GENETIC POLYMORPHISM OF METHYLENETETRAHYDROFOLATE REDUCTASE AS A RISK FACTOR FOR CONGENITAL HEART DEFECT IN ROMANIAN CHILDREN

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BACKGROUND

Congenital heart defects (CHD) are the most common congenital abnormalities - 30% of the total abnormalities. Low folate intake as well as alterations in folate metabolism as a result of polymorphisms in the enzyme methylenetetrahydrofolate reductase (MTHFR) have been associated with an increased incidence of neural tube defects, vascular disease, CHD. Within the MTHFR gene, several single nucleotide polymorphisms (SNPs) have been described.

The MTHFR gene encodes for the enzyme that catalyses the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Two SNPs in MTHFR, 677C→T and 1793G→A, are associated with decreased enzyme activity.

Case-control study: to determine whether the C677T and G1793A variants in the MTHFR gene is associated with CHD susceptibility.

METHODS

DNA samples were isolated from peripheral blood samples and genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), with the digestion of restriction endonuclease.

Genotyping of SNP C677T in the MTHFR gene

Primers used: 5'-CATCCCTATTGGCAG GTTAC-3' (forward) and 5'-GACGGTGCGGTGAG AGTG-3' (reverse). PCR amplicons were digested with restriction enzyme HinfI. The wild type homozygote (CC), heterozygote (CT) and mutant homozygote (TT) showed one band (265 bp), three bands (265, 171 and 94 bp) and two bands (171 and 94 bp), respectively.

Genotyping of SNP G1793A in the MTHFR gene

For the PCR reactions we used the following primer pairs: forward primer 5‘ - GGGACAGGAGTGGCTCCAACGCAGG-3' and reverse primer 5'-CTCTGTGTGTGTGTGCATGTGT GCC-3‘. The amplified fragment of 310 bp was digested with BsrbI endonuclease. The G1793A mutation abolishes a BsrbI restriction site. Digestion of the 310-bp fragment of the 1793 GG genotype gives two fragments of 233 and 77 bp, whereas the 1793AA genotype results in a single fragment of 310 bp. The MTHFR 1793GA heterozygotes demonstrated 3 DNA bands (310, 233 and77 bp).

The restricted products were analyzed on 2% agarose gel.

RESULTS AND DISCUSSION

We evaluated for the first time the possibility of an association between the G1793A SNP and C677T SNP in the MTHFR gene and CHD in a Romanian population group.

Subjects: 20 patients with CHD and 20 healthy controls respectively.

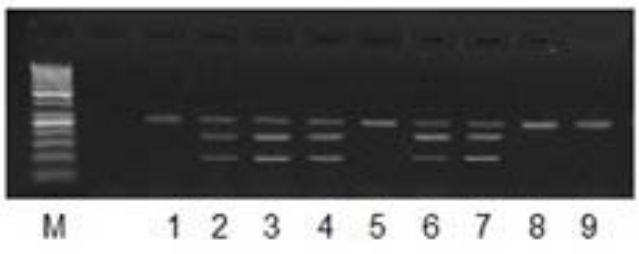

Figure 1. DNA electrophoresis of MTHFR C677T on agarose gel: M - DNA ladder 50 bp; 1, 5, 8, 9 - homozygotes CC; 2- 4, 6, 7 - heterozygotes CT;

	Patients group (n= 20)	Control group (n= 20)	P value	OR (95% CI)
MTHFR C677T				
Genotypes				
CC	9 (45%)	15 (75%)	P = 0.052 (TT+CT vs CC)	OR=3.66 (0.95-14.02)
CT	9 (45%)	4 (20%)		
TT	2 (10%)	1 (5%)		
Alleles				
C	27 (67.5%)	34 (85%)	P=0.056 (T vs. C)	OR=2.72 (0.91-8.12)
T	13 (32.5%)	6 (15%)		
MTHFR G1793A				
Genotypes				
GG	15 (75%)	18 (90%)	P=0.20 (AA+GA vs. GG)	OR=3 (0.50-17.74)
GA	5 (25%)	2 (10%)		
AA	0	0		
Alleles				
A	5 (12.5%)	2 (5%)	P=0.21 (A vs. G)	OR=2.71 (0.49-14.90)
G	35 (87.5%)	38 (95%)		

In patients, the genotypes frequencies of the MTHFR G1793A polymorphism were 25% and 75% for the GA and GG genotype respectively, whereas the genotypes frequencies of the MTHFR C677T polymorphism were 45%, 45% and 10% for CC, CT and TT genotypes.

The MTHFR 677 T allele was more common in the CHD group than in control group (p=0.056), but there are no significant differences. It was associated with a high probability of association of CHD – odds ratio [TT+CT vs CC] = 3.66; 95% CI: 0.95-14.02, p= 0.052).

The MTHFR 1793 A allele was more common in the CHD group than in control group (p=0.21), but there are no significant differences. It was associated with a high probability of association of CHD – odds ratio [AA+GA vs GG] = 3; 95% CI: 0.50-17.74, p= 0.20).

CONCLUSIONS

The MTHFR C677T and G1793A polymorphism may influence CHD, but the MTHFR polymorphisms need to be studied further for confirmation in larger studies.

The study was realized in the research project MAMI no 41-042/2007 financed by the Romanian Ministry of Education, Research and Youth.