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Original Research Article

Polymorphisms in folate-metabolizing genes as risk factors for congenital heart defects in Down syndrome in Egypt

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Abstract

Objectives: Down syndrome (DS) may be associated with congenital heart defects (CHD). Folate-metabolizing genes have been suspected as risk factors in DS and in CHD. We investigated the role of methylenetetrahydrofolate reductase (*MTHFR*) C677T and A1298C polymorphisms and methionine synthase (*MTR*) A2756G polymorphism as risk factors for CHD in DS offspring.

Material and Methods: This study included 116 DS children and their Egyptian mothers. Atrial septal defects, ventricular septal defects and patent ductus arteriosus were the defects considered. Mothers were divided into CHD-DS mothers and normal hearts-DS mothers. The analysis of *MTHFR* C677T and A1298C polymorphisms and *MTR* A2756G polymorphism was performed by PCR-RFLP. Allele/genotype frequencies were determined. Odd ratios and 95% confidence intervals were measured.

Results: The distribution of different genotypes and allele frequencies of the three polymorphisms showed no significant differences between both groups of DS mothers, however, the genotypes of both *MTHFR* 677T and *MTR* 2756A together showed a significant *p*-value (0.035), but with an odd ratio of 0.39 (95% CI: 0.09-1.4).

Conclusion: According to the results, *MTR* A2756G polymorphism and *MTHFR* C677T and A1298C polymorphisms could not be considered as maternal risk factors in Egyptian mothers for CHD in DS offspring. Further studies in different populations are needed.

Keywords: Down syndrome; Congenital heart defects; *MTR* A2756G polymorphism; *MTHFR* C677T and A1298C polymorphisms.

1. Introduction

Congenital heart defects (CHD) are among the leading causes of infant mortality in the whole world; however the etiology is still not fully established [1]. Deficiency of folic acid was suspected as a risk factor for CHD. Researchers investigated the role of polymorphisms in genes which are involved in folate metabolism as potential risk factors or maternal risk factors for CHD. *MTHFR*A1298C and C677T polymorphisms were the commonest studied polymorphisms, but results were contradictory and meta-analyses, still did not solve this controversy [2-8]. In 2013, the frequencies of *MTHFR* 1298AC and CC genotypes were significantly higher in Egyptian mothers with CHD affected children [9].

Down syndrome is one of the most common chromosomal abnormalities. It was observed in 1/733 live births in United States [10], and 1/600 in Egypt [11]. Atrioventricular septal defects were the most common lesions occurring roughly in 1: 5 live births with DS [12, 13]. Folate genes polymorphisms as potential risk factors and maternal risk factors for Down syndrome have been studied [14, 15]. Some reports studied the role of folate-metabolizing genes as potential risk factors and as maternal risk factors for CHD in Down syndrome [16, 17]. The aim of the present study was to evaluate the role of *MTHFR* C677T and A1298C polymorphisms and *MTR* A2756T polymorphism as maternal risk factors for CHD in Egyptian DS offspring.

2. Materials and Methods

2.1 Subjects

This study was carried out over one year 2014. It included 116 Egyptian mothers of cytogenetic proven non-disjunction trisomy 21 offspring. The children with Down syndrome attended the Clinical Genetics out-patient clinic, National Research Centre, and Children's Hospital (Abou-

Elreesh) out-patient clinics, Cairo, Egypt. The age range of children with Down syndrome was 7 months – 3 years. All mothers and their offspring underwent detailed clinical examination. Children with Down syndrome (DS) were subjected to pelvi-abdominal sonographic and echogardiographic examinations to detect any congenital anomaly. DS children with any other congenital anomalies apart from atrial septal defect (ASD), ventricular septal defect (VSD), patent ductus arteriosus (PDA) or any combination of them were not included in the study.

Mothers enrolled were subjected to full history taking with emphasis on suffering from chronic disease or long-term intake of any drug or vitamin. Mothers suffering from any chronic disease or taking any vitamins or drugs were excluded from the study. All enrolled mothers had no periconceptional folic acid. Subjects participated in this study after the importance of the investigation was explained to them and informed consents were obtained. Four ml of peripheral blood were sampled and collected on EDTA from each mother for molecular analysis. The ethical committee of National Research Centre approved this research according to "World Medical Association Declaration of Helsinki".

2.2 Molecular analysis

DNA was extracted from the collected blood according to the standard procedures of Qiagene extraction Kit. Polymorphisms *MTHFR* C677T, A1298C and *MTR* A2756G were analyzed by polymerase chain reaction (PCR) with specific primers and protocols as described by Forsst *et al* [18]. The PCR-amplified fragments were digested with fast digest Hinf1, MboII and HaeIII restriction enzymes (Fermentase) to delineate the *MTHFR*- 677C>T, 1298A>C and *MTR* 2756A>G polymorphisms, respectively. The digested products were analyzed after separation by gel electrophoresis in three per cent agarose gel with ØX174 ladder.

2.3 Statistical analysis

Data was analyzed by IBM-SPSS statistical software version 20. The chi square, chi square for trend and odd ratio (OR) with 95% confidence interval (CI) for quantifying risk were used. Gene counting method was used to measure allele frequencies and Pearson X^2 test was performed to test significance of the results. The frequencies of three genotypes and alleles were represented with 95% CI. The association between either the combined genotypes or haplotypes of the three studied polymorphisms and the risk of having CHD in the DS offspring was evaluated by logistic regression analyses. The interactions between the three polymorphisms were measured by using multivariate logistic regression methods. The statistical tests were two-sided, and $p \leq 0.05$ was considered of statistical significance.

3. Results

A total of 116 mothers of Down syndrome children (MDS) were studied. Each one of them had one child with non-disjunction trisomy 21. Fifty one DS children (43.9 %) had ASD, VSD, PDA or any combinations of these. Statistical analysis revealed that all genotypes, combined genotypes distribution and alleles haplotypes distribution were statistically non-significant (p>0.05) (Tables 1, 2, & 3). The combined genotypes of both *MTHFR* 677T and *MTR* 2756A was an exception, showing a significant *p* value of 0.035, but with an odd ratio of 0.39 (95% CI 0.09-1.4) indicating that this polymorphism has no genetic impact as a risk factor (table 2). Statistical analysis of genotypes, combined genotypes and alleles haplotypes distribution showed no increase maternal risk factor for CHD among DS, as the odd ratios ranged from 0.2 to 1.72 (Tables 1,2, & 3). Table 3 shows an odd ratio of 3.3 (95% 0.6-17.3) with alleles haplotype *MTHFR* 667C, *MTHFR* 1298C and *MTR* 2756G, but the *p*-value was statistically non-significant.

Table 1: Genotype frequencies of *MTHFR* C667T, *MTHFR* A1298C and *MTR* A2756G among mothers of DS children with CHD and those with normal hearts

Genotype	MDS with congenital heart defects (no=51)		MDS with normal hearts (no=65)		P value	Odd Ratio (95% CI)
	N	%	N	%		
C677T						
C/C	21	41.2	21	32.3	0.2403	1
C/T	15	29.4	32	49.2	0.0622	0.47(0.4-2.5)
T/T	15	29.4	12	18.5	0.9814	1.25(0.6-3.7)
T/T and C/T	30	58.8	44	67.7	0.6955	0.68(0.2-2.2)
A1298C						
A/A	16	31.4	25	38.5	0.5522	1
A/C	21	41.2	25	38.5	0.6213	1.36(0.7-4.1)
C/C	14	27.4	15	23.0	0.4952	1.45(0.8-3.6)
C/C and A/C	35	68.6	40	61.5	0.5389	1.37(0.8-2.4)
A2756G (*MTR*)						
A/A	27	52.9	41	63.1	0.3647	1
A/G	21	41.2	21	32.3	0.5009	1.52(0.6-3.7)
G/G	3	5.9	3	4.6	0.5188	1.51(0.5-3.9)
G/G and A/G	24	47.1	24	36.9	0.3227	1.51(0.6-3.8)

* P ≤ 0.05 is significant

Table 2: Combined genotype frequencies of *MTHFR* C667T, *MTHFR* A1298C and *MTR* A2756G among mothers of DS children with CHD and those with normal hearts

Combined Genotype	MDS with congenital heart defects (no=51)		MDS with normal hearts (no=65)		P value	Odd Ratio (95% CI)
	N	%	N	%		
C677T and A1298C						
C/C and A/A	9	17.6	11	16.9	0.918	1
C/C and C/C + A/C	12	23.5	10	15.4	0.267	1.46(0.2-3.6)
T/T + C/T and A/A	7	13.7	14	21.5	0.278	0.6(0.09-1.2)
T/T + C/T and C/C + A/C	23	45.1	30	46.1	0.910	0.94(0.4-2.6)
C677T and A2756G (*MTR*)						
C/C and A/A	16	31.4	15	23.1	0.316	1
C/C and G/G + A/G	5	9.8	6	9.2	0.917	0.78(0.2-1.8)
T/T + C/T and A/A	11	21.6	26	40	0.035*	0.39(0.09-1.4)
T/T + C/T and G/G + A/G	19	37.3	18	27.7	0.273	0.99(0.2-1.9)
A1298C and A2756G (*MTR*)						
A/A and A/A	13	25.5	17	26.2	0.935	1
A/A and G/G + A/G	3	5.9	8	12.3	0.241	0.49(0.3-1.4)
C/C + A/C and A/A	14	27.5	24	36.9	0.281	0.76(0.6-4.2)
C/C + A/C and G/G + A/G	21	41.2	16	24.6	0.057	1.72(1.01-3.8)
C677T and A1298C and A2756G (*MTR*)						
C/C and A/A and A/A	8	15.7	7	10.8	0.433	1
C/C and A/A and G/G + A/G	1	2	4	6.2	0.270	0.2(0.01-0.89)
C/C and C/C + A/C and A/A	8	15.7	8	12.3	0.600	0.8(0.2-1.6)
C/C and C/C + A/C and G/G + A/G	4	7.8	2	3	0.250	1.75(0.5-4.6)
T/T + C/T and A/A and A/A	5	9.8	10	15.4	0.374	0.4(0.2-3.6)
T/T + C/T and A/A and G/G + A/G	2	3.9	4	6.2	0.590	0.4(0.1-3.5)
T/T + C/T and C/C + A/C and A/A	6	11.8	16	24.6	0.080	0.3(0.09-2.1)
T/T + C/T and C/C + A/C and G/G + A/G	17	33.3	14	21.5	0.154	1.06(0.8-6.2)

P ≤ 0.05 is significant

Table 3: Haplotype frequencies of the three studied polymorphisms (*MTHFR* C667T, *MTHFR* A1298C and *MTR* A2756G) among mothers of DS children with CHD and those with normal hearts

Haplotype	MDS with congenital heart defects (no=102)		MDS with normal hearts (no=130)		P value	Odd Ratio (95% CI)
	N	%	N	%		
$C_{677} - A_{1298} - A_{2756}$	37	36.3	52	40	0.562	0.8(0.5-1.5)
$T_{677} - C_{1298} - G_{2756}$	16	15.7	16	12.3	0.458	1.3(0.6-2.7)
$T_{677} - C_{1298} - A_{2756}$	14	13.7	22	16.9	0.504	0.8(0.4-1.6)
$T_{677} - A_{1298} - G_{2756}$	5	4.8	5	3.8	0.694	1.3(0.4-4.6)
$T_{677} - A_{1298} - A_{2756}$	11	10.8	14	10.8	0.997	1(0.4-2.3)
$C_{677} - C_{1298} - G_{2756}$	5	4.8	2	1.5	0.137	3.3(0.6-17.3)
$C_{677} - C_{1298} - A_{2756}$	13	12.7	15	11.5	0.779	1.1(0.5-2.5)
$C_{677} - A_{1298} - G_{2756}$	1	0.9	4	3.1	0.205	0.3(0.02-2.4)

* P ≤ 0.05 is significant

4. Discussion

Congenital heart defects are present in 40-60% of individuals with Down syndrome, with ASD, VSD and PDA being the most common lesions in the studied DS populations [19-22]. The present report detected CHD in 43.9% of enrolled DS cases. A population based study by Bean *et al*. [23] reported that lack of maternal supplementation with folic acid was associated with increased atrioventricular septal defects in their offspring having Down syndrome. Few studies investigated the role of maternal folate-metabolizing genes as risk factors for Down syndrome having congenital heart defects, but the results were controversial. Brandalize *et al*. [16] and Coppede [24] suggested that maternal *MTHFR* 677T allele may be associated with increased CHD in Down syndrome children. They suggested periconceptional folic acid supplementation in mothers with this polymorphism. The present study did not detect similar results. Ethnic variability may have a role, however periconceptional folic acid is a good recommendation. In Croatia, Vraneković *et al*. [14] and Bozovic *et al*. [25] studied the role of allele/genotype or genotype combinations of *MTHFR* C677T and A1298C polymorphisms as maternal risk factors for DS-related CHD. Both studies showed no evidence to support the possibility that *MTHFR* polymorphisms were linked with risk of having a DS child suffering from CHD in the Croatian population. These findings are in concordance with our results. The conflicting results found in the different populations may also reflect the interactions present between genetic and environmental factors involved in folate metabolism. Risk factors may depend on the effect of different genetic polymorphisms, and/or on the gene-environment interaction.

In conclusion, our results suggested that *MTR* A2756G polymorphism and *MTHFR* C677T and A1298C polymorphisms were not associated with Egyptian maternal risk factors for congenital heart defects in Down syndrome offspring. Further studies in different populations are needed and periconceptional folic acid supplement could be recommended.

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