

A missense mutation (Y1702C) in the coagulation factor V gene is a frequent cause of factor V deficiency in the Italian population

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Background and Objectives. Factor V (FV) deficiency is a rare bleeding disorder whose molecular bases are poorly characterized. We have recently described a FV missense mutation (Y1702C) predicting reduced FV levels in a thrombophilic patient and in a healthy individual. The aim of the present work was to assess the prevalence of the FV Y1702C mutation among subjects with FV deficiency.

Design and Methods. Carriership of the FV Y1702C mutation was tested in 8 patients with severe FV deficiency (FV:C <8%), in 16 individuals with asymptomatic partial FV deficiency (mean FV:C 38.0%, SD 11.6%) and in 9 patients with pseudo-homozygous APC-resistance (mean FV:C 46.2%, SD 3.6%). An AclI-restriction protocol was employed for rapid mutation screening.

Results. The FV Y1702C mutation was detected in two unrelated patients with unmeasurable FV levels (one being homozygous and the other doubly heterozygous for a still unknown mutation) and in one subject with partial FV deficiency (FV:C 30%). A striking difference in bleeding phenotype was observed between the homozygous patient and her asymptomatic brother with the same FV genotype. A multi-point FV haplotype analysis was performed in all unrelated carriers of the FV Y1702C mutation. Three haplotypes were found to underlie the mutation in different individuals, suggesting that it might have arisen independently more than once.

Interpretation and Conclusions. FV Y1702C is a common cause of FV deficiency in the Italian population and might be a recurrent mutation.

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Key words: factor V deficiency, VNTR, haplotype, recurrence.

Deficiency of coagulation factor V (FV) is a rare bleeding disorder which is inherited as an autosomal recessive trait within families.¹ Partial FV deficiency, characterized by a mild reduction of FV levels, is usually completely asymptomatic. In contrast, a wide spectrum of symptoms, ranging from occasional epistaxis to life-threatening post-traumatic bleeding, is observed in individuals with FV levels below 10%. On the whole, there is poor correlation between FV levels and clinical phenotype² and one individual with unmeasurable FV levels has been reported to have remained asymptomatic until the age of seven years.³

The elucidation of the molecular bases of FV deficiency has been hampered for a long time by the low prevalence of the disorder and by the large size of the FV gene (~100 kb). Only a few intragenic mutations have been reported to cause FV deficiency, mainly nonsense mutations⁴⁻⁶ and short insertions/deletions predicting a translation frame-shift and a premature stop codon.^{3,7-9} Most of these mutations are private, having been detected only in one patient (and his/her family members). Only the R712Stop⁴ and the R506Stop^{5,6} mutations have each been reported to account for FV deficiency in two unrelated individuals. Therefore, the underlying genetic defect remains unknown in the vast majority of FV deficient patients.

Co-inheritance of quantitative FV deficiency and the thrombophilic FV R506Q (FV Leiden) mutation results in a strongly procoagulant condition, known as pseudo-homozygous APC-resistance.¹⁰ Since individuals with pseudo-homozygous APC-resistance often develop thrombosis and thereby come to clinical attention, several mutations causing FV deficiency have been identified in such patients.

While studying the molecular bases of FV deficiency in a thrombophilic family, we detected a novel FV gene mutation (5279 A/G) predicting a remark-

able amino acid substitution (Y1702C) in the A3 domain.¹¹ The propositus of the family, whose FV antigen and activity levels were reduced by half, was heterozygous for this mutation and the mutant protein could not be demonstrated in his plasma by Western blot analysis. An additional carrier of the FV Y1702C mutation, also showing reduced FV levels, was detected by screening a general population sample.¹¹ The causative role of the FV Y1702C mutation is supported by the absolute conservation of the affected residue in all three A domains of FV and of homologous factor VIII and ceruloplasmin, and by the disrupting structural effects of the Y1702C substitution.¹¹ We report here that this mutation is a common cause of FV deficiency in the Italian population.

Design and Methods

Patients

Patient A. A female patient from Southern Italy with a history of recurrent epistaxis. The diagnosis of severe FV deficiency (FV:C 1%) was made after a serious hemorrhage following a dental extraction. Subsequently, the patient experienced traumatic hemothorax after thoracentesis and hemoperitoneum following rupture of a follicular cyst. Her asymptomatic brother was subjected to a coagulation screening in view of a dental extraction and was also found to carry severe FV deficiency (FV:C 1%), which had been previously undetected. Their parents are consanguineous (second-degree cousins).

Patient B. A 17-year old female patient from Central Italy. She has suffered from epistaxis, gum bleeding and melena since the age of 3. At 8 years she presented with post-traumatic hemarthrosis, and at 15 with recurrent menorrhagia. On that occasion, the diagnosis of congenital FV deficiency (FV:C <1%) was made. Afterwards, she had two episodes of hemoperi-

toneum following follicular cyst rupture. A number of relatives, not available for this study, showed FV levels compatible with heterozygous FV deficiency.

Patient C. A male patient from Northern Italy. The occurrence of a hemorrhage following tonsillectomy prompted coagulation screening that revealed the status of partial FV deficiency (FV:C 30%). Measurement of FV levels in his parents (not available for DNA analysis) showed that the propositus had inherited the FV defect from his mother (FV:C 46%), whereas his father had virtually normal FV levels (FV:C 90%).

Patient D. A 51-year old man from Northern Italy. He came to clinical attention because of recurrent thromboses, attributable to pseudo-homozygous APC-resistance. In fact, in addition to the FV Y1702C mutation, which accounts for his low FV levels (FV:Ag 50%, FV:C 50%), he is a carrier of the FV R506Q mutation on the counterpart FV allele. This patient is the propositus of the thrombophilic family described in ref. #11.

Coagulation laboratory investigations

FV activity (FV:C) levels were measured by a one-step biological assay, based on the ability of the test plasma to normalize the prothrombin time in a congenitally FV-deficient plasma sample. FV antigen (FV:Ag) levels were measured by an enzyme-linked immunosorbent assay (ELISA), essentially as described elsewhere.¹⁰

DNA studies

The FV Y1702C transition was ascertained by AclI-restriction analysis after polymerase-chain reaction (PCR) amplification of a 120-bp fragment by means of a mutagenized primer.¹¹ Carriership of the mutation was confirmed by direct sequencing in all patients.

Both biallelic (single-nucleotide polymorphisms, SNPs) and multiallelic (variable number of tandem

Table 1. FV gene haplotyping in carriers of the FV Y1702C mutation.

Location	IVS 2	EX 8	IVS 9	EX 13			EX 15	EX 16	IVS 19	
Polymorphism	(AT) _n	1328 T/C	-164 C/T	2298 T/C	2325 C/T	2391 G/A	4070 A/G	5279 A/G	5380 G/A	+122 T/G
Aminoacid	-	385 Met/Thr	-	708 Ile	717 Asn	739 Ser	1299 His/Arg	1702 Tyr/Cys	1736 Val/Met	-
Patient A	25/25	T/T	C/C	C/C	T/T	A/A	A/A	G/G	A/A	G/G
Patient B	23/25	C/C	C/C	T/C	C/T	G/A	A/A	A/G	G/A	T/G
Patient C	18/21	T/T	T/T	C/C	T/T	A/A	A/A	A/G	A/A	G/G
Patient D*	10/21	T/T	C/T	C/C	T/T	A/A	A/A	A/G	A/A	T/G
C43	19/21	T/T	C/T	C/C	T/T	A/A	A/A	A/G	G/A	T/G

*Carriership of the FV R506Q mutation, which resides on a single conserved FV gene haplotype, made it possible to determine the phase between markers in this patient unambiguously. C43, asymptomatic carrier of the FV Y1702C mutation detected by a general population screening.¹¹

repeats, VNTR) markers in the FV gene were employed for the haplotype analysis of subjects carrying the mutation (Table 1). Genotyping for biallelic polymorphisms was performed by allele-specific amplification (exon 16 and intron 19 markers) or by PCR-amplification followed by endonuclease restriction analysis (exon 8, intron 9 and exon 13 markers). In order to characterize the genotype for the VNTR marker, the relevant portion of intron 2 was amplified in the presence of ³⁵S-dATP. The PCR products were run on a high resolution denaturing polyacrylamide gel and visualized by autoradiography.

Results

Detection of carriers of the Y1702C mutation

Carriership of the FV Y1702C mutation was investigated in 8 patients with severe FV deficiency (six had FV:C levels ≤ 1%), in 16 subjects with asymptomatic or very mildly symptomatic partial FV deficiency (mean FV:C levels 38.0%, SD 11.6%) mostly detected in pre-surgery coagulation screenings, and in 9 patients with pseudo-homozygous APC-resistance (mean FV:C levels 46.2%, SD 3.6%). As shown in Figure 1, the mutation was found in two patients with severe FV deficiency, in the homozygous (patient A) and in the heterozygous (patient B) condition; and in one asymptomatic subject with mild FV deficiency (patient C), who was heterozygous. None of the patients with pseudo-homozygous APC-resistance was a carrier, apart from the propositus of the family described in ref. #11 (hereafter referred to as patient D). The virtual absence of FV activity in the plasma of patient B, who is heterozygous for the FV Y1702C mutation, indicates that this patient carries a second, as yet unknown, null mutation on the counterpart FV allele. No candidate gene variation was observed in either exons or splicing junctions, suggesting that the FV defect may be concealed in some intronic region.

Extension of the molecular studies to patient A's relatives available for study revealed that her brother, who also carried severe FV deficiency (FV:C 1%), was homozygous for the mutation, whereas both parents, who are second-degree cousins, turned out to be heterozygous (Figure 1).

In summary, the FV Y1702C mutation was detected in three unrelated patients with FV deficiency (this paper), one patient with pseudo-homozygous APC resistance¹¹ and one out of 252 healthy individuals from the general population,¹¹ all carriers coming from different regions of Italy. These findings indicate that the FV Y1702C mutation is a widespread cause of FV deficiency in Italy.

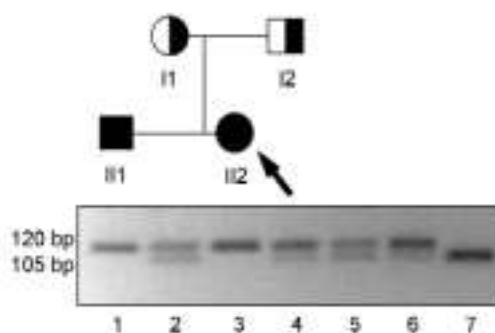


Figure 1. Top: pedigree of patient A. The proposita is indicated by an arrow. Closed symbols, homozygous FV deficiency. Half-open symbols, heterozygous FV deficiency. Bottom: detection of carriers of the FV Y1702C mutation by Accl-restriction analysis. The mutated allele gives rise to a 120-bp undigested band, whereas the wild-type allele gives rise to a 105-bp band.¹¹ Lane 1, patient A; lanes 2-4, patient A's mother, brother and father, respectively; lane 5, patient B; lane 6, patient C; lane 7, normal subject.

Haplotype analysis in carriers of the FV Y1702C mutation

To find out whether the ample distribution of the FV Y1702C mutation results from multiple mutational events at the same site or rather reflects the spreading of a single mutated FV allele, a multi-point FV gene haplotype analysis was performed in all carriers of the mutation (Table 1). In addition to a number of biallelic polymorphisms scattered all over the FV gene, a highly polymorphic (>90% heterozygosity) intragenic (AT)_n repeat located in intron 2 (manuscript submitted) was also included in the haplotype analysis. Three partially related haplotypes, differing for the exon 8 and intron 9 polymorphisms, as well as for the VNTR marker, turned out to underlie the FV Y1702C mutation in this small sample of carriers (Table 1). Such variability of the genetic background suggests that FV Y1702C is either a recurrent mutation or a very ancient allele.

Discussion

The FV Y1702C mutation has been previously reported to predict CRM- FV deficiency and to be rare (allele frequency 1/504=0.002) in the general Italian population.¹¹ The present finding of three additional unrelated carriers among 24 subjects with FV deficiency confirms its relationship with reduced FV levels and indicates that this mutation is a common cause of FV deficiency in Italy. According to a ceruloplasmin-based structural model,¹¹ the FV Y1702C substitution causes protein instability by disrupting

the A3 domain scaffold. In addition, the exposure of a novel Cys residue at position 1702 may interfere with correct disulfide bridge formation between nearby Cys residues (Cys¹⁶⁹⁷-Cys¹⁷²³).

It has long been observed that FV levels are a poor predictor of the bleeding phenotype. Although life-threatening intracranial bleeding has been reported in newborns with very low FV levels,¹²⁻¹⁴ most cases of severe FV deficiency show a mild-to-moderate bleeding diathesis,² and at least one patient has remained completely asymptomatic for several years.³ Our sample of severely FV-deficient patients was also characterized by considerable phenotype heterogeneity, patient B showing definitely more severe hemorrhagic symptoms than patient A, in spite of their comparable FV levels. Even more striking was the difference between the moderately affected patient A and her asymptomatic brother, who shared identical FV genotypes and equally unmeasurable FV levels. The reason for such phenotypic discrepancies is not known, but it may rest with the existence of additional genetic components and environmental factors that modulate the expressivity of FV-deficient mutations.

Our molecular genetic data indicate that the FV Y1702C mutation is associated with at least three different FV gene haplotypes, which differ for the exon 8 and intron 9 polymorphisms, as well as for the intron 2 microsatellite marker (Table 1). Although the variability at the VNTR locus could have developed after the occurrence of the mutation, given its high mutation rate, it is noteworthy that SNPs are sufficient to define the three different haplotypes, since the genetic information provided by the VNTR marker is redundant with that deriving from the intron 9 polymorphism. Therefore, if one assumes that the FV Y1702C mutation has arisen only once, at least two subsequent events of intragenic recombination and/or mutation are required to account for the extant variability in the underlying genetic background. Since a long time would be necessary to accumulate the observed genetic variability (even taking into account that both exon 8 and intron 9 polymorphisms are located in highly mutable CpG sites), the FV Y1702C must be either very ancient or recurrent. The defect may be one of the most common causes of FV deficiency in Italy. Its ancient origin and/or recurrence suggest that it might be present in other populations as well.

Contributions and Acknowledgments

EC played a major role in the mutation screening and haplotype analysis of factor V deficient patients and wrote the paper. BL and FM set up the mutagenized restriction analysis and microsatellite genotyping thus extensively contributing to the molecular investigations. GM, RR and GM recruited the FV deficient patients, collected clinical histories, evaluated the clinical phenotypes and measured FV levels. FB supervised the whole work and was responsible for the final interpretation of results.

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Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

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Potential implications for clinical practice

It is worthwhile to screen FV deficient patients and families for the FV Y1702C mutation potentially responsible for severe deficiency and bleeding diatheses.

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