

Factor VII polymorphisms associated with plasma factor VII coagulant activity levels in healthy Tunisians

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تعدّد أشكال جين العامل السابع المترابط مع مستويات نشاط عامل التخثر السابع بالبلازما بين الأصحاء التونسيين

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الخلاصة: قد يساهم تعدّد أشكال جين العامل السابع VII في ارتفاع مستويات العامل السابع التخثيري VIIc، التي تترافق باختطار حدوث الأمراض القلبية الوعائية. وقد قام الباحثون بدراسة المترابط بين نمطين من تعدّد الأشكال هما: تعدّد أشكال المنطقة R353Q على الرامزة 353 وهي المسؤولة عن الناحية الحفازية وتعدّد أشكال انغراز الزوج القاعدي 10 (0/10) وهي المسؤولة عن الناحية المعزّزة بين مستويات العامل السابع التخثيري، في 176 شخصاً من بين التونسيين الأصحاء. وكان تواتر الأليل من الضرب Q 0.021 ± 0.213 بينما كان تواتر أليل انغراز الزوج القاعدي 10 هو 0.023 ± 0.250 . وكانت مستويات العامل السابع التخثيري في الأشخاص من النمط الجيني R/R مرتفعة جداً بالمقارنة مع الزيجوتات المتغايرة الألائل والزيجوتات المتماثلة الألائل (96.36) Q353 مقابل (59.52). ولم تلاحظ فروق يُعتدّ بها إحصائياً في مستويات العامل السابع التخثيري التي تتضمن تعدّد أشكال انغراز الزوج القاعدي 10. وترافق وجود الأليل Q353 لتعدّد أشكال جين العامل السابع بانخفاض في مقدار العامل السابع، مما قد يجعله مفيداً في الوقاية من الأمراض القلبية الوعائية.

ABSTRACT Factor VII gene polymorphisms may contribute to elevations in factor VII coagulant (FVIIc) levels that have been associated with cardiovascular risk. We therefore studied the association of two polymorphisms – R353Q polymorphism at codon 353 involving the catalytic region and the 10 base pair (bp) insertion polymorphism involving the promoter region – with FVIIc levels in 176 healthy Tunisians. The variant Q allele had a frequency of 0.213 (SD 0.021) whereas the frequency of the 10 bp insert allele was 0.250 (SD 0.023). Subjects with R/R genotype had significantly higher FVIIc levels than Q353 heterozygote and homozygote subjects (96.36 versus 59.52). FVIIc levels with the 10 bp insertion polymorphism were not significantly different. The Q353 allele of the factor VII gene polymorphism is associated with decreased factor VII and could be protective against cardiovascular disease.

Les polymorphismes du gène du facteur VII associés à la concentration plasmatique en activité coagulante du facteur VII chez des Tunisiens en bonne santé

RÉSUMÉ Les polymorphismes du gène du facteur VII peuvent contribuer à l'élévation de la concentration en activité coagulante du facteur VII (FVIIc) qui a été associée au risque cardio-vasculaire. Nous avons donc étudié l'association de deux polymorphismes – R353Q au niveau du codon 353 intervenant sur la région catalytique et le polymorphisme d'insertion de 10 paires de bases (pb) impliquant la région du promoteur - avec les taux de FVIIc chez 176 Tunisiens en bonne santé. L'allèle Q variant avait une fréquence de 0,213 (E.T. 0,021) tandis que la fréquence de l'allèle d'insertion de 10 pb était de 0,250 (E.T. 0,023). Les sujets ayant un génotype R/R avaient une concentration en FVIIc significativement plus élevée que les sujets hétérozygotes et homozygotes porteurs de l'allèle Q353 (96,36 vs 59,52). La concentration en FVIIc avec le polymorphisme d'insertion de 10 pb n'était pas significativement différente. L'allèle Q353 du polymorphisme du gène du facteur VII est associé à la diminution du facteur VII et pourrait constituer une protection contre les maladies cardio-vasculaires.

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Introduction

Arterial and venous thrombosis, which clinically manifest as stroke, myocardial infarction or pulmonary embolism are a major cause of death [1]. Genetic factors contribute significantly to the development of these diseases. Since the advent of molecular genetics, it has been a focus of interest to elucidate the role of mutations in candidate genes and their impact on haemostatic disorders such as arterial and venous thrombosis. The genes encoding proteins affecting haemostasis are intriguing factors that may increase cardiovascular disease. Recently several reports have focused on the association between the factor VII of the cascade coagulation and cardiovascular risk [1].

Human factor VII (FVII) is a vitamin K-dependent glycoprotein, synthesized in the liver and secreted in the blood as an inactive zymogen. Upon contact with tissue factor exposed by vascular injury, FVII is cleaved to its two-chain active form, which then activates factors IX and X leading to the generation of thrombin [2]. The importance of FVII in normal haemostasis is illustrated by the severe bleeding diathesis associated with low plasma levels of the protein [3]. It also appears to play an important role in atherothrombosis [4]. Moreover, several studies have demonstrated positive correlations between plasma FVII level and ischaemic cardiovascular events [5]. Controversially, Iacoviella et al. suggested that certain polymorphisms of FVII may protect against familial myocardial infarction, possibly by modifying FVII coagulant (FVIIc) levels [6].

The FVII gene contains nine exons and is located on the chromosome 13q34 [7,8]. The clinical presentation of patients with FVII deficiency is variable and consists of asymptomatic patients as well as patients

with severe to very severe bleeding tendencies [9]. The relationships between the clinical presentation and FVII levels and the associated molecular genetic defects lack apparent consistency [7,10]. Plasma FVII levels vary significantly in the general population and are influenced by environmental factors [11,12]. In addition, several polymorphisms within the FVII gene can affect either the function or its expression level, i.e. a replacement of arginine by glutamine in codon 353 (R353Q) arising from a single nucleotide substitution (G to A) at position 10976 in exon 8 in the catalytic region of the FVII gene and an insertion of a decanucleotide (designated as 0/10 bp) in the promoter region of the gene at position -323 [13,14].

To our knowledge, the prevalence of factor VII gene polymorphisms has not been studied previously in any country of North Africa. It has been reported that differences in FVII activity levels and in genotype frequencies depend on the ethnic groups [15]. We studied FVII gene polymorphisms in healthy Tunisians with the objectives of determining the frequencies of these gene polymorphisms in the population and clarifying the genotype association of the R353Q and 0/10 bp insertion polymorphisms with plasma FVIIc levels.

Methods

We recruited 176 unrelated healthy blood donors who were living in Tunisia for our study. All 112 men and 64 women were aged 17–63 years and were ethnic Arabs. No women were taking oral contraceptives or other drugs that might affect FVII levels. No one had cardiovascular disease, diabetes or thromboembolic diseases.

Blood was collected by standard atraumatic venepuncture technique using 0.1

mol/L citrate buffer as anticoagulant. FVIIc was measured by a one-stage semi-automated bioassay in an ST4 coagulometer (Diagnostica Stago, Asnieres, France). Results were expressed as a percentage of activity of the standard plasma supplied by the manufacturers.

To prepare DNA specimens and polymerase chain reaction (PCR) assays for polymorphism detection, DNA was extracted from ethylenediamine tetra-acetic acid (EDTA)-anticoagulant white blood cells by standard methods [16]. PCR amplification of the promoter region (10-bp insertion) and exon 8 (R353Q) of the factor VII gene was performed using the oligonucleotide primers and amplification conditions as described by Marchetti et al. and Green et al., respectively [13, 14]. The conditions of restriction enzyme digestion for the R353Q were as given by the manufacturers (Promega Corporation, Madison, Wisconsin, United States of America). The amplified products of the 0/10-bp promoter polymorphism did not require enzyme digestion. This polymorphism contains two alleles, the 0-bp allele and the 10-bp allele; the latter corresponds to the presence of a decanucleotide insertion. The PCR fragment size of the 0-bp allele is 214-bp whereas that of the 10-bp allele is 224-bp. The PCR fragment size of the R353Q polymorphism is 312-bp and required MspI digestion. Enzyme digestion generated in all PCR products 40-bp which is added by either: one fragment of 272-bp in homozygote R/R; two fragments, one of 205-bp and the other 67-bp in homozygote Q/Q; or three fragments of 272-bp, 205-bp and 67-bp in heterozygote R/Q. The amplified products of the 0/10-bp promoter polymorphism and enzyme digestion fragment of R353Q polymorphism were separated by 8% polyacrylamide gel.

Allelic frequencies were calculated by gene-counting method for statistical analysis. Chi-squared goodness of fit was used to determine if genotype distributions were as expected by Hardy–Weinberg proportions. Variable means of FVIIc were compared by Student *t*-test. The level of significance was set at 0.05.

Results

We determined FVII levels in a sample of 176 Tunisian blood donors and studied the relationships between 2 polymorphisms of the FVII gene. The promoter decanucleotide insertion (–323 0/10-bp) and the R353Q polymorphisms of FVII gene were evaluated. For the exon 8 polymorphism, 66 of 176 individuals carried the Q353 allele (57 heterozygotes and 9 homozygotes), whereas 110 carried homozygotes for the R353 allele. There were 81 individuals with 10-bp insertion (74 heterozygotes and 7 homozygotes) and 95 lacked the insertion.

The allele frequency derived from the analysis of 352 chromosomes was 0.213 (standard deviation = 0.021) for the Q353 allele and 0.787 (SD = 0.021) for the R353 allele. The corresponding frequency of the 10-bp insertion allele was 0.25 (SD = 0.023) and that of the 0 allele (absence of insertion) was 0.75 (SD = 0.023).

The genotype distribution of the R353Q or 0/10 bp promoter polymorphism in the Tunisians did not deviate significantly from a population at Hardy–Weinberg equilibrium ($P > 0.05$). Table 1 shows FVIIc levels as a function of genotypes for each polymorphism without the concomitant presence of the other. Table 2 shows FVII levels as a function of a combination of the two polymorphisms.

Table 1 Adjusted plasma factor VII coagulant (FVIIc) activity levels as a percent of standard by genotype

Genotype	Number	FVIIc Mean (SD)
<i>R353Q polymorphism</i>		
R/R	110	96.36 (32.14)
R/Q	57	54.08 (33.27)
Q/Q	9	75.83 (30.02)
Presence of 1 Q allele or 2 Q alleles	66	59.52 (33.41)
<i>Promoter polymorphism</i>		
0/0 bp	95	88.34 (33.03)
0/10 bp	74	71.97 (41.44)
10/10 bp	7	73.80 (21.50)
Presence of 1 or 2 decanucleotide allele(s)	81	72.08 (40.34)
<i>Total</i>	176	

SD = standard deviation.

For the R353Q polymorphism, the presence of allele Q353 in heterozygous or homozygous state was associated with FVII levels lower by 20%–40% than the

Table 2 Plasma factor VII coagulant (FVIIc) activity levels in combined genotypes of R353Q and 0/10 bp promoter polymorphisms of the factor VII gene among Tunisians

Genotype	Number	FVIIc Mean (SD)
R/R/0/0	66	95.65 (27.90)
R/R/0/10	34	97.35 (42.82)
R/R/10/10	2	88.50 (2.12)
R/Q/0/0	20	68.87 (44.88)
R/Q/0/10	38	46.68 (24.12)
R/Q/10/10	0	–
Q/Q/0/0	12	77.00 (34.35)
Q/Q/0/10	2	84.50 (3.54)
Q/Q/10/10	2	59.00 (2.83)
<i>Total</i>	176	

SD = standard deviation.

R353 allele ($P < 0.0001$). The genotypes with the 10-bp insertion were associated with lower FVII levels ($P > 0.05$).

Discussion

During the past 20 years, substantial progress has been made in understanding the multifactorial and multigenic nature of haemostatic diseases. It is well established that interactions between environmental and genetic factors determine the risk for a thrombotic episode [17]. During the last decade, evidence has accumulated that indicates that raised plasma factor VII levels increase the risk of ischaemic heart disease, even though in some reports this association was not seen [18]. Conflicting results have been reported about the role of common polymorphisms in influencing FVII plasma levels [19,20]. We, therefore, explored the frequencies of two FVII gene polymorphisms among healthy Tunisians. Our investigation was the first of its kind in Tunisia to attempt to determine the associations of these polymorphisms with FVII coagulant levels.

The observed frequency of the Q allele (0.213) among normal Tunisians was higher than among Europeans (0.099) or Chinese (0.045) [20,21,15]. The 10 bp insertion allele frequency in healthy Tunisians (0.25) was also higher than those of Europeans (0.139) and Chinese (0.036) [20,21].

We also evaluated the FVII genotype-phenotype relationships by the determination of two polymorphisms (four alleles) in different functional regions of the gene. There was a trend towards lower FVIIc in individuals carrying the Q allele. These findings were consistent with other previous reports in which heterozygous or homozygous individuals for the Q allele have 10%–38% lower FVIIc levels than their

homozygous (R/R) counterparts [20,22]. However, we found that the 10 bp insert allele was not associated with significantly lower FVIIc levels.

From our data, it was apparent that the effect of genotypes was more pronounced at the codon 353 than at the promoter locus. For the latter, no significant genotype associations with FVIIc levels were observed. However, others have found associations between them [21,23]. It is possible that the association of factor VII polymorphisms with plasma levels varies with race and ethnicity. In our study, the effect of the R353Q polymorphism on plasma FVIIc levels appeared to be dominant over the promoter polymorphism. The Q allele may interfere with biological activity of the factor VII protein leading to the decreased plasma FVIIc levels.

The best way to establish which gene polymorphism affects plasma factor VII would be to study it *in vitro*, in which each polymorphism is expressed in mammalian cells alone or in combination with others, and the amount of factor VII produced by the different constructs are compared. At the moment, studies on single polymorphisms without the concomitant presence of others in the constructs are not available, making it difficult to assign a functional effect on FVIIc levels to any [24].

Genetic polymorphisms underlie the diversity of any species. Most of such inherited changes in DNA structure are neutral, but others could affect the function of proteins and with more or less severity, the efficiency of whole physiological system, thus modifying susceptibility to a particular

disease. Genetic changes could have particular strength in very sensitive systems like the haemostatic system. Because of the dichotomy of this system, polymorphic changes affecting haemostatic factors could have mild but opposite effects in the pathogenesis of thrombotic and haemorrhagic disorders. It has been reported that the frequencies of the Q353 allele association with lower FVII levels in patients who had a myocardial infarction were less frequent than in control subjects. These findings suggested that genetic protection from myocardial infarction is provided by this allele [6,25].

We observed significant genotype associations with FVIIc levels among Tunisians with more pronounced and consistent effect in the R353Q polymorphism. If the Q allele is indeed the functional mutation that causes reduction of FVII activity in Tunisians, it might confer some protection against thrombosis and coronary artery disease in this ethnic group. Future work should seek to determine the action mechanism of polymorphism, alone or in association with others, on factor VII levels and evaluate its frequency in other ethnic groups and in patients with atherothrombotic disease.

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Genetics, genomics and the patenting of DNA. Review of potential implications for health in developing countries

This new report, *Genetics, genomics and the patenting of DNA*, addresses the important ethical, legal, social and health issues raised by the patenting of DNA sequences – not only for the industrialized world, but also for developing countries. Science is now at a critical moment as it attempts to translate voluminous genetic data into practical health tools. It is therefore timely to consider the various incentives and barriers that exist for the creation of practical health interventions of value in poorer settings. The report emphasizes that genomics has the potential to offer great benefit to public health on a global scale, notes the present ambiguity in international agreements on intellectual property rights on the legal status of genetic “inventions”, and highlights the ongoing controversy surrounding the patenting of genetic sequences. The report proposes areas of further exploration that could provide a foundation for the establishment of informed policies. This document is available free on line at: <http://www.who.int/genomics/FullReport.pdf>