex way where there is increasing link evidence that gene-environment correlation interaction could play an important role in the psychopathological etiology.1

ADHD is a complex disorder influenced by many genes, genetic and environmental risk factors co-occurring in a non-random fashion because of the heritable characteristics of the individual or parents carrying the risk of exposure to certain environmental risk factors. The genetic element may increase the ADHD risk via influencing the exposure to risk or protective environmental factors.2

The MTHFR gene C677T and A1298C polymorphisms affect both nucleotide synthesis and DNA methylation.3 The MTHFR C677T polymorphism is associated with reduced folate bioavailability and folate metabolites that "mimics" low dietary folate intake.4 MTHFR polymorphisms A1298C allele, especially in its homozygous expression, can result in a disturbance in the biochemical tetra-hydrobiopterin (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 the behavioral symptoms that define ADHD.5

Accordingly, the aim of current study was to determine the relationship between MTHFR gene mutation C677T and ADHD as phenotype in Egyptian children sample, a disorder that genetic factors play an important role in its etiology.

2. Study design and research methodology

Outpatient Clinic Hospital - based, case control study was conducted within regular working hours to study children during the period from January to August, 2015.

The plan was subjected to study a sample of children with ADHD from those who regularly attending the Psychiatric clinic, Faculty of postgraduate studies for Childhood - Ain Shams University, Cairo, Egypt, and an equal number of normal control children. The patients were selected by simple random sample. This study was conducted on 30 cases of well diagnosed patients with ADHD with age of 6-12 years and 30 healthy controls who were matched for age and sex, randomly recruited, and with normal developmental and psychiatric evaluation.

Cases and controls were studied properly and completed during year 2015. Data were collected from patient's parents.

The purpose of the study was explained to parents, they were informed that participation in this study was voluntary, and they could exit from the study at any time

without providing any explanation, the results of the study would be used for the scientific purposes. Verbal and written consent was obtained from parents. This study was approved by the ethical Committee of Faculty of postgraduate studies for Childhood, Ain Shams University, as this work was carried out in concordance the Ethics Code of the World Medical Association (Declaration of Helsinki).

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

- Diagnostic criteria of the DSM-IV .

-The IQ was assessed by using Stanford-Binet Intelligence Scale V5

- Pediatric symptom checklist (PSCL)

- Conner's rating scale.

- ADHD Rating Scaling-IV

-Genetic Analysis

Genomic DNA of the patients and control was isolated from peripheral blood samples and the extracted genomic DNA sample underwent assay for methylene tetrahydrofolate reductase (MTHFR) gene polymorphic locus (C677T) detection. Analysis was done via polymerase chain reaction (PCR) and reverse hybridization to screen for and amplify two MTHFR gene mutation, MTHFR C677T, Allele was assayed according to instructions of MTHFR Stripassay™ kits obtained from Vienna lab diagnostics® GmbH, Vienna, Austria.

3-Ethical consideration

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

4-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.

3. Results

The descriptive data in terms of mean age, gender distribution, family history and parent's consanguinity of the ADHD and the control groups are presented in table 1. The mean age and gender distribution of the ADHD cases and controls showed statistically insignificant difference. The control group revealed statistically significant normal family history compared to the ADHD cases who presented history of medical illness (10%) and psychiatric illness (8.33%). Both ADHD cases and controls showed statistically insignificant difference regarding their parent's consanguinity. As regards MTHFR gene C677T allele in ADHD and control groups, there was heterozygous advantage (Heterosis) between C677T allele genotype, a statistically significant association was found in control group compared to ADHD cases ($p=0.0159$). It was presented in table 2.

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4. Discussion

ADHD is a very complex disorder. The number of factors contributing to symptoms are vast- and growing. MTHFR is a genetic abnormality that's somewhat common in individuals with ADHD, and its presence could explain a lot and offer additional treatment.6

Methylenetetrahydrofolate reductase (MTHFR) is important for folate chemical reactions. MTHFR gene is located at the end of the short arm of the chromosome 1p 36.3.7 The enzyme methylenetetrahydrofolate reductase (MTHFR) helps to convert folate in folate metabolic cycle into metabolites that may be used for cellular processes, which include 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. This reaction is required for a multistep process which converts homocysteine to another amino acid methionine, methyltetrahydrofolate donates its methyl group to convert homocysteine into methionine in the presence of methionine synthetase and vitamin B12 in the generation of S-adenosyl methionine, which is a major source of methyl group in the brain.8

MTHFR polymorphisms C677T and A1298C affect nucleotide synthesis and DNA methylation. Several studies link folate/ homocysteine levels with cognitive functions, as patients with folate deficiencies in the CNS exhibited cognitive deficits.9

The present study revealed that male predominance in ADHD cases was 86.7% close to 84.3% reported by Novik et al., (2006); who studied the influence of gender on 1478 ADHD child in Europe. The ratio of female to male in the current study was 1:6.5, this ratio is in accordance with a ratio range 1:3 to 1:16 formerly mentioned by Novik et al., (2006). 10

In addition, another study conducted in Fayoum city, Egypt among ADHD school aged children by Abouel-ata and Amin, (2015); revealing a ratio of 1:5.11

However, another study in Menoufia Governate, Egypt among ADHD primary school children by Farahat et al., (2014) reported a ratio of 1:3.5, this ratio difference could be attributed to the hospital-based sample in the current study versus a community based last mentioned study.12

In the current study, ADHD cases age ranged from 6- to 12-year-old. The mean age value found to be 8.147 which agrees with other study by Cheon et al., (2007); with a mean age of 8.4.13

This study revealed that among children having a positive family history of medical and psychiatric illness, there was 20% and 16.67% chance respectively of being ADHD phenotype compared to another study in Menoufia Governate which showed 62.8% and 66% chance respectively, however; both studies demonstrated significant positive family history of medical and psychiatric illness.

The current study showed a consanguinity rate of 16.67%. This is comparable to a study in Jordan which indicated a prevalence of 34.8% .14

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 in ADHD cases compared to controls ($p=0.005$), which in-concordance with a study by von Gontard and Equit, (2014).15

The present study revealed that IQ score mean value among ADHD cases was 94.1±SD 10.19 that is inconsistent with a study by Cheon et al., (2007); showing an IQ mean value of 111.8±SD 17.4.13

As is the norm for most psychiatric phenotypes, many etiological studies have primarily focused on the genetic and environmental factors interplay. Family, twin and adoption studies provided an overwhelming evidence for an inherited contribution to the ADHD pathogenesis.16

The current study aimed to compare children with – and children without ADHD according to the presence of MTHFR mutant allele. The results revealed a significant presence of MTHFR C677T allele wild-, hetero-type with the absence of mutant alleles in the control group (p=0.016), showing no association between C677T genotype and ADHD phenotype expression. This result is in concordance with a study conducted by Gokcen et al., 2011; that showed no difference in respect to MTHFR C677T allele (p=0.678).8 Similarly, another study by Krull et al., 2008; showed absence of association between C677T genotype and inattentive symptoms.17

These findings point to heterozygous advantage (Heterosis) between ADHD and MTHFR C677T gene mutation.

The most important limitation of this study was the small size sample. In addition, a limited literature that focus the MTHFR gene mutation, C677T; and the ADHD syndrome.

5. Conclusion

Under the limitations of the present study, it can be concluded that:

This study demonstrated a heterozygous advantage (Heterosis) regarding C677T allele genotype and ADHD cases and there was absence of association between MTHFR C677T gene polymorphism in the ADHD group.

Conflict of interest

The authors received no financial support and declare no conflicts of interest with respect to authorship and/or publication of this article.

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Table 1: Descriptive data of ADHD and control groups.

Variable	ADHD	Control	P value
Age	8.147±1.75	9.04±2.02	0.073
Gender			
Male	26 (43%)	21 (35%)	0.21
Female	4 (7%)	9 (15%)	
Family History			
Normal	19 (31.67%)	27 (45%)	0.0248
Medical Illness	6 (10.00%)	3 (5%)	
Psychiatric Illness	5 (8.33%)	0 (0.00%)	
Consanguinity			
Non-cognate	25 (41,67%)	26 (43.33%)	0.838
First Degree	2 (3.33%)	1 (1.67%)	
Second Degree	3 (5%)	3 (5%)	

Table 2: MTHFR geneC667T allele of ADHD and control groups.

Gene Allele		ADHD	Control	P value
C677T	CC	16 (27%)	6 (10%)	*0.0159
	CT	14 (23%)	24 (40%)	

	TT	0 (0%)	0 (0%)

*statistically significant