Prevalence of thrombophilic gene polymorphisms in an Azari population of Iran

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Abstract

There is several evidence suggests that thrombophilic gene polymorphisms may influence susceptibility to thromboembolic events. The prevalence of these polymorphisms is different in various races and ethnics. Accordingly, we studied the prevalence of Factor V (G1691A and A4070G), prothrombin G20210A and PAI-1 4G/5G in healthy northwest population of Iran. In this prospective study, 500 healthy individuals, who had no history of both personal and family history of thromboembolic disorders, were selected as a sample of healthy population in northwestern Iran. Genotyping of these polymorphisms was performed using the amplification refractory mutation system-polymerase chain reaction method. No significant differences were detected between the expected and observed frequencies of FV G1691A and A4070G, prothrombin G20210A polymorphisms (P>0.05), while the expected frequency of 4G allele was significantly more than observed frequency in the studied population (P<0.01). These findings were compared with other reports from various populations. In conclusion, the allele frequency for FV G1691A and PAI-1 4G/5G polymorphisms showed relative consistency compared to those of previous studies, while the incidence pattern of FV A4070G polymorphism in Northwestern population of Iran showed conflicting results regarding other studied population. The prothrombin G20210A polymorphism was observed at a higher frequency than other studied populations.

Introduction

Functional polymorphisms in genes involved in hemostasis are effective in increasing the risk of thrombotic events. However, the occurrence probability of a single polymorphism is highly variable, even in families' members with a history of thrombosis. This variation in different arrangements of polymorphisms in several genes may increase the risk of thrombosis via reinforcing one another. The FV G1691A polymorphism causes a substitution of arginine by glutamine at codon 506, resulting in reduce its sensitivity to inactivation by activated protein C due to loss of 506 cleavage site. Therefore, it leads to increase thrombin production raising the risk for venous thrombosis.[1]

The A4070G polymorphism of the FV gene causes amino acid replacement of histidine for arginine at the codon 1299. This polymorphism which marked by the HR2 haplotype affects plasma level of FV and contribute to the activated protein C (APC) resistance.[2] Double heterozygous individuals for FV G1691A and FV A4070G have a high risk of thromboembolism with respect to FV G1691A alone.[3] The prothrombin G20210A polymorphism is a single G to A nucleotide transition in the 3' untranslated region of the prothrombin gene resulting in change in stability of prothrombin mRNA and elevated plasma prothrombin levels. Therefore, it disturbs the delicate equilibrium between coagulation cascade and fibrinolysis and increase the risk for thrombosis 3- to 4-fold.[4]

Plasminogen activator inhibitor-1 (PAI-1) is the principle inhibitor of tissue plasminogen activator (tPA) and urokinase (uPA) and therefore inhibits the fibrinolysis process. Elevation of PAI-1 serum level could be lead to a thrombotic tendency. The PAI-1 (-675 I/D, 5G/4G) polymorphism affects the binding of nuclear proteins involved in the regulation of *PAI-1* gene transcription. The 4G allele appears to bind only an enhancer, whereas the 5G allele binds both an enhancer and a suppressor. So, the 4G allele is associated with higher rates of PAI-1 synthesis and thromboembolism.[5]

Previous studies have shown that the prevalence of FV G1691A, FV HR2 (4070A/G), prothrombin G20210A and PAI-1 (-675 I/D, 5G/4G) polymorphisms is different in various races and ethnics (populations). Hence, this study aims to evaluation of these polymorphisms in an Azari population of Iran to provide the rate of these polymorphisms.

Materials and Methods

Subjects

Regarding the outbreak of G1691A polymorphism with relatively high rate (5.5%) in central part of Iran,[6] we investigated five hundred individuals (275 males and 225 females) with the ages averaged between 46.27±5.82 years (ranging between 36 and 59) from northwest states population of Iran, including East Azarbaijan, West Azarbaijan and Ardebil. Excluded from this study were individuals with both personal and family history of thromboembolic disorders. All samples were collected following the informed consent of the participants and this study has been approved by the ethical committee of Tabriz University of Medical Sciences, Tabriz, Iran.

DNA extraction and genotyping

Peripheral blood samples were taken from all participants and genomic DNA was extracted from leukocytes using GIAamp DNA Blood Mini kit (Qiagen, Germantown, MD USA), according to the manufacturer's instruction. Genotyping was carried out using amplification refractory mutation System-polymerase chain reaction (ARMS-PCR) technique. Amplification of DNA was performed using three primers for each polymorphism, one forward primer and two reverse primers specific for the wild type and mutant alleles (Table 1). PCR was done using 100 ng of genomic DNA in a 25 μL PCR reaction, which contained 2.5 μL PCR buffer 1X, 10 pmol of each primers, 10 nmol each deoxyribonucleotide triphosphates, 1.5 mM Mg^{2+} and 1U *Taq* polymerase. The PCR conditions consisted of an initial denaturation step (96°C, 2 min) was followed by 10 cycles of denaturation (96°C, 15 s) and annealing/extension (65°C, 60 s), followed by a final 20 cycles of denaturation (96°C, 10 s), annealing (62°C, 50 s), and extension (72°C, 30 s).[7] The PCR products were separated on 2% agarose gel and visualized with ethidium bromide.

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Statistical analysis

We used SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA) for statistical analysis. The expected allele frequencies were computed by Hardy Weinberg Equilibrium. The chi-square (χ^2) tests were used to evaluate the differences between observed and expected allele frequencies.

Results

Our results showed that the frequencies of mutant allele for FV G1691A, FV HR2 (4070A/G), prothrombin G20210A and PAI-1 (-675 I/D, 5G/4G) polymorphisms were 2.1, 2.8, 3.0 and 20.3% respectively (Table 2). When the expected and observed frequencies of mutant allele were compared, we found no statistically significant differences for FV 1691A, FV 4070G and prothrombin 20210A alleles (P>0.05). However, the observed frequency of 4G allele in the studied population was significantly higher than its expected frequency (P<0.01) (Table 2).

Discussion and Conclusions

The frequency of FV 1691A allele is more common (1.0 to 8.5%) among European people compared to that in African, Chinese, Japanese, and North/South America.[1,4,8,9] FV G1691A polymorphism is also relatively common in healthy individuals of European population where the average reported allelic frequency is 2.7%.[10]

Among Arab ethnic, the highest allelic frequencies are related to people of Lebanese (7.88%), Tanzania, (3.5%), Bahrain (1.5%), Saudi Arabia (1%), Egypt (0.09%) and north of India (1.9%),[11-14] respectively (Figure 1).

Considering previous studies, it is concluded that the homozygosity for HR2 haplotype is less than 0.5% in different populations.[2,3] This is completely consistent with our results in the present study. The study of simultaneous occurrence of FV G1691A and A4070G polymorphisms showed that both polymorphisms are not on a common allele, it is therefore expected that such polymorphisms have a high risk of thrombosis with respect to FV G1691A polymorphism alone.

In support of this case, it has been found that the resistance of activated C factor is far more in individuals with double polymorphisms in FV G1691A and FV A4070G, compared to the presence of any of these mutations alone.[3] A4070G polymorphism pattern in haplotype HR2 shows conflicting results in the population of Azerbaijan when compared with other populations. Turkey's population with frequency of 4.2% shows an almost identical pattern in this case, while in European popula-

Table 1. Nucleotide sequence of primers used for genotype screening by amplification refractory mutation system-polymerase chain reaction method.

Alleles	Sense primer 5'→3'	Antisense primer 5'→3'	PCR product (bp)
Factor V (1691G)	CAGATCCCTGGACAGGCG	ATCACACTCTAGACTTGCCTTCGG	249
Factor V (1691A)	CAGATCCCTGGACAGGCA	ATCACACTCTAGACTTGCCTTCGG	249
Factor V HR2 (4070A)	ACCTCTCTCCAGAACTCAGCCATAT	GAAGTCTAGAGAAAGGGTTGTAT	125
Factor V HR2 (4070G)	ACCTCTCTCCAGAACTCAGCCATAT	GTCTAGAGAAAGGGTTGTAC	122
Prothrombin (20210G)	AATAAAAGTGACTCTCAGCG	TACCAGCGTGCACCAGGTG	131
Prothrombin (20210A)	CAATAAAAGTGACTCTCAGCA	TACCAGCGTGCACCAGGTG	132
PAI-1 (-675 insertion, 5G)	CACTGCTCCACAGAATCTATCGG	CTGACTCCCCCACGT	356
PAI-1 (-675 deletion, 4G)	CACTGCTCCACAGAATCTATCGG	GCTGACTCCCCACG	355

Table 2. Expected and observed frequencies of Factor V (G1691A and A4070G), Prothrombin G20210A and PAI-1 4G/5G polymorphisms among an Azari population living in northwest of Iran.

	Observed (%)	Expected (%)*	χ^2	Degrees of freedom	P
FV 1691 G/A					
GG	95.8	95.84			
GA	4.2	4.11	0.23	1	0.901
AA	0.00	0.04			
Frequency of A allele	2.1	4.19			
FV HR2 4070 A/G					
AA	96.4	94.47			
AG	3.6	5.44	0.41	1	0.798
GG	0	0.07			
Frequency of G allele	2.8	5.58			
F II 20210 G/A					
GG	94	94.09			
GA	6	5.82	0.48	1	0.789
AA	0	0.09			
Frequency of A allele	3	5.91			
PAI-1 (-675 I/D, 5G/4G)					
5G/5G	70.6	63.52			
5G/4G	18.2	32.35	95.72	1	0.000
4G/4G	11.2	4.12			
Frequency of 4G allele	20.3	40.59			

*The expected genotype frequencies were calculated using Hardy Weinberg Equilibrium

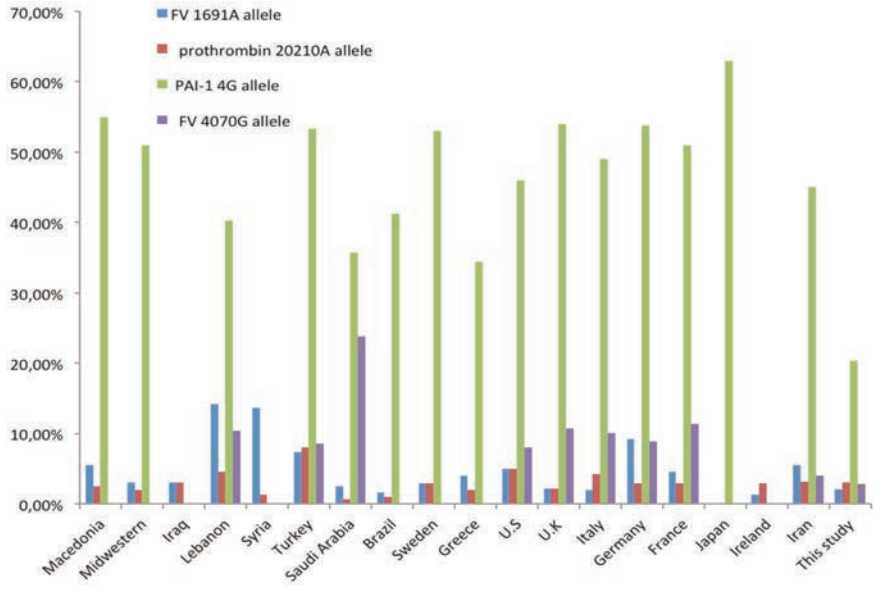

Figure 1. Comparison of the prevalence of FV (1691A and 4070G), prothrombin 20210A and PAI-1 4G allele polymorphisms among different populations.

tion the corresponding frequencies are completely different (10.4%) and sometimes often quite similar (5.8%).2,10,14,15 The frequency of A4070G mutation for HR2 haplotype of coagulation factor V has been reported 6.2, 8.0 and 10% in European-Asian population of Australia, India and Somalia, respectively.[3,10,15] Mari *et al.* have reported different patterns of polymorphism in the haplotype HR2 with frequency of zero percent in Han population, China.[15] Another study conducted in the Arab population resulted in a much higher prevalence rate than those observed in the present study (16.5%) (Figure 1).[6,13] Italian and Greek population has the highest prevalence of G20210A mutations of prothrombin gene, while the black population shows very low allele frequencies. It is reported the allele frequency of 1.0 to 1.9% for European population, such as Ireland, Netherland, England, Australia and Croatia.[9,10,13] Brazil, Korea and Japan population lack this mutation.[1,4, 9,13]

Based upon various reports, the frequency of this polymorphism has been found 0.5 to 1.5% in different parts of Iran, which is higher than that reported for East Asia (0.5%), India (0.5%) and Arabic countries, such as Lebanon (0.7%), Tunisia (1.2%), Bahrain (0.5 to 1%), Saudi Arabia (0 to 0.2%). Also, according to the available reports, the Jews of Iran with an allele frequency rate of 0.01% shows a lower frequency of the mutations, compared to Iraqi Jews (0.02%) (Figure 1). Although there are many research case-control studies in the literature, however it has been counted as a main risk factor in different countries. So, it is necessary to study on the frequency rate of PAI-1 (4G/5G) polymorphism in healthy population of different countries. A relatively similar pattern has been reported on high frequency of this kind of polymorphism in several studies in which healthy individuals are used as the control group. Meanwhile in most studies, the observed frequency is higher than expected rate from Hardy Weinberg law.[5,13] The *PAI-1* gene 4G/5G polymorphic variations of 50.5% have been observed in population of Italy in a case-control study.[13] Such polymorphism frequency is also considerably high in East Asian population such as China (48.2%).[5]

In the present study, the frequency rate of PAI-1 (4G/5G) polymorphism showed no significant difference between northwestern people of Iran and a population of Italy or China (P>0.05) (Figure 1).

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