

## Prevalence of factor XIII VAL34LEU polymorphism in the Iranian healthy females (West Azerbaijan Province, Iran)

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### Abstract

**Background & Aims:** Factor XIII controls hemostatic, bleeding and thrombotic state. Inherited Factor XIII deficiency leads to an increased thrombotic tendency. Present study investigates the factor XIII gene Val34Leu mutation in the Iranian Turkish healthy females.

**Materials and Methods:** Totally 100 unrelated healthy Turkish females entered the study. 3-5 ml whole blood was collected in tube containing EDTA. Salting out method was used for isolation of genomic DNA from blood samples. Dde I based RFLP-PCR was performed to determine the factor XIII gene Val34Leu mutation in tested samples.

**Results:** Our results showed that frequencies of G (Val) and T (Leu) alleles, and also, G/G (Val/Val), G/T (Val/Leu) and T/T (Leu/Leu) genotypes of factor XIII were 165(84.18%) and 31(15.82%), and also, 67(68.37%), 31(31.63%) and 0(0%) in the tested group. Factor XIII gene Val34Leu genotypes showed an excellent fit to Hardy-Weinberg equilibrium in tested population ( $\chi^2 = 3.45 < 3.84$ , P-value (with degree of freedom=2) = 0.177 > 0.05). In this study, factor XIII G (Val) and T (Leu) allele frequencies were 0.84 and 0.16, respectively.

**Conclusion:** Our findings suggested that Factor XIII Val34Leu mutation has a very low prevalence in the tested population. These results from the Iranian Turkish females are the first official report and could be considered as control group for studies relating the prevalence of factor XIII gene Val34Leu mutation with human primary hemostatic, spontaneous bleeding and thrombotic disorders.

**Key words:** factor XIII, Iranian, healthy females

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### Introduction

Factor XIII as transglutaminase controls human primary hemostatic, bleeding and thrombotic state. More than 60 mutations were identified in factor XIII gene (1). A large body of studies indicated that coagulation factor XIII gene mutations have been associated with human disorders such as myocardial infarction (2-5), coronary artery disease (5-7), thromboembolism (8-11), cerebrovascular disease (12), retinal artery occlusion (13), recurrent miscarriage (14), cardiovascular disease (15), aneurysmal subarachnoid hemorrhage (16). Presence a mutation in exon 2 of

factor XIII gene makes a transition of G to T nucleotide resulting substitution of Val to Leu at amino acid 34 (17). It has been demonstrated that factor XIII has wide range of normal functions that have never been clearly explained and factor XIII gene Val34Leu mutation in the A subunit leads to a significant increased level of transglutaminase activity (17). The frequency of factor XIII sequence variations and kind of these mutations are dissimilar in different populations (17). The identification of factor XIII gene Val34Leu mutation is very helpful in target genetic consulting in a population. Present study was designed to evaluate the frequency of

factor XIII gene mutation (Val34Leu) in the Iranian Azeri Turkish healthy females.

## Materials and Methods

Committee on Human Research approved the study in Urmia University of Medical Sciences. Totally 100 unrelated healthy Turkish females were randomly selected among individuals referred to the Department of Genetics at Motahari Hospital (Urmia, West Azerbaijan, Iran). The participants were females with an average age of 29 years (range, 18 to 40 years). Females were chosen based on their past medical history and exclusion of any congenital disorders. This group of females had experienced a live singleton birth without any medical problems (Urmia, Iran). After obtaining a written informed consent from all individuals, 3-5 ml whole blood was collected in 15 ml tube containing EDTA as anticoagulant. A simple salting out procedure was used for extracting DNA from blood samples (18). Dde I based RFLP-PCR was performed to determine the factor XIII gene Val34Leu mutation using a set of forward and reverse primers as follow: catgccttttctgtgtcttc and taccttgacaggttgacccccggggcacta (14). Observed genotype and allele frequencies were estimated by direct

counting. We used the Hardy-Weinberg principle to test the deviations of alleles and genotypes from Hardy-Weinberg equilibrium. % frequency, expected genotype frequency and genotype/allele frequency were calculated. Regarding  $P$ -value  $<0.05$  as statistically significant level, genotype frequencies were compared to expected genotype frequencies and  $\chi^2$  and  $P$ -value (with degree of freedom=2) were computed. For all statistical analysis Excel 2013 were used.

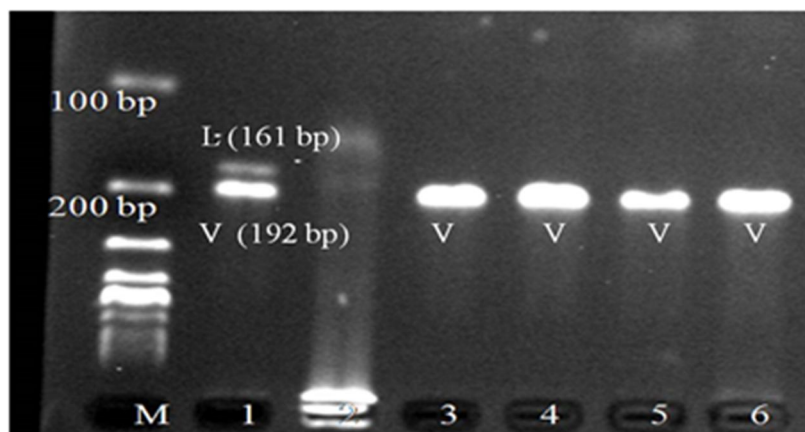
## Results

Our findings were summarized at table 1 and showed that frequencies of G (Val) and T (Leu) alleles, and also, G/G (Val/Val), G/T (Val/Leu) and T/T (Leu/Leu) genotypes of factor XIII were 165(84.18%) and 31(15.82%), and also, 67(68.37%), 31(31.63%) and 0(0%) in the tested group. T/T (Leu/Leu) genotype of factor XIII gene Val34Leu was not found in this study. Factor XIII gene Val34Leu genotypes showed an excellent fit to Hardy-Weinberg equilibrium in tested population ( $\chi^2 = 3.45 < 3.84$ ,  $P$ -value (with degree of freedom=2) = 0.177 > 0.05). In present study, factor XIII G (Val) and T (Leu) allele frequencies were 0.84 and 0.16, respectively. Figure 1 shows a representative gel image in 5 samples.

**Table 1.** Hardy-Weinberg equilibrium test in this population

FXIII	F	% F	F (Expected)
Val/Val (G/G)	67	68.37	69.5
Val/Leu (G/T)	31	31.63	26.1
Leu/Leu (T/T)	0	0	2.45
Val (G)	165	84.18	
Leu (T)	31	15.82	

F: Frequency,  $\chi^2 = 3.45 < 3.84$ ,  $P$ -value (with degree of freedom=2) = 0.177 > 0.05



**Figure 1.** G (Val:V) and T (Leu:L) allele and G/G (Val/Val(V/V)) and G/T (Val/Leu(V/L)) genotypes of factor XIII Val34Leu in 5 samples (Lane 1: VL; Lanes 3-6:VV).

## Discussion

Factor XIII gene were genotyped using Dde I based RFLP-PCR method in this study. Presence a mutation in exon 2 at amino acid 34 of factor XIII gene leads to transition of G (Val) to T (Leu) nucleotide and creates restriction enzyme sites for Dde I (17). Our findings reports the first official data regarding frequencies of G (Val) and T (Leu) alleles and the distribution of G/G (Val/Val), G/T (Val/Leu) and T/T (Leu/Leu) genotypes of factor XIII gene Val34Leu in a group of Iranian Turkish healthy females. The prevalence of factor XIII gene Val34Leu mutation in different general populations shows variable frequencies in ethnic groups (19). Frequency of factor XIII gene 34Leu mutation is more frequent in control groups from United Kingdom and Brazil, Portuguese, representing 0.48-0.49 (20,21) and 0.44 (19), respectively. In our group, frequency of factor XIII gene 34Leu mutation is approximately 0.16 and imply that factor XIII 34Leu mutation has a very low prevalence comparing to other Caucasian population from Italy (22), Austria (23), Brazil (19,24), Australia (17), Finland (25), United Kingdom (21) (ranging from 0.20 to 0.49) and Black population from Cameroon, Zaire, Angola (0.29) (19); but that is more frequent in our population comparing to Asian populations as Korean (0) (26) and Japanese (0.02) (19). Factor XIII Leu34Leu genotype was not found in this study that is consistence with others (26, 19). There is a

higher trend of factor XIII 34Leu mutation (T allele) frequency from east to west. Although the results of several investigations shows controversial findings (27-29),but others suggested that 34Leu mutation of factor XIII have been associated with thromboembolic diseases such as deep venous thrombosis, myocardial infarction (20,24,25, 30,31), increased risk for haemorrhagic stroke (21), and a protective effect against development of some of thrombotic disorders (8,9,23), coronary artery disease and cardiovascular events (7,20,24, 25). Since the interaction between individual specific genetic biomarkers and acquired factors play a role in human disease pathogenesis, screening for the Val34Leu mutation of factor XIII will be very helpful to estimating the risk of human disease predisposition such as thrombosis and classical metabolic risk factors.

Our findings suggested that Factor XIII Val34Leu mutation has a very low prevalence in tested population. These results could be considered as control group for studies relating the prevalence of factor XIII gene Val34Leu mutation in this population.

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## Declaration of interest

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