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CLINICAL INVESTIGATION

Prothrombin 20210A Allele May Not Be an Independent Risk Factor for Myocardial Infarction

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Abstract: The G->A transition at position 20210 in the prothrombin gene is a genetic risk factor for venous thrombosis. Nevertheless, it is not clear if the prothrombin 20210A allele is also a genetic risk factor for arterial thrombosis.

We studied 124 patients with myocardial infarction (MI) and 182 healthy individuals to assess the possible correlation between prothrombin gene polymorphism and MI. In addition, we analyzed 42 MI patients, who were selected as having no other secondary risk factors and who thus have a potential genetic predisposition to infarction.

The frequencies of heterozygous carriers of the 20210A polymorphism of the gene (% GA genotype) were calculated as follows: among the 124 patients with MI 3.23%, 42 selected patients 9.53% and among the 46 selected controls 4.35%. The prothrombin 20210A allele was detected by allele specific polymerase chain reaction.

We found no correlation between the prothrombin 20210A allele and MI. This study supports the view of that prothrombin 20210A polymorphism is not an independent risk factor for MI.

Key Words: Prothrombin 20210A, Myocardial infarction, Heart, Turkey

Introduction

Thrombotic tendency increases the risk of myocardial infarction (MI) in some individuals, especially in young adults. Local thrombotic occlusion in coronary arteries plays a pathogenetic role at the site of a ruptured plaque (1). There are several risk factors known to be causative in the development of MI, such as hyperlipidemia, hypertension, diabetes mellitus, obesity and cigarette smoking. Additionally, there are some genetic markers whose roles are still poorly understood (2).

Prothrombin (factor II) is the final effector of the clotting cascade that leads to the formation of fibrin. Prothrombin is the precursor of the serin protease thrombin which plays a central role in the control of blood coagulation by exerting both procoagulant and anticoagulant effects (3). The prothrombin gene is localized on chromosome 11 near the centromere, spans approximately 21000 bp, and is composed of 14 exons and 13 introns. The cDNA encodes a preproleader sequence similar to that found in other vitamin K dependent proteins (4).

Recently, a genetic polymorphism located in the 3'-untranslated region of the prothrombin gene was described. The polymorphism is due to a single base pair substitution at position 20210 of a guanine (G) for an adenine (A) nucleotide. As reported previously (5) the prothrombin 20210 A allele is present in all white subpopulations. It is thought to be more prevalent in Mediterranean countries, especially in Israel (5.4%) and Italy (4.5%) (5). In Turkey, the incidence (percentage) of the 20210A allele has been reported at 2.7% (6). Blood prothrombin levels in people with the GG genotype are 1.05 U/ml, compared with 1.32 U/ml in heterozygous carriers of the 20210A allele. An elevated prothrombin level in blood alone is a risk factor for venous thrombosis (1,2,3,7,8,9,10) which indicates a likelihood that the prothrombin 20210A allele acts through elevated plasma prothrombin levels (5,11).

Previous research in to the correlation between prothrombin 20210A polymorphism and MI has been controversial. Some researchers reported 20210A polymorphism to be a risk factor for MI (12,14,15),

while others did not observe a positive correlation (1,8,13). In the present study, we examined the prevalence of the recently described 20210 G->A substitution in the prothrombin gene in 42 men and women with MI and 46 healthy control subjects who were carefully chosen in order to exclude other secondary risk factors except for smoking (19% of the MI patients and 17% of the healthy controls were smokers). Additionally, we analyzed 124 patients with MI having secondary risk factors.

Materials and Methods

Study and Control Subjects

The study included 124 unselected and 42 selected patients with MI admitted to İstanbul University Hospital. It was approved by the institutional review committee and informed consent was given by subjects. The diagnosis of MI was based on chest symptoms, typical electrocardiographic changes and serum enzymes. The study also included 46 control subjects, chosen from healthy individuals. Patients and control subjects were selected on the basis of having had no other known secondary risk factors for MI as determined by the National Cholesterol Education Program (NCEP) such as hypertension, hypercholesterolemia, diabetes mellitus or old age.

Coronary Risk Factors

Hypertension was defined by a history of several blood pressure measurements elevated either systolically (> 140 mmHg) or diastolically (> 90 mmHg). Diabetes mellitus was defined by elevated blood glucose levels after fasting (> 7.8 mmol/l-140 mg/dl) or 2 h after 75 g of oral glucose loading (> 11.1 mmol/l-200 mg/dl). Hypercholesterolemia was defined by elevated total serum cholesterol levels (> 200 mg/dl).

Laboratory Analysis

20210G->A polymorphism of the prothrombin gene was determined from DNA extracted from peripheral blood using the ammonium acetate method. We used allele specific amplification to discriminate the prothrombin allele. Two external primers (1 and 2) and 2 internal forward (nested) primers (3 and 4) were used. Primer 1 was a forward primer, 5'TCCGCCTGAAGAAGAGTGGATA3' (nt 20064 to 20085); primer 2 was a reverse primer, 5'GAGTGCTCGGACTACCAGCGTGC3' (nt 20333 to 20311); primer 3 was a forward primer, 5'TTCCCAATAAAAGTGACTCTCAGCA3' (nt 20186 to 20210) and primer 4 was a forward primer 5'TTCCCAATAAAAGTGACTCTCAGCG3' (nt 20186 to 20210). The 2 internal primers, 3 and 4, only differred in the last nucleotide (specific to G->A substitution). The external primers, 1 and 2, amplified the 270 bp DNA fragment. Primers 3–2 and 4–2 amplified the148 bp fragment. For every subject, 2 tubes were used; the first tube with primers 1, 2 and 3 and the second tube with primers 1, 2, 4 (Figure 1). Amplification was carried out in 25 µl containing 100 ng of genomic DNA, 1.5 mmol/l of $MgCl_2$, 750 mM Tris-HCl (pH 8.8 at 25 °C), 200 mM

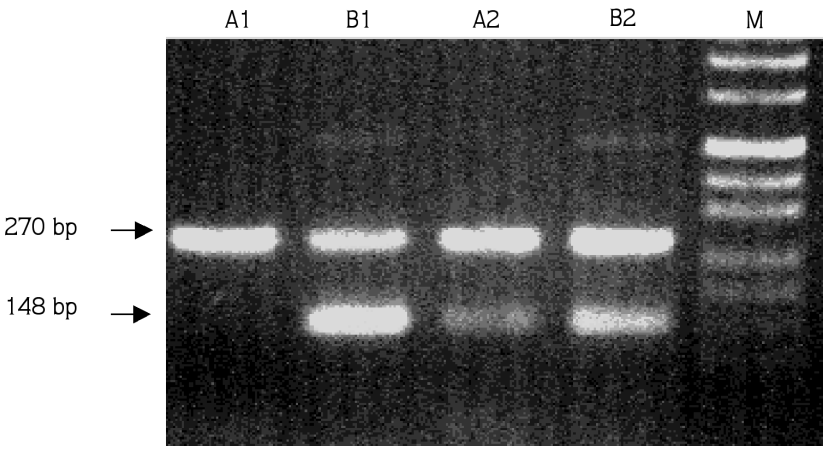

Figure 1. Detection of prothrombin 20210A polymorphism by allele specific polymerase chain reaction. A_1B_1: Homozygote GG, A_2B_2: Heterozygote GA, M: Molecular Weight Marker (Puc Mix 8).

$(NH_4)_2SO_4$, 0.1% Tween 20, 2 mM dNTPs, 400 nmol of each primer and 1 unit of Taq polymerase. A first denaturation step at 94 °C for 4 min was followed by 36 cycles of 94 °C for 45 s, 62 °C for 1 min and 72 °C for 1 min (16). Finaly,15 µl of each PCR product was loaded onto 2% agarose gel.

Statistical Analysis

The frequencies of the alleles and genotypes were compared among patients and the control groups using Fisher's exact test when appropriate. The genotype frequencies in control subjects, patients and the overall study groups – as determined using Fisher's exact test – were in accordance to the distribution predicted by the Hardy-Weinberg equilibrium model. SPSS for Windows 10.0 and Microsoft Excel were used for statistical analysis.

Results

Prothrombin 20210A polymorphism was examined in a subgroup of Turkish patients angiographically proven to have MI. We first examined 124 MI patients for the presence of the GG and/or GA genotype. We determined that the patients with MI had a frequency 96.77% GG genotype and a 3.23% frequency GA genotype ($P > 0.005$; $X^2 = 0.808$) (Table 1). As published previously, the GA genotype frequency was determined to be 2.7% in Turkey (6).

We further analyzed the data and selected 42 patients having no other secondary risk factors (Table 2), but one or more of whose family members had a previous history of MI and compared these with 46 no-risk controls. Of the selected patients 90.47% were GG and 9.53% were GA genotypes and of the in selected controls 95.65% were GG and 4.35% GA genotypes (Table 1) ($P > 0.005$; $X^2 = 0,926$).

In conclusion, we did not observe any statistically significant increase in the frequency of the prothrombin 20210 GA genotype in either the unselected and selected patient groups with MI, compared with healthy individuals.

Discussion

Prothrombin gene polymorphism is characterized by the single base pair substitution of guanine for an adenine nucleotide at position 20210. The prothrombin level of A allele carriers is higher than that of non-carriers (14). The higher level of prothrombin causes more growth of fibrin clots and may plug the blood vessels. Therefore, this mutated allele had been described as a moderate risk factor for venous thrombosis with a frequency level of 0.7-4% in healthy individuals from different countries (13). When the gene was investigated for arterial thrombosis, controversial results emerged: some groups reported a positive correlation between this mutation and MI (12,14,15), whereas others did not (1,8,13).

Our main goal in this study was to clarify the question of whether there is a correlation between prothrombin gene polymorphism (nt20210A) and the MI in a subgroup of the Turkish populations.

Table 1. Statistically, there is no difference in the distribution of the prothrombin GG and GA genotypes between patients with MI and the healthy controls.
Prevalence of the prothrombin gene 20210 G A variant among MI patients and controls.

		Genotype GG	GA	p	x^2
Unselected MI patients	n = 124	120	4	0.531	0.808
	%	96.77	3.23		
Turkish Population*	n = 182	177	5		
	%	96.3	2.7		
Selected patients group	n = 42	38	4	0.336	0.926
	%	90.47	9.53		
Control group	n = 46	44	2		
	%	95.65	4.35		

* Reference 6
$P > 0.05$

Table 2. Selected patients and healthy controls were matched with cholesterol, diabetes, ldl, hdl, triglyceride, bmi and gender. Family history and age were not matched.
Major cardiovascular risk factors in selected patients and controls.

Risk Factors	Patients (n: 42)	Controls (n: 46)	P
Cholesterol (mg/dl)	168.69 ± 24.05	165.74 ± 24.12	0.25
HDL (mg/dl)	43.40 ± 9.69	47.22 ± 10.44	0.675
LDL (mg/dl)	103.31 ± 19.45	87.57 ± 24.06	0.217
Glucose (mg/dl)	93.31 ± 6.56	86.96 ± 10.21	0.033
Triglyceride (mg/dl)	122 ± 40.72	119.54 ± 45.52	0.526
BMI	24.5 ± 2.23	24.4 ± 2.28	0.165
Age	38.58 ± 11.3	49.82 ± 17.04	
Female/Male	36.78 ± 13.17	61.28 ± 12.37	0.082
MI history			
Positive % (n)	66.7 (28)	6.52 (3)	
Negative % (n)	3.33 (14)	93.48 (43)	P < 0.05
Gender			
Female % (n)	40.48 (17)	41.31 (19)	
Male % (n)	59.52 (25)	58.69 (27)	0.937

Although our findings are consistent with some of the previously published results, they differ significantly from a few others. For example Doggen et al. found that prothrombin 20210A allele carriership was associated with a 50% increase in risk of MI in 560 men aged < 70 years (14). Another group reported that the prothrombin 20210A allele increased the risk of MI in young women, especially in those with other major risk factors for coronary heart disease (15). In our study, there were 124 unselected patients with MI and 42 selected MI patients 46 control cases and most of them were male. This could be one of the reasons for the discrepancy among the several groups.

Interestingly, there have been some reports that showed a synergistic effect of several thrombogenetic risk factors such as FV G1691A, prothrombin G20210A and MTHFR 677C->T which increase the risk of MI 9-fold (18). We are currently in the process of exploring of a possible additive correlation of several molecular markers as being genetic inducers for MI.

In conclusion, it appears that there was no correlation between prothrombin 20210A mutation and MI in the patient group we examined. After this initial study, we plan to continue analyzing the synergistic effects of the suggested genetic risk factors by increasing the number of patients examined.

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