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Full Length Research Paper

Detection of FXIII gene V34L and fibrinogen β-gene -455G/A polymorphisms among Saudi Arabia population via polymerase chain reaction-reverse hybridization technique

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FXIII gene Val34Leu variant appears to be associated with decreased risk of myocardial infarction and venous thromboembolism as well as with increased risk of intracerebral hemorrhage. Fibrinogen β-gene SNP -455G/A are associated with differences in the plasma levels of fibrinogen and severity of arterial disease. The aim of the present work was to study the prevalence of FXIII gene V34L and Fibrinogen β-gene -455G/A SNPs in Saudi population. Among 200 blood samples randomly collected from unrelated healthy Saudi subjects, FXIII gene V34L and Fibrinogen β-gene -455G/A SNPs were genotyped via cardiovascular disease (CVD) StripAssay (ViennaLab, Austria. Homozygous (V/V) and heterozygous (V/L) genotypes were detected with 96 and 4%, respectively, among FXIII gene V34L genotypes, whereas (L/L) genotype was not found. The allele frequency was 0.98 for V allele and 0.02 for L allele. Three genotypes of Fibrinogen β-gene -455G/A SNP (GG, GA and AA) were obtained and its prevalence (%) was 70, 25 and 5, respectively. The frequency of G allele was 0.825 and 0.175 for A allele. Prevalence of FXIII gene Vl34L polymorphism and its allele frequency are in line with other Asian populations. Distribution of β-gene -455G/A genotypes and allele frequency are in accordance with previous reports in different ethnic groups. This is the first time to report these polymorphisms in Saudi Arabia population. This study provides valuable information on Saudi genetic background in comparison with other populations. In addition, it serves as a template for future clinical research involving cardiovascular and cerebrovascular diseases.

Key words: FXIII gene V34L, fibrinogen β-gene -455G/A, polymorphisms, Saudi Arabia.

INTRODUCTION

Cardiovascular disease (CVD) is one of the main causes of illness and death in both developed and developing countries (Lin et al., 1998; Angeline et al., 2005). CVD is responsible for about 30% of all deaths worldwide, and is projected to cause 24 million deaths by 2020 (Mathers et al., 2006). A number of genetic and environmental risk factors have been found or suspected to predispose to cardiovascular disease (CVD). Genetic susceptibility of CVD may be caused by mutations and polymorphisms in a variety of genes mainly involved in blood coagulation, regulation of blood pressure, and metabolism of lipids, glucose, homocysteine or iron. Among the genetic factors are, single nucleotide polymorphisms (SNPs) in the genes for blood coagulation factors V (FV), II (prothrombin), and XIII (FXIII), ß-fibrinogen (FGB), platelet glycoprotein IIIa (GPIIIa), plasminogen activator inhibitor-1 (PAI-1), 5,10- methylenetetrahydrofolate reductase (MTHFR), angiotensin-converting enzyme (ACE), as well as apolipoproteins B (Apo B) and E (Apo E) resulting in CVD.

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Coagulation factor XIII (FXIII) play an important role in clot stabilization by crosslinking fibrin chains during the final steps of the blood coagulation (Greenberg et al., 1991; Gemmati et al., 2001). It is a tetrameric structure consisting of two A (active) and two B subunits. More than 70 FXIIIA or B subunit gene mutations have been identified. The FXIII gene has several common single nucleotide sequence variations, which encode amino acid substitutions. Five common coding polymorphisms have been identified in the FXIII-A subunit: Val34Leu, Tyr204Phe, Pro564Leu, Val650Ile and Glu651Gln. The Val34Leu variant is the most studied polymorphism, with the amino acid substitution occurring in the activation peptide sequence, three amino acids upstream from the thrombin-cleavage site (Hsieh and Nugent, 2008). Several studies were conducted to evaluate the role of FXIII mutations in cardiovascular and cerebrovascular diseases.

The (FXIII Val34Leu) polymorphism in exon 2 of the coagulation factor XIII A- subunit gene was reported (Mikkola et al., 1994). This genetic variation results in increased transglutaminase activity (Anwar et al., 1999) and paradoxically appears to be associated with decreased risk of myocardial infarction (Kohler et al., 1998, McCormack et al., 1998, Franco et al., 2000) and venous thromboembolism (Catto et al., 1999) and with increased risk of intracerebral hemorrhage (Catto et al., 1998). The mutant allele of FXIII Val34Leu seems to be linked to increased activation of FXIII and enhanced fibrin cross-linking (Anwar et al., 1999; Kohler et al., 1998).

An elevated plasma fibrinogen level has been established to be an independent predictor of coronary artery disease, stroke and peripheral vascular disease in Whites (Maresca et al., 1999; Kain et al., 2002) There is evidence that up to 51% of the variation in fibrinogen levels may be due to genetic factors, and a relation between the Bβ Arg448Lys, β-455G/A and AαThr312Ala polymorphisms and fibrinogen level has been reported (Behague et al., 1996, Kain et al., 2002). Variation of the β-fibrinogen gene (β-455G/A polymorphisms) was associated with the severity of arterial disease (Scarabin et al., 1993; Behague et al., 1996; Martiskainen et al., 2011).

Several studies have been conducted to estimate the prevalence of different cardiovascular disease related polymorphisms among different populations (Castro et al., 2000). Different molecular genetic techniques are adopted to detect these SNPs such as PCR-RFLP or allele-specific amplification techniques (Kirschbaum and Foster, 1995; Poort et al., 1996; Hezard et al., 1998; Angeline et al., 2005). SNPs can also be detected by post amplification techniques which are based on allele-specific oligonucleotide probes and in which the use of biotin labeled probes is required (Mahfouz et al., 2006; Zaatari et al., 2006; Sabbagh et al., 2009; Argyri et al., 2010; Awad and El-Tarras, 2012). The prevalence of these SNPs varies depending on the geographical location and the ethnic background as well as genetic makeup of the population (Franco et al., 1998; Cho et al., 2002). However, in general, limited data are available on the prevalence of these polymorphisms among Asian populations (Cho et al., 2002) as well as Arabian populations. So, it should encourage the search for distribution of this polymorphism inside Asia and among Arab populations. To our knowledge, there has been no information on the prevalence of these polymorphisms in Saudi Arabia until now and this is the first report on this polymorphism in Saudi Arabia population. The aim of this study was to report the prevalence of the FXIII gene-V34L and Fibrinogen β-gene -455G/A SNPs in the healthy Saudi population residing at Taif governorate which belongs to western region in Kingdom of Saudi Arabia.

MATERIALS AND METHODS

Samples collection

In the present study, two hundred unrelated healthy and non-smoking Saudi subjects were recruited. The recruited subjects are residing in Taif city, 5600 feet above sea level and from the western region in Kingdom of Saudi Arabia. The study sample consisted of 120 males (60%) and 80 females (40%). The age of subjects ranged from 55 to 60 years. Whole blood samples were collected into EDTA-anticoagulated vacutainer tube. Non Saudi subjects as well as cardiovascular diseases were excluded from the study. No thrombotic events were identified. Verbal consent was obtained from all participants prior to blood samples collection and all institutional requirements were met.

DNA extraction, PCR amplification and reverse hybridization

The protocol of CVD StripAssay (ViennaLab, Austria) (http:// www.viennalab.com) was used according to the manufacturer's instruction to genotype the FXIII gene-V34L and Fibrinogen β-gene -455G/A SNPs. CVD StripAssay protocol includes three steps: 1) DNA extraction 2) PCR amplification using biotinylated primer pairs and 3) hybridization of amplified products with test strips carrying allele-specific oligonucleotide probes immobilized in parallel lines. Bound biotinylated sequences are detected by streptavidin – alkaline phosphatase and color substrates. Briefly, PCR amplifications were carried out in two separate reactions A (amplification mix A) and B (amplification mix B) that differ in primers pairs and 5 µl DNA template for each reaction. PCR reactions A and B were carried out with the same thermal profile as follows: Initial step of 94°C for 2 min and followed by 35 cycles of 94°C for 15 s, 58°C for 30 sand 72°C for 30 s, final extension was at 72°C for 3 min. PCR products from reaction A and B were mixed together with hybridization buffer, incubated for 5 min at room temperature and hybridized to the detection test strip. Hybridization was accomplished at 45°C. After series of stringent washes (according to the protocol of provider), the reaction was detected by color development directly on test strip. Results were evaluated from test strips using provided scale included in the kit (Mahfouz et al., 2006; Zaatari et al., 2006; Sabbagh et al., 2009; Argyri et al., 2010; Awad and El-Tarras, 2012).

RESULTS

Two hundred Saudi subjects were recruited in the

Table 1. Genotype and allele frequencies for Val34Leu and β-gene - 455G/A polymorphisms.

Polymorphism	Genotypes (%)			Allele frequency	
FXIII Val34Leu	**GG (V/V)**	**GT (V/L)**	**TT (L/L)**	**V (G)**	**L (T)**
	192 (96%)	8 (4%)	0	0.98	0.02
β-gene -455G/A	**GG**	**GA**	**AA**	**G**	**A**
	140 (70%)	50 (25%)	10 (5%)	0.825	0.175

Table 2. Distribution of genotypes and allele frequency of the FXIII Val34Leu polymorphism among different ethnic groups.

Ethnic group	Number of individuals	Genotypes (%)			Allele frequency		Reference
		GG (V/V)	GT (V/L)	TT (L/L)	V (G)	L (T)	
Spain	236	65.2	31.8	2.9	0.81	0.19	Corral et al., 2001
Italy	240	57.9	35.8	6.2	0.76	0.24	Gemmati et al., 2001
Australian Caucasian	150	51.3	42.7	6	0.73	0.27	Kangsadalampai and Board, 1998
United kingdom	252	48.8	42.5	8.7	0.7	0.3	Maresca et al., 1999
Brazilians	49	53	32.7	14.3	0.69	0.306	Castro et al., 2000
Portuguese	48	58.3	35.4	6.25	0.76	0.24	Castro et al., 2000
Zaire	33	60.6	30.3	9.1	0.757	0.243	Castro et al., 2000
Cameron	34	76.5	23.5	0	0.88	0.118	Castro et al., 2000
Angola	32	71.9	18.7	9.4	0.812	0.188	Castro et al., 2000
Japanese	40	97.5	2.5	0	0.987	0.013	Castro et al., 2000
Peru	52	48.1	44.2	7.7	0.7	0.298	Castro et al., 2000
Lebanese	205	74.2	22.4	3.4	0.86	0.14	Mahfouz et al., 2008
Saudi Arabia	200	96	4	0	0.98	0.02	Present study

present study. FXIII Val34Leu and β-gene -455G/A polymorphisms were genotyped via CVD StripAssay. According to the principles of this assay, one of three possible staining patterns may be obtained for each subjects as follows, 1) wild type probe only: normal genotype, 2) wild type and mutant probe: heterozygous genotype carrier and 3) mutant type probe only: homozygous mutant type. Obtained results are summarized in Table 1 and illustrate the genotype prevalence and allele frequency for FXIII Val34Leu and β-gene -455G/A. Based on previously published reports, Tables 2 and 3 shows the distribution of genotypes and allele frequency of the FXIII Val34Leu and β-gene -455G/A polymorphisms among different ethnic groups. Our results summarized in Table 1 and indicate that only two types of FXIII Val34Leu polymorphism (GG and GT) were obtained whereas, TT genotype was absent. The prevalence (%) of GG (|V/V) genotype was 96 and 4 for GT (V/L) genotype. The V allele frequency was 0.98 and L allele frequency was 0.02. Genotyping of β-gene -455G/A revealed three genotypes (GG, GA and AA). The genotype distribution was 70, 25 and 5% for GG, GA and AA genotypes, respectively. The G allele frequency was 0.825, while it was 0.175 for A allele.

DISCUSSION

The FXIII Val34Leu polymorphism was originally described by Mikkola et al. (1994) in a Finnish population with a allele frequency of 0.23. Several studies among different population were followed to estimate the allele frequencies of FXIII Val34Leu (Suzuki et al., 1996, Kohler et al., 1998; Castro et al., 2000; Renner et al., 2000). There was significant ethnic heterogeneity linked to FXIII Val34Leu polymorphism (Castro et al., 2000).

Our data showed complete absence of homozygous (L/L) genotype in Saudi studied sample and relatively low frequency of heterozygous (V/L) carriers (4%) with Lucien allele frequency of 0.02. These results are in line and concordance with previous reported results illustrated in Table 2. In Asia, studies showed that FXIII Val34Leu was absent in Korean population (Cho et al., 2002) or had a very low frequency (0.013) as well as complete absence of homozygous (L/L) genotype among Japanese population (Kangsadalampai and Board, 1998; Castro et al., 2000). Among Arab populations, only one study was conducted with Lebanese population and itreported that, the allele frequency of Lucien allele was 0.14 (Mahfouz et al., 2008). While in Turkish population, the frequency of V

Table 3. Genotypes distribution and allele frequency of the FGB -455 G/A polymorphism among different races.

Race	Number of individuals	Genotypes (%)			Allele frequency		Reference
		GG	GA	AA	G	A	
South Asians of United Kingdom	100	66	26	8	0.79	0.21	Kain et al., 2002
Whites of United Kingdom	100	56	37	5	0.745	0.235	Kain et al., 2002
Chinese Han population	503	64.6	32.2	3.2	0.8	0.2	Xiang-feng et al., 2008
Chinese	402	55.9	39.3	4.8	0.75	0.245	Liu et al., 2002
Egypt	17	35.3	29.4	35.3	0.5	0.5	Hamdy 2011
Finland	249	69.9	24.9	5.2	0.82	0.18	Martiskainen et al., 2011
Lebanese	160	60.6	31.9	7.5	0.77	0.23	Shammaa et al., 2008
Korean	267	70.4	25.8	3.7	0.83	0.17	Lee et al., 2008
Greek	121	69	29	2	0.83	0.17	Xenophontos et al., 2002
Saudi Arabia	200	70	25	5	0.825	0.175	Present study

and L alleles were 0.805 and 0.195, respectively (Hancer et al., 2005). Another report indicated that the Leu 34 allele has a high prevalence in Brazilian and Portuguese Caucasians (allele frequencies of 0.27 and 0.240, respectively). The data from the present study confirmed and extended the previous findings by demonstrating a lower prevalence of FXIII Val34Leu in Asian and Arab population. This difference in allele frequency of FXIII Val34Leu polymorphism might be due to ethnic heterogeneity and/or geographic regions (Castro et al., 2000; Cho et al., 2002; Franco et al., 1998; Pepe et al., 1997). Contrary to this explanation, Amerindians showed high prevalence of the leucine allele polymorphism (0.29). It cannot be explained by racial admixture, since the Amerindian tribes here investigated exhibit minimal degree of racial admixture. Thus, the finding of an allele frequency of 0.293 in Amerindians is an indication that FXIII Val34Leu may be present in other Asian populations (Castro et al., 2000). Worldwide, there are heterogeneous distribution of venous and arterial thrombosis in different ethnic groups and geographic regions. The risk of the venous and arterial thrombosis may vary in different human populations as a result of different combinations of genetic and environmental risk factor. Variation of the prevalence of FXIII Val34Leu polymorphism might be a contribution of this differential thrombotic risk (Castro et al., 2000).

The G/A variability in the -455 locus of the B β-fibrinogen (FGB) promoter region have previously been shown to be associated with elevated fibrinogen levels and a risk of cardiovascular diseases and stroke (Green et al., 2001; Martiskainen et al., 2003). The genotype distribution and allele frequency of β-gene -455G/A among studied Saudi population was in agreement with previous studies with different populations illustrated in Table 3 such as in Greek Cypriot population (Xenophontos et al., 2002), Korean population (Lee et al., 2008), Japanese population (Nishiuma et al., 1998), Chinese population (Liu et al., 2002), Caucasian population (Lee et al., 2008) and Finnish population (Martiskainen et al., 2011). Only one Egyptian study reported that allele frequency was relatively high (0.5), this might be due to small recruited sample size (17 individual) (Hamdy et al., (2011). The obtained results showed distribution of genotypes and allele frequency which is similar to previous reports from South Asians in the United Kingdom (Kain et al., 2002) and Arab Lebanese population (Shammaa et al., 2008). As compared to other ethnic groups, the Saudi carriers of the A allele of the FGB-455 G/A polymorphism may be predisposed to development of severe coronary artery stenosis (Martiskainen et al., 2011). The homozygous for -455A allele might lead to the development of coronary artery disease, stroke and peripheral vascular disease which might be due to higher plasma fibrinogen level than individuals with homozygous for -455G allele (de Maat et al., 1997).

Conclusion

This is the first report from Saudi Arabia population on the prevalence of FXIII gene V34L and Fibrinogen β-gene -455G/A SNPs. The obtained results suggested that the prevalence of FXIII gene Vl34L polymorphism was as in other south Asian populations. The prevalence and allele frequencies of Fibrinogen β-gene -455G/A SNP are in accordance with previous reports in different ethnic groups in different parts of the world. This report will prospectively serve as a baseline statistical data for future investigations of the prevalence of Factor XIII V34L and Fibrinogen β-gene -455G/A mutations in association with various clinical entities, notably cardiovascular diseases.

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