Clinical and Molecular Study of Common Thrombophilia Mutation Prothrombin G20210A

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Abstract

Background: One of the most common genetic causes associated with thrombophilia is mutation G20210A of the coagulation factor II (*F2*) gene.

Materials and methods: Data collected from 355 unrelated Greeks examined for the mutation G20210A over a period of two decades were anonymously analyzed.

Results: The statistical analysis confirmed the importance of *F2* G20210A in thrombosis and the significance of a positive family history of thrombosis. An interesting finding was the increased prevalence of G20210A in men with thrombotic events aged >40 years.

Conclusions: This study highlighted the great value of a positive family history of thrombosis and the importance of testing for this common mutation as a putative prevention strategy and a future biomarker for thrombophilia.

Keywords

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1 Introduction

Thrombophilia (OMIM 188050) is a multifactorial tendency for thrombosis due to inherited blood hypercoagulation. The spectrum of thrombotic events includes brain stroke, myocardial infarction, pulmonary embolism, deep vein thrombosis, and about 60% spontaneous pregnancy miscarriages; therefore, thrombophilia has a high rate of morbidity and mortality in the general population [1–5]. Thrombophilia is mainly associated with variants in genes of coagulation factors, which are functioning alone, or in association with other genetic and environmental factors [2–6]. The Global Burden of Disease Study 2010 reported that thrombotic disorders were responsible for 1 in 4 deaths worldwide and are thus a leading cause of mortality [5–8].

Genetic causes are present in approximately one-third of unselected thrombosis cases and up to two-thirds of familial cases [5, 9]. One genetic factor most commonly associated with thrombophilia in Europeans is the G20210A mutation in the 3′ untranslated region of gene *F2* encoding coagulation factor II (FII) [5, 10, 11]. FII, also known as prothrombin, is a plasma glycoprotein which is activated to thrombin by FXa and FVa. The G20210A mutation causes alternative polyadenylation of the resultant mRNA transcript, which is more efficiently translated producing higher plasma FII levels amplifying the risk of venous thrombosis [10, 12, 13]. The allelic frequency of this mutation is 1.3–4.5% in Caucasian populations [5, 10, 11, 14].

Based on a plurality of evidence, the above genetic defect has a dominant predisposition effect [10, 12, 13]. Heterozygotes with the *F2* G20210A mutation have a two- to fourfold higher risk of venous thrombotic incidents compared with individuals without these mutations, and homozygotes have a 50- to 100-fold increased risk compared to individuals with respectively normal genotypes [4, 11].

Although thrombotic diseases present a significant morbidity and mortality burden in adults, they are mostly preventable. Personalized preventive medicine based on genetic counseling seems to be the solution [13, 15]. Prevention in thrombosis may include lifestyle choices (healthier diet, more exercise, less anxiety, no smoking or heavy drinking, etc.), anti-embolism stockings, pneumatic devices, and prophylactic pharmacological treatments.

Herein we present the analysis of existing data of 20 years of thrombophilia screening for the *F2* G20210A mutation in a sample of the Greek population. The findings of this analysis may be useful for better understanding of thrombophilia, future research, and, eventually, prevention and treatment of thrombosis.

2 Material and Methods

2.1 Subjects

Data regarding demographic characteristics, health status, and molecular testing of a Greek population sample were anonymously analyzed in this study. The protocol of the study was approved by a University Department Ethics Committee (27022019) in accordance with the standards of the 1964 Declaration of Helsinki.

The studied cohort included 355 unrelated Greeks who were genetically examined within two decades for a common thrombophilia-associated mutation, namely, *F2* G20210A, in two diagnostic centers of Athens under the supervision of Prof. C. Yapijakis. DNA tests were performed at the Department of Molecular Genetics of Bioerevna Center from 1999 to 2008, and the Department of Molecular Genetics of Cephalogenetics Center from 2008 to 2017. The molecular methodology used in the two diagnostic centers was the same, involving restriction analysis of PCR products [13, 16], and results are considered reliable since they had been randomly verified by DNA sequencing. All individuals had

given their informed consent signing a form in order to be tested.

Data collected for analysis from each individual anonymously were nationality (all were Greeks), gender, age, results of DNA testing *F2* G20210A mutation, and health status. Regarding the health status, some individuals had presented with a thrombotic event at the time of DNA testing, while for others information regarding their health was collected until 2018. A family history of thrombosis in 3–4 generations was known for 233 individuals out of the whole cohort of 355 people (65.6%). For the remaining 122 individuals, no information regarding their family history was available.

In addition, a group of 2243 blood donors of Greek origin was tested for *F2* G20210A mutation at the Department of Molecular Genetics of Cephalogenetics Center, using the same molecular methodology of PCR product restriction analysis [15, 16].

2.2 Molecular Analysis

DNA was extracted from blood or saliva samples with the use of NucleonSpin™ kit (Macherey-Nagel GmbH & Co, Dfiren, Germany). Molecular analysis was done using a combination of polymerase chain reaction, incubation with a restriction enzyme, and agarose gel electrophoretic analysis. The thrombophilia-associated G20210A in the gene of coagulation factor II abolishes a sequence recognized by Taq I; therefore it may readily be distinguished from the normal allele that retains the enzyme recognition sequence [10, 16].

2.3 Statistical Analysis

Observed *F2* genotypes in the whole cohort of studied individuals (n = 355) as well as in those with positive family history of thrombosis in (n = 233) were first examined for Hardy-Weinberg equilibrium at the level of importance of 0.05, in order to assess whether the studied cohort was genuinely representative of the general population, and, therefore, further statistical analysis would be legitimate. Demographic, health, and genetic data were furthermore analyzed with Fisher's exact test by comparing health status and genotypes in the whole cohort and in several subgroups. In order to analyze small samples, Fisher's exact test assesses the null hypothesis of independence applying hypergeometric distribution of the numbers in the cells of stratified 2 × 2 and 2 × 4 tables [17]. We performed statistical analysis using 2 × 2 tables in the whole cohort and in individuals with family history of thrombosis by comparison of numbers of patients and healthy individuals according to observed genotypes in subgroups regarding gender (males or females) and age (0–40 or 41–82). The age division at 40/41 years was selected because the average age of the whole cohort of subjects was 41 and the median age was 39.

3 Results

A random sample of the population of Greece including 2243 blood donors was tested for *F2* G20210A mutation and a prevalence of 3% (135/4486 chromosomes) was detected. The observed frequencies of the mutation are within the range previously reported in Greeks and other European populations (1.5–4.5%) [14].

Furthermore, a cohort of 355 subjects of Greek origin (120 males and 235 females), who had been examined for the common thrombophilia associated G20210A mutation over a period of twenty years, was analyzed. Regarding their health status, 278 of them were healthy and 77 were patients who presented with at least one thrombotic incident until 2018. DNA testing was performed in the patients because they had one or more of thrombosis-related conditions: heart attack, coronary artery disease, phlebitis, vascular stroke, pulmonary thrombosis, first trimester miscarriage, brain aneurysm with thrombotic complications, and a couple of cases of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. Reasons for DNA testing of healthy individuals included a positive family history with thrombosis, a prena-

tal examination, or a routine checkup. In the total selected cohort, the prevalence G20210A mutation was 39/355 (11%), increased multiple times compared to the prevalence in the general population, as expected.

In about two-thirds of all subjects (n = 233, 101 males and 132 females), a positive family history of thrombosis was collected. The subgroup of people with a positive family history included 159 healthy individuals and 74 patients. In the family thrombosis subgroup, the prevalence of G20210A mutation was 33/233 (14.2%). Interestingly, the prevalence of the subgroup with a positive family history was comparable with the abovementioned prevalence of the total cohort for the mutation. It appears that people with unknown family history to us had their reasons to ask for a checkup genetic test for thrombophilia. Possibly, at least for some of them, thrombotic events had occurred in their family.

In the whole cohort and in the subgroup with a family history of thrombosis, the observed and expected genotype frequency did not differ significantly for the selected gene (Table 1). Consequently, the populations under study were in Hardy-Weinberg equilibrium for these variants; they were representative of the general population and further analysis was valid.

Statistical analysis was performed in the whole cohort by comparing genotypes in subgroups regarding health status, gender, and age (Table 2). Significant differences were observed between men aged 41–82 for G20210A mutation (p = 0.02).

In a similar manner, statistical comparison of genotypes in subgroups regarding health status, gender, and age was also performed in the group with positive family history with thrombosis (Table 3). Genotypes of patients and healthy individuals were significantly different in the subgroups of men aged 41–82 (p = 0.02).

4 Discussion

Thrombophilia poses a huge direct burden to the general population, as it is linked with high morbidity and mortality rates [1, 5]. Major genetic causes of thrombophilia include G20210A mutation in the gene *F2*, displaying a dominant predisposition effect. Despite a wealth of research data regarding this mutation for almost three decades, a deeper understanding of thrombophilia causing mechanisms is still needed and prevention of thrombosis in the at-risk fraction of the general population is still lacking [18].

Table 1 Analysis of Hardy-Weinberg equilibrium comparison of genotypes in the whole cohort and in individuals with positive family history to thrombosis

FII genotype frequencies in the whole cohort						
	Genotype		Observed		Expected	p-value[a]
Heterozygotes		29	(8.2%)	34	(9.6%)	
G20210A$^+$/G20210A$^-$						
Normal	Homozygotes	324	(91.2%)	320	(90.1%)	0.1636
G20210A$^-$/G20210A$^-$						
Mutant	Homozygotes	2	(0.6%)	1	(0.3%)	
G20210A$^+$/G20210A$^+$						
FII genotype frequencies in individuals with positive family history to thrombosis						
	Genotype		Observed		Expected	p-value[a]
Heterozygotes		22	(9.4%)	26	(11.2%)	
G20210A$^+$/G20210A$^-$						
Normal	Homozygotes	209	(89.7%)	206	(88.4%)	0.1473
G20210A$^-$/G20210A$^-$						
Mutant	Homozygotes	2	(0.9%)	1	(0.4%)	
G20210A$^+$/ G20210A$^+$						

[a]Two-tailed Fisher's exact test

Table 2 Statistical comparison of prevalence of G20210A in healthy individuals and patients from the whole cohort

The whole cohort									
		Male 0–40					Female 0–40		
	G20210A$^+$	G20210A$^-$	*p*-value	Phi		G20210A$^+$	G20210A$^-$	*p*-value	Phi
Patients	1	7	0.6	−0.04	Patients	4	15	0.15	−0.12
Healthy	3	29			Healthy	13	117		
		Male 41–82					Female 41–82		
	G20210A$^+$	G20210A$^-$	*p*-value	Phi		G20210A$^+$	G20210A$^-$	*p*-value	Phi
Patients	7	24	**0.02**	−0.29	Patients	4	14	0.12	−0.17
Healthy	1	47			Healthy	6	62		

Phi is the coefficient of association. Significant *p*-values are shown in bold

Table 3 Statistical comparison of prevalence of G20210A in healthy individuals and patients from the subgroup with positive family history to thrombosis

Individuals with positive family history to thrombosis									
		Male 0–40					Female 0–40		
	G20210A$^+$	G20210A$^-$	*p*-value	Phi		G20210A$^+$	G20210A$^-$	*p*-value	Phi
Patients	1	7	1	−0.07	Patients	4	15	0.49	−0.04
Healthy	2	24			Healthy	9	42		
		Male 41–82					Female 41–82		
	G20210A$^+$	G20210A$^-$	*p*-value	Phi		G20210A$^+$	G20210A$^-$	*p*-value	Phi
Patients	7	24	**0.02**	−0.3	Patients	3	13	0.68	−0.07
Healthy	1	35			Healthy	6	40		

Phi is the coefficient of association. Significant *p*-values are shown in bold

In this study, we statistically analyzed data (gender, age, health status, and DNA testing results) obtained anonymously from a cohort of 355 unrelated Greeks, who were genetically investigated for the common abovementioned mutation. This mutation was detected in significantly higher prevalence in the selected cohort than that of a larger sample of 2243 people from the general Greek population. Most of the tested individuals of the selected cohort were referred to us or they alone asked for DNA testing for thrombophilia because they either had a positive family history or a thrombotic condition.

This study has confirmed and expanded on the previously observed key role of *F2* G20210A in thrombotic events in men [19, 20] and the importance of positive family history of thrombosis in the pathogenesis of thrombophilia [21, 22]. In addition, there was an observed tendency of the G20210A mutation to be significant in men of >40 years, confirming a previous observation [23]. A positive family history of thrombosis seems to be important in male patients with brain aneurysm, as we previously observed in another study [24]. There are reports of cases of thrombophilia-associated mutation found in patients with deep vein thrombosis, intracranial aneurysm, and a positive family history of thrombotic incidents [25]. This study highlighted the great value of testing for the *F2* G20210A common mutation epecially in cases with positive family history as a prevention strategy for thrombophilia.

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