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Association Study of Polymorphisms in Folate Metabolism and Mothers of Down Syndrome Offsprings in the Southwest of Iran

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Abstract

Background: Down syndrome (DS) is a complex genetic disease that is caused by having three copies of chromosome 21. A possible association between polymorphisms in maternal folate metabolism genes and DS has been evaluated.
Objectives: It was aimed to first investigate the influence of C677T and A1298C polymorphisms in the methylenetetrahydrofolate reductase gene (*MTHFR*) and plasma homocysteine (Hcy) on the maternal risk for DS in the southwest of Iran.
Methods: The *MTHFR* C677T and A1298C polymorphisms were genotyped using restriction fragment length polymorphism and Sanger sequencing, respectively. Allele and genotype frequencies and the dominant model of the *MTHFR* C677T and A1298C polymorphisms were evaluated in 80 mothers of children with DS and 80 control mothers. Eventually, the ELISA test was used to compare the concentration of plasma Hcy in both groups.
Results: A significant association was observed in the 677T and 1298C alleles between the mothers of DS and control groups ($P = 0.00077$ and $P = 0.01248$, respectively). Further, the median concentrations of Hcy were significantly higher in mothers with DS babies compared to the control group ($P < 0.05$).
Conclusions: There was an association between *MTHFR* C677T, A1298C, and plasma Hcy concentrations as the maternal risk of mothers with DS children.

Keywords: Down Syndrome, Methylenetetrahydrofolate Reductase, Polymorphisms, Association Study

1. Background

Down syndrome (DS) is the most common and popular chromosomal disease in humans. Approximately 95% and 2 - 4% of people with DS have 47 chromosomes and translocation, respectively, and the rest of them are mosaicism. More than 90% of extra chromosome 21 is the result of maternal nondisjunction during meiosis (1). Nonetheless, the mechanism underlying the meiotic nondisjunction is not yet realized, and it is thought to have a multifactorial etiology that is affected by both genetic and acquired factors. The association between chromosomal nondisjunction and folate metabolism has acquired attention. Aberrant chromosome segregation is the result of chronic folate and methyl deficiency. The risk of chromosome nondisjunction may be enhanced by abnormal folate metabolism and DNA hypomethylation (2, 3).

The human *MTHFR* gene contains 11 exons situated on the short arm of chromosome 1 at position 36.3. It encodes a crucial enzyme in folate and Hcy metabolism, methylenetetrahydrofolate reductase (MTHFR). MTHFR catalyzes the biologically irreversible reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, making Hcy remethylated by the methyl group to methionine (4). In the *MTHFR* gene, some single nucleotide polymorphisms (SNP), including C677T and A1298C, which are the two most significant variations, can influence folate and total Hcy status. The *MTHFR* C677T is due to the C/T transition at nucleotide 677,

which produces an alanine-to-valine substitution. Also, it is associated with declining concentrations of folate in the serum, plasma, and red blood cells and slightly increased plasma total Hcy concentration. It further causes impaired folate binding and reduced activity of the *MTHFR* enzyme. The *MTHFR* A1298C, which includes an adenine (A) to cytosine (C) substitution at position 1298, converts glutamate to alanine in the enzyme and is correlated with reduced enzyme function in vitro (5). The C677T and A1298C are located in the catalytic and regulatory domains, respectively. Several studies focused on examining C677T and A1298C polymorphisms in the *MTHFR* gene and were related to DS in different ethnic groups worldwide, yielding contradictory results in this regard.

2. Objectives

This study sought to first determine the association of *MTHFR* C677T and A1298C polymorphisms in Iranian mothers with DS babies and compare them with control groups in southwestern Iran. Furthermore, the association of Hcy concentration was compared in case and control groups.

3. Methods

3.1. Study Population

In general, 80 women giving birth to children with DS were included in this study. The exclusion criteria were disorders related to abnormal folate status in DS mothers, including psychiatric disorders, cleft lip and palate, neural tube defects, cardiovascular diseases, late pregnancy complications, and neurodegenerative. The mothers of children with mosaic and Robertsonian translocation type of DS were not included in this study. Moreover, 80 control mothers had at least two intact children enrolled in this study. They had no frequent abortions and no children with DS in their relatives. In addition, informed consent was obtained from case and control groups.

3.2. Polymorphism Genotyping

Genomic DNA was extracted from the EDTA whole blood of the case and control groups using the YEKTA TAJHIZ kit (cat. No: FABGK001). For genotype analysis, the C677T *MTHFR* gene was amplified by PCR using previously described primers and conditions, followed by restriction enzyme digestion with HinfI, which was then analyzed by electrophoresis in 3% agarose gel (6). Additionally, the genotype analysis of A1298C in the *MTHFR* gene was performed by Sanger sequencing with suitable primers, which were described earlier (7).

3.3. Plasma Total Hcy Concentrations

Blood samples were collected into EDTA-containing tubes from overnight fasted individuals and centrifuged at 3000 rpm for 15 minutes. The plasma was stored at 70 °C until assaying. Finally, the total plasma Hcy concentration was measured by the ELISA test.

3.4. Statistical Analysis

Data were reported as means, numbers, or frequency. The analysis of allele and genotype frequencies of the *MTHFR* C677T and A1298C polymorphisms were assessed for Hardy-Weinberg equilibrium using the X^2 test. In addition, a *t*-test was used to calculate the Hcy level of blood samples. The dominant model was employed to evaluate C677T and A1298C polymorphisms as DS risk. Eventually, statistical analysis was performed using SPSS (version 16.0 and Microsoft Excel 2003).

4. Results

A total of 160 women were enrolled in this study and divided into 80 cases and 80 controls. For the case group, the mean maternal age at conception of DS babies was 31.5 ± 3.5 years (in the range of 22 - 35). For the control group, the mean maternal age at conception (the latest pregnancy) was 33 ± 3 years (in the range of 26 - 36). Each group contained 51 Bakhtiari and 29 Fars ethnicity mothers. The mean body mass index (BMI) for the case and control groups was 25.8 and 28.3, respectively.

The allele and genotype frequencies of *MTHFR* C677T and *MTHFR* A1298C are presented in Table 1. Based on the data, the frequencies (percentages) at position 677 for C and T alleles were 120 (75%) and 40 (25%), as well as 143 (89%) and 17 (11%) in the case and control groups, respectively. Further, the corresponding frequencies at position 1298 for A and C alleles were 83 (52%) and 77 (48%), as well as 105 (66%) and 55 (34%) in the case and control groups, respectively. Additionally, *MTHFR* 677T and the 1298C alleles were more frequent in DS mothers compared to control mothers. There was a significant association in these allele frequencies (*MTHFR* 677C allele versus *MTHFR* 677T allele, P = 0.00077 and *MTHFR* 1298A allele versus *MTHFR* 1298C allele, P = 0.01248) between the case and control groups. Furthermore, the results demonstrated a significant association in genotype frequencies (P = 0.00088 and P = 0.03 for *MTHFR* C677T and *MTHFR* A1298C, respectively) between mothers with DS children and control groups. Using the dominant model, a

significant association was observed between *MTHFR* C677T (CC versus TT+CT, P = 0.000205) and A1298C (AA versus AC+CC, P = 0.02742) polymorphisms and the risk of DS. Our results for the two polymorphisms are in line with the Hardy-Weinberg equilibrium in both groups.

The comparison of different genotypes (Table 2) revealed that no mothers with DS children had a double homozygous mutant genotype (677TT/1298CC) for these polymorphisms. The highest combined genotype (30%) was C677C/A1298C, followed by CT/AC (26.25%), which was heterozygous for both genotypes. A significant positive interaction was found for the *MTHFR* 677CC and *MTHFR* 1298 genotypes (P = 0.000014), confirming the role of allele-allele interactions in the assessment of genetic susceptibility to DS. The amounts of Hcy in the case and control groups were examined, and the ELISA results indicated that the mean concentrations of Hcy were significantly higher ($P < 0.05$) in DS mothers with 677CT and 677TT (4.17 and 4.76 μmol/L, respectively) compared with the control group (4.25 and 4.02 μmol/L, respectively). In addition, the mean concentrations of plasma Hcy levels for 1298AC and 1298CC significantly differed ($P < 0.05$) between case (4.30 and 4.12 μmol/L, respectively) and control groups (4.17 and 4.10 μmol/L, respectively), the details of which are provided in Table 3.

5. Discussion

According to the findings of the present study, a significant association was identified between *MTHFR* C677T and A1298C in the mothers of DS children compared to healthy control groups of the same age in the southwest of Iran. Based on our statistical analysis, the 677T and 1298C alleles run a significant risk of delivering DS babies.

In agreement with our findings, Rai et al. reported a significant association between the mothers of DS and the healthy groups in terms of *MTHFR* C677T using meta-analysis from 34 articles (8). Similarly, Kaur and Kaur published a meta-analysis article using 37 case-control studies and supported the idea that the *MTHFR* C677T genotype was associated with increased risk for the mothers of DS babies (9). Some studies also showed that *MTHFR* A1298C had a significant association in Egyptian (10), Iranian (in Tehran; article in Persian), and Jordanian (11) mothers with DS children and control groups, which corroborates with the findings of the present study. In contrast, some studies reported no significant association between the mothers of DS and control groups regarding C677T and A1298C polymorphisms. For instance, the results of studies performed in southern Brazil (12), Poland (13), Iran (in Tehran), and southern China (7) represented that the *MTHFR* C667T was not a risk factor between the mothers of DS and control groups. Moreover, no relationship was found between the occurrence of *MTHFR* A1298C polymorphism in the mothers of DS and control groups in Brazil (14), Poland (13), and Southern China (7). Additionally, Wu et al. conducted a meta-analysis including 21 studies. The comparison results demonstrated no significant association between the mothers of DS and the healthy group regarding *MTHFR* A1298C (15).

Although most studies indicated the correlation between these SNPs and DS, some of them reported contradictory results. For instance, Brandalize et al. investigated *MTHFR* C677T and A1298C on 239 Brazilian mothers with DS children and 197 mothers with normal babies using polymerase chain reaction (PCR)-restriction fragment length polymorphism and found a significant correlation between 1298AA, 677CT, and 677TT genotypes and the risk of having DS children (16). Additionally, Coppedè et al. in 2009 investigated C677T and A1298C in 94 Italian mothers of DS children and 113 matched control mothers; in the total population, they observed a significant correlation between micronucleated blood cells and both MTHFR C677T and A1298C polymorphisms (17). In another study, Chandel and Kedar examined the prevalence of the C677T polymorphism in 118 Indian mothers of DS children and 118 control mothers and confirmed the association of *MTHFR* C677T with DS risk (5). The findings of meta-analyses revealed that C677T polymorphism has often been associated with the risk of having DS babies (18). However, Tayeb evaluated *MTHFR* C677T on 70 Saudi females. There was a null association between the C677T polymorphism and the risk of having DS babies (19). Another meta-analysis examined C677T and A1298C polymorphisms in mothers of DS babies and control groups, and it was found that C677T, but not A1298C, had a significant association with mothers with DS babies (20).

Many factors could explain the conflicting results from different studies, including distinct population characteristics (i.e., sample size and ethnic differences). In the present study, 160 Bakhtiari and Fars ethnicity mothers (cases and controls) were examined, while the studies with contrast achievements analyzed populations with different ethnic groups.

In general, the prevalence of C677T alleles was observed to be significantly higher than that of A1298C among mothers with DS children, indicating that maternal *MTHFR* C677T polymorphism is likely to play a crucial role in causing DS. As described earlier, more contradictory reports were released for *MTHFR* A1298C polymorphism.

Halder et al. evaluated C677T and A1298C polymorphisms for their association with meiotic

Table 1. Allele and Genotype Frequencies of the *MTHFR* C677T, A1298C Polymorphisms in the Mothers of Children with DS (n = 80) and Control Mothers (n = 80)

Polymorphism	DS Mothers, No. (%)	Control Mothers, No. (%)	X^2	P-Value	OR (95%CI)
***MTHFR* 677**					
C	120 (75)	143 (89)	11.2921	0.00077	2.8039 (1.5126 to 5.1975)
T	40 (25)	17 (11)			
CC	43 (53.7)	65 (81.2)	14.0645	0.00088	
CT	34 (42.5)	13 (16.2)			
TT	3 (3.8)	2 (2.5)			
CC [a]	43 (53.7)	65 (81.2)	13.7892	0.000205	3.7287 (1.8277 to 7.6069)
CT+TT [a]	37 (46.3)	15 (18.7)			
***MTHFR* 1298**					
A	83 (52)	105 (66)	6.2411	0.01248	1.7711 (1.1292 to 2.7780)
C	77 (48)	55 (34)			
AA	19 (24)	32 (40)	7.0215	0.0298	
AC	45 (56)	41 (51)			
CC	16 (20)	7 (9)			
AA [a]	19 (24)	32 (40)	4.8642	0.02742	2.1404 (1.0821 to 4.2336)
AC+CC [a]	61 (76)	48 (60)			

Abbreviations: OR, odds ratio; CI, confidence interval.
[a] Dominant model.

Table 2. Allele-Allele Interactions of C677T and A1298C in Control Mothers and Those with DS

C677T/A1298C	DS Mothers, No. (%)	Control Mothers, No. (%)	P-Value	OR (95%CI)
TT/AA	3 (3.75)	2 (2.5)	-	-
TT/AC	0 (0)	0 (0)		
TT/CC	0 (0)	0 (0)		
CT/AC	21 (26.25)	13 (16.25)	-	-
CT/CC	0 (0)	0 (0)		
CT/AA	13 (16.25)	0 (0)		
CC/AC	24 (30)	28 (35)	0.000014	0.6207 (0.2011 to 1.9160)
CC/CC	16 (20)	7 (8.75)		
CC/AA	3 (3.75)	30 (37.5)		

errors in oocyte and DS birth in India and analyzed 730 controls and 1019 mothers having DS children. They showed a significant association between *MTHFR* A1298C, but not *MTHFR* C677T variants, and maternal meiosis II nondisjunction in a maternal age-independent manner. These findings from the largest sample population tested ever bring a significant step closer to understanding the relationship between meiotic errors and DS birth (21). Considering the previously described MTHFR enzyme dimer, each polypeptide (monomer) has a catalytic and regulatory domain, harboring the (677) and (1298) positions, respectively. It was proposed that the increased sensitivity to folate intervention for the increase of Hcy in 677TT homozygotes could be clarified by the stabilization of the enzyme dimer (18).

Hcy is an amino acid that is released during folate metabolism, and an enhanced concentration of Hcy, due to the *MTHFR* gene mutation, has been associated with an increased risk for DS, indicating changes in this metabolic pathway. Our results confirmed that the Hcy concentrations were significantly higher in DS mothers. In agreement with our results, several studies reported that the maternal Hcy level was a risk factor in DS mothers (7, 22).

Table 3. Correlation Between Plasma Homocysteine Concentrations (μmol/L) and *MTHFR* C677T, A1298C Genotypes in Control Mothers and Mothers of Children with DS [a]

Genotype	DS Mothers	Control Mothers	P-Value [b]
***MTHFR* 677**			
CC	4.30 ± 0.35	4.24 ± 0.29	0.174016
CT	4.17 ± 0.09	4.25 ± 0.11	0.009691
TT	4.76 ± 0.01	4.02 ± 0.03	0.02234
***MTHFR* 1298**			
AA	4.31 ± 0.36	4.36 ± 0.39	0.323766
AC	4.30 ± 0.29	4.17 ± 0.08	0. 005047
CC	4.12 ± 0.004	4.10 ±0.03	0.015368

[a] Homocysteine data are reported as means ± SD
[b] *t*-test.

On the other hand, the indirect effect of *MTHFR* gene C677T polymorphism on the risk of DS babies, due to changes in blood factors Hcy, 677T, and 1298C polymorphisms, is the strongest predictor of the blood Hcy level. Homozygous people 677TT, 1298CC, and heterozygous 677CT, 1298AC have an increase in the Hcy level in their blood. The obtained results for the genotype analysis of the mothers of DS babies conform to those from the analysis of the Hcy factor. The mutation of *MTHFR* 677 damaged the activity of enzymes involved in folate metabolism to increase the level of Hcy. Similarly, Liew and Gupta indicated that the homozygous mutations of *MTHFR* had higher Hcy levels, while the heterozygous mutations mildly raised Hcy levels compared with the controls (23).

5.1. Conclusions

In this study, we first determined two polymorphic alleles (i.e., *MTHFR* C677T and *MTHFR* A1298C) and Hcy concentrations are maternal risk factors for DS in southwest Iran. These data could be helpful for the prognosis and early diagnosis of pregnant mothers who possibly have children with DS. Finally, these observations suggest that genetic polymorphisms involved in folate metabolism may have population specificity in determining the susceptibility of having DS babies.

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Footnotes

Authors' Contribution: Atefeh Heydari prepared the manuscript and implemented the main part of the laboratory experiments. In addition, Majid Aminzadeh performed the main part of clinical studies on the patients and prepared the manuscript. Moreover, Ali Akbar Momen, Maryam Tahmasebi-Birgani, Reza Azizi Malamiri, Ata A. Ghadiri, and Neda Farajnezhad participated in gathering some parts of the samples and clinical studies in the patients. Finally, Pegah Ghandil conceived and developed the presented idea, supervised the experiments, conducted the analysis, and wrote and supervised the manuscript. All authors read and approved the final manuscript.

Conflict of Interests: I declare the authors have no competing interests as defined or other interests that might be perceived to influence the interpretation of the article.

Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication.

Ethical Approval: Ahvaz Jundishapur University of Medical Sciences (IRAJUMS.REC.1395.516).

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Informed Consent: Informed consent was obtained from case and control groups.

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