



## MTHFR, prothrombin and Factor V gene variants in Turkish patients with coronary artery stenosis

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### Abstract

Many epidemiological studies have reported an association between hemostatic factors and risk of both coronary and peripheral artery diseases. Using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) analysis, we investigated the association between coronary artery disease and polymorphisms in the methylenetetrahydrofolate reductase (*MTHFR* C677T and A1298C), prothrombin (G20210A), and factor V (A4070G) genes. We screened these gene variants in 174 subjects who had undergone coronary angiography - 115 patients with patent coronary artery disease (grade 3 vessel disease, *i.e.*, significant coronary stenosis), and 59 healthy controls with grade 0 vessel disease. The analysis of our data did not show any statistically significant association between coronary artery disease (CAD) and the investigated polymorphisms.

**Key words:** genetic polymorphism, coronary disease, *MTHFR* gene, prothrombin gene, Factor V gene.

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Many epidemiological studies have reported an association between hemostatic factors and risk of both coronary and peripheral artery diseases. The blood coagulation system is thought to be involved in the pathogenesis of atherosclerotic diseases, and inherited prothrombotic risk factors are supposed to be predisposing to thrombus formation and vascular occlusions (Folsom, 2001). We screened patients with coronary artery disease (CAD) for polymorphisms in the *MTHFR* (C677T and A1298C), Prothrombin (G20210A), and Factor V (A4070G; Leiden) genes, because they were suggested to be probable inherited risk factors for CAD (Alhenc-Gelas *et al.*, 1999; Redondo *et al.*, 1999; Varela *et al.*, 2001).

This case-control study involved 174 individuals who had undergone coronary angiography. The patient group included 115 subjects with grade 3 vessel disease, *i.e.*, significant coronary stenosis, and the control group was formed by 59 subjects with grade 0 vessel disease. The distributions of patients and controls according to gender, family history of coronary disease, and comorbidities are

shown in Table 1. No statistically significant associations between gender or traditional risk factors and the presence of coronary artery stenosis were detected, except in relation to hypercholesterolemia.

Genomic DNA was extracted from venous blood samples by the standard phenol-chloroform method. Polymorphisms in the candidate genes were investigated by polymerase chain reaction followed by restriction fragment

**Table 1** - Comparison between patients with coronary disease and controls.

	Patients (n = 115)	Controls (n = 59)	p
Gender			
Male	86 (74.8%)	37 (62.7%)	0.595
Female	29 (25.2%)	22 (37.3%)	0.143
Mean age ± SD	56 ± 12	54 ± 16	0.729
Family history	38 (33%)	20 (33.9%)	0.832
Smoking	46 (40%)	18 (30.5%)	0.219
Diabetes mellitus	27 (23.5%)	10 (16%)	0.319
Hypercholesterolemia	46 (40%)	14 (23.7%)	0.033*
Hypertension	52 (45%)	25 (42.4%)	0.721

**Table 2** - Allele and genotype distributions of *MTHFR*, Prothrombin and Factor V gene variants in patients with coronary stenosis and controls.

Variants investigated	Genotypes and alleles	Patients n = 115	Controls n = 49	OR	95% CI	p
Factor V A4070G	AA	108 (93.9%)	53 (91.4%)	1.0	0.893-1.082	0.724
	AG	6 (5.2%)	5 (8.6%)	0.8	0.244-2.668	
	GG	1 (0.9%)	0			
	A	0.966	0.957			
	G	0.044	0.053			
Prothrombin G20210A	GG	114 (99.1%)	56 (96.6%)			
	AG	0	2 (3.4%)			
	AA	1 (0.9%)	0			
	G	0.991	0.983			
	A	0.009	0.017			
MTHFR C677T	CC	56 (48.7%)	33 (55.9%)	1.0	0.795-1.399	0.715
	CT	46 (40.0%)	24(40.7)	1.129	0.587-2.174	
	TT	13 (11.3%)	2 (3.4%)	3.83	0.813-18.04	
	C	0.687	0.76			
	T	0.313	0.24			
MTHFR A1298C	AA	50 (43.5%)	21(35.6%)	1.0	0.676-1.081	0.497
	AT	58 (50.4%)	31 (52.5%)	0.78	0.402-1.537	
	TT	7 (6.1%)	7 (11.9%)	0.42	0.131-1,347	
	A	0.687	0.618			
	T	0.313	0,381			
MTHFR C677T	CC	56 (48.7%)	339 (55.9%)	1.0	0.795-1.399	0.715
	CT	46 (40.0%)	24 (40.7%)	1.129	0.587-2.174	
	TT	13 (11.3%)	2 (3.4%)	3.83	0.813-18.04	
	C	0.687	0.76			
	T	0.313	0.24			
MTHFR C677T and A1298C	677T and 1298C	25 (21.7%)	13 (22.03%)	0.98	0.460-2.099	0.555

length polymorphism analysis (PCR-RFLP) as follows: C677T (Frosst *et al.*, 1995) and A1298C (van der Put *et al.*, 1998) of the *MTHFR* gene, G20210A of the prothrombin gene (Poort *et al.*, 1996), and A4070G of the Factor V-Leiden gene (Alhenc-Gelas *et al.*, 1999). No differences were found between patients and controls in terms of genotype and allele frequencies in relation to the four genetic polymorphisms studied (Table 2).

Our results are consistent with those of Yilmaz *et al.* (2006), who did not find an association between *MTHFR* C677T and increased risk for CAD in Turkish patients either. In contrast to these results, two other studies showed a significant increase in the frequency of the *MTHFR* C677T polymorphism among patients with CAD (Almawi *et al.*, 2004, in a sample from the Bahrain population; Ghazouni *et al.*, 2008, in Tunisians). However, concerning the *MTHFR* A1298C polymorphism, Ghazouni *et al.* (2008) did not find a significant difference between patients and controls, in line with the present study.

The carrier frequency of the factor V-Leiden (G1691A) and prothrombin G20210A gene polymor-

phisms did not differ between patients and controls in the study of Almawi *et al.* (2004), in accordance with our results. Similarly, Baykan *et al.* (2001) did not find an increased frequency of factor V-Leiden in patients presenting with CAD either.

In conclusion, although the results concerning the association of the *MTHFR* C677T polymorphism with CAD are still controversial, the factor V-Leiden and prothrombin G20210A polymorphisms do not seem to confer an increased risk for CAD in the different populations studied.

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