

Blood Coagulation, Fibrinolysis and Cellular Haemostasis

Clinical phenotypes and factor VII genotype in congenital factor VII deficiency

Guglielmo Mariani¹, Falko H. Herrmann², Alberto Dolce³, Angelika Batorova⁴, Daniela Etro⁵, Flora Peyvandi⁶, Karin Wulff², Jean F. Schved⁷, Günter Auerswald⁸, Jorgen Ingerslev⁹, Francesco Bernardi⁵ for the International Factor VII Deficiency Study Group (see appendix)

¹Dipartimento di Medicina Interna e Sanità Pubblica, Università de L'Aquila, Italy, ²Institut für Humangenetik, E. M. Arndt Universität, Greifswald, Germany, ³Istituto Nazionale di Statistica, Rome, Italy, ⁴Haemophilia Centre, Bratislava, Slovak Republic, ⁵Dipartimento di Biochimica e Biologia Molecolare, Università di Ferrara, Italy, ⁶IRCCS Ospedale Maggiore di Milano, Angelo Bianchi Bonomi, University of Milan, Italy ⁷Laboratoire Central d'Hématologie, CHU Montpellier, France, ⁸Kinderklinik, Zentral Krankenhauses, Bremen, Germany, ⁹Haemophilia Centre, Department of Clinical Biochemistry, University Hospital of Aarhus/Skejby, Denmark

Summary

To investigate the relationship between clinical phenotype, clotting activity (FVIIc) and FVII genotype, a multi-center study of factor VII (FVII) congenital deficiency with centralized genotyping and specific functional assays was carried out. FVII mutations characterized in patients (n=313) were extremely heterogeneous (103 different, 22 novel). Clinical phenotypes ranged from asymptomatic condition, including 15 homozygotes and 14 double heterozygotes, to patients with a severe disease characterized by life-threatening and disabling symptoms (CNS, GI bleeding and hemarthrosis) strongly associated with an early age of presentation. Based on type and number of symptoms we classified 90 'severe' (median FVIIc 1.4%, IQR [Interquartile Range] 0.9–3.8), 83 'moderate' (FVIIc 3%, IQR 1–21.7), and 140 'mild' bleeders (FVIIc 14%, IQR 3–31). The significantly different FVIIc levels, and the decreasing prevalence of homozygotes or

double heterozygotes among severe (98%), moderate (84%) and mild (56%) bleeders, further support our classification. The excess of females among moderate bleeders (female/male ratio =2.6) is attributable to menorrhagia. There was no evidence for modulation of clinical features by frequent functional polymorphisms. Homozygotes for the same mutation (Ala294Val; 11125delC) with similar FVIIc and FXa generation levels, showed striking differences in clinical phenotypes. Our study depicts the ample clinical picture of this rare disorder, proposes a severity classification and provides arguments for the early management of the disease in the severe cases. Genotype-phenotype relationships indicate the presence of major environmental and/or extragenic components modulating expressivity of FVII deficiency.

Keywords

Bleeding disorders, FVII deficiency, bleeding symptoms, gene mutations, polymorphisms

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Introduction

Inherited FVII deficiency is the commonest of the rare congenital coagulation disorders with an estimated prevalence of the symptomatic individuals of 1:500,000 (1). Numerous mutations (<http://europium.csc.mrc.ac.uk>) (2) causing this autosomal recessive disorder underlie considerable heterogeneity in genotype and phenotype (i.e. FVIIc) (3). Indeed, clinical heterogeneity is a feature of this haemorrhagic disorder, which ranges in severity

from lethal to mild, or even asymptomatic forms. FVII deficiency is not believed to be associated with complete absence of functional FVII and this fact accords with data from knock-out mice studies, which suggest that a complete absence of FVII is incompatible with life (4). The minimal FVII level able to interact with tissue factor to prevent lethal bleeding in human subjects has not yet been defined (5, 6).

Numerous publications, focusing on the molecular genetics, FVII structure/function analysis and pathophysiology of FVII

Correspondence to:
Prof. Bernardi
Dipartimento di Biochimica e Biologia Molecolare
Università di Ferrara,
Via Fossato di Mortara 74
44100, Ferrara, Italy
Tel.: +39 0532 424425, Fax: +39 0532 424484
E-mail: ber@dns.unife.it

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deficiency, have appeared in the literature (7) and are based on a noteworthy series of patients (8–13). However, a comprehensive approach, particularly one based on clinical issues and involving a very large patient population, has not yet been forthcoming. Hence, no systematic analyses on disease presentation and diagnosis are available and issues, such as the relationship between gender and bleeding tendency, that are important in the context of an autosomal disease, remain largely unsolved.

The absence of a systematic approach to classifying FVII deficiency in groups phenotypically homogeneous has precluded the evaluation and comparison of treatments; this is mainly attributable to the lack of a clear relationship between factor clotting activity and bleeding phenotype (2). It is known that FVII levels are modulated by FVII gene polymorphisms (14), but their impact on the bleeding tendency awaits verification (9). The availability of an integrated clinical and genetic data set would provide the opportunity for evaluating the role of molecular genetics to understand the clinical heterogeneity of the disease. To fill this void, we carried out an extensive analysis of 515 subjects with FVII activity from less than 1% up to 50% of normal.

Methods

Patient enrollment

A total of 515 subjects (264 females [age range 1–90], 251 males [age range 2–86]) were referred from 59 centers from Europe, Asia, Australia-New Zealand and the Americas (see list of participants in the Study Group) because a FVII deficiency (FVIIc < 50% and/or a causative mutation) was diagnosed. Of these, 202 subjects were asymptomatic and 313 symptomatic.

Initially, data collection was carried out with suited questionnaires which included the following items: FVIIc activity and antigen levels; family history; dates of laboratory diagnosis; dates of onset of bleeding and subsequent bleeding episodes (up to 22 clinical variables). The initial database was then completed to include data from DNA analyses, specific phenotypic assays and missing clinical information. Attending physicians were

asked to fill in file blanks and individual subject files were checked and validated. In total, the database comprises 41 primary and 18 derived variables/file-patient. Twenty-two files, not suitable for the analysis, were discarded.

The research protocol was approved by the Review Board of Palermo University (the original institution of GM) and by the boards of each of the participating institutions. A common form for the patient informed consent and study information was developed and used.

Genetic and coagulation studies

For all DNA samples available mutations in the FVII gene were investigated by DNA sequencing of all coding regions, exon/intron boundaries and the promoter region, according to previously described methods (12, 15).

FVIIc levels were assayed using a standard one-stage method in the lab of each participating center (lowest detection limit 1%). High sensitivity thromboplastins (International Sensitivity Index [ISI] close to 1, i.e. recombinant and human-derived reagents) were employed for 82% of the sample assays. Rabbit brain and bovine thromboplastins were also used to screen for specific variants. FVII antigen (FVIIAg) was measured with a commercial immunoenzymatic method (Asserachrom FVIIAg-Stago, Asnières, France) and results expressed as percentage of pooled standard plasma.

The generation of activated factor X (FXa) in plasma, or using the recombinant molecules, was estimated using the FXa fluorogenic substrate, MeSO₂-D-CHA-Gly-Arg-AMCacOH (American Diagnostica, CT, USA), as previously described (16). Briefly, the reaction of FXa generation was initiated by adding an excess (30 microL) of thromboplastin (Thromborel S) (Behring, Marburg, Germany) to 50 microL of diluted plasma (1:20, 1:40, 1:80 in 20 mM HEPES, 150 mM NaCl, 0.1% PEG 8000, 5 mM CaCl₂, pH 7.4). The reaction was quenched with 30 microL 20 mM HEPES, 150 mM NaCl, 0.1% PEG 8000, 50 mM EDTA, pH 7.4 after incubation of 5 minutes at ambient temperature. FXa fluorogenic substrate (MeSO₂-D-CHA-Gly-Arg-AMCacOH) (American Diagnostica, Greenwich, CT) was added (200 microM), and fluorescence (360 nm excitation, 465 nm emission) was measured on SpectraFluorPlus microplate reader (Tecan, Salzburg, Austria).

rFVII in 2 nM conditioned medium was incubated 5 minutes at 37°C with 1 nM human factor Xa (hFXa) (Sigma, St Louis, MO) in 50 microL final volume. After addition of 30 microL thromboplastin, FXa generation was started with 40 nM human FX (Sigma). Reaction was quenched and fluorescence was measured as described above.

The assays were standardized with dilutions of pooled normal plasma (PNP) or of recombinant wild-type (rWt) FVII. The assay was calibrated using dilutions of pooled normal plasma or wild-type recombinant FVII, respectively. At least three independent sets of experiments were carried out for each assay.

Recombinant FVII expression

Recombinant FVII molecules were obtained (16) by transient transfection of baby hamster kidney (BHK) cells with the expression vectors for the wild type-FVII and Ala294Val; 11125delC-FVII variant. The latter was created by site-directed

Table 1: Symptoms by gender and presentation.

Symptoms (*)	Males		Females		Prevalence as presenting symptom (%) (n=228)
	n	% patients (n=139)	n	% patients (n=174)	
Epistaxis	92	66.2	98	56.3	36.7
Easy bruising	60	43.2	83	47.7	7.9
Gum bleeding	36	25.9	59	33.9	4.8
Muscle hematoma	29	20.9	28	16.1	4.4
Hemarthrosis	30	21.6	28	16.1	4.4
GI bleeding	20	14.4	24	13.8	4
Hematuria	17	12.2	9	5.2	1.3
CNS bleeding	9	6.5	8	4.6	4.4
Post-operative bleeding	38	30.4†	40	29.8†	7.9
Menorrhagia	/	/	100	62.9‡	15.6

* Only most frequent symptoms are reported. † Prevalence in surgical patients (♂ = 125; ♀ = 134).

‡ Frequency found in females aged > 10.4 (age of the first episode recorded in the database).

Abbreviations: CNS = central nervous system; GI = gastrointestinal.

mutagenesis (Stratagene, CA, USA) of the human FVII cDNA cloned in pcDNA3.1, using the following oligonucleotides: 5'CCGTGGCGCCACGGTCCTGGAGCCATG- G-3' and 5'GGAGTCCTCCTGCGAGCCATTCCCTAGC-CCAGC3'. Transfections were performed using the LipofectAmine 2000 Reagent (Life Technologies, ME, USA).

Statistical methods

Descriptive statistics for ordinal and counting variables were used (median, range and interquartile range -IQR). Since distributions were not normal, non-parametric statistical analyses were used (17, 18): for the skewed distribution, non-parametric Wilcoxon test and χ^2 test (association and homogeneity) were used, and for $n > 2$ groups Kruskal-Wallis one-way analysis of variance with *post-hoc* pair-wise comparisons (Dunn's test) were performed (18). Odds Ratio (OR) with (1- α %) Confidence Interval (CI) were used to estimate bleeding risk. Multivariate regression analysis (ordinal proportional odds logistic regression models) was performed to assess the significance of variables after a backward selection (19). P-value (p) <0.05 was considered statistically significant. All analyses were carried out using the statistical packages SAS 8.12 and S-PLUS 2000.

Table 2: Proposed severity classification for FVII deficiency.

Class of Severity	Clinical Phenotype
Severe	CNS bleeding and/or GI bleeding and/or Hemarthrosis with or without other bleeding symptoms (Table 1)
Moderate	Three or more symptoms (Table 1) (GI bleeding, CNS bleeding or Hemarthrosis excluded)
Mild	One or two symptoms (Table 1) (GI bleeding, CNS bleeding or Hemarthrosis excluded)

ed statistically significant. All analyses were carried out using the statistical packages SAS 8.12 and S-PLUS 2000.

Results

Symptoms and disease presentation

We observed an excess of symptomatic females (n=174/313, 56%) as compared to males (n=139/313, 44%). The distribution of symptoms for gender in patients with FVII deficiency is shown in Table 1.

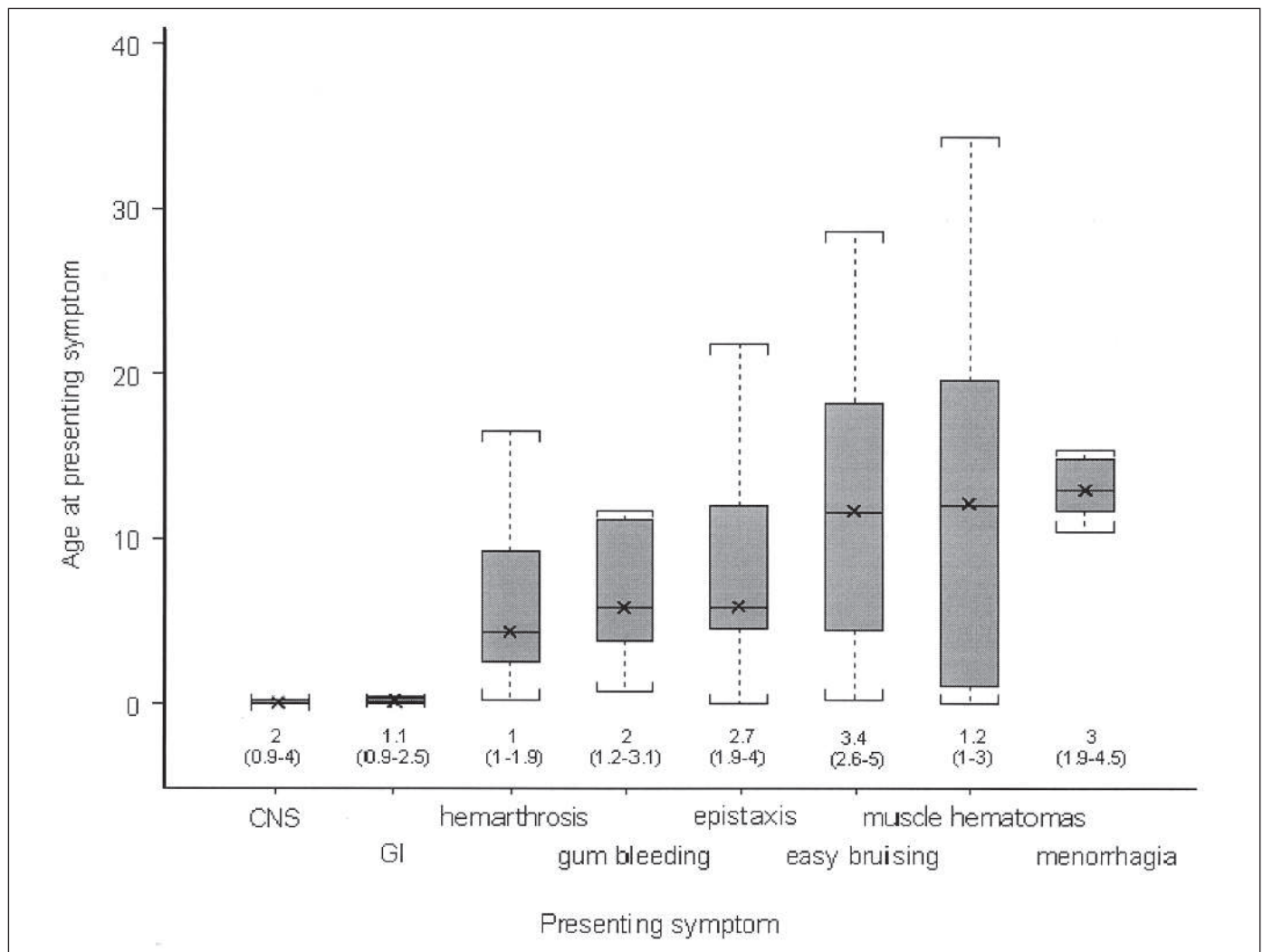


Figure 1: Ranking of symptoms by age at presentation. Gray area in the Box and Whisker plot comprises 50% of cases (25th-75th percentiles). x = median values of age; caps denote extreme values. The values under the boxes denote median FVIIc values and (CI).

Age at symptom onset was compared with age at laboratory diagnosis in the 209 patients for whom both time points were available. In 50.5% of subjects, diagnosis of FVII deficiency was made at the same time or within 6 months of symptom onset. The median age of symptom onset (7.2 y) was different from that at diagnosis (11.6 y; $p < 0.0001$).

The most frequent presenting symptoms, as recorded in at least 10 patients, were ranked in order of median age at presentation (Fig. 1). Comparison of the median age at presentation for specific symptoms yielded a statistically significant difference ($\chi^2 = 26.6$, $p = 0.0004$). In particular, median age of patients presenting with either central nervous system (CNS) or gastrointestinal (GI) bleeds were lower (post hoc Dunn's test) than those of patients presenting with bleeds in other sites, with the exception of hemarthrosis.

Classification of clinical phenotypes

In hemophilia A and B a reliable classification of severity can be obtained by plasma factor VIII or IX activity levels. We found, instead, that 24 subjects with FVIIc levels below 2% (tested with human or recombinant thromboplastins in 18 and with rabbit thromboplastin in 6 subjects) were either asymptomatic or had a very mild phenotype. By contrast 16 subjects with FVIIc levels above 5% , most of them (70%) tested with human or recombinant thromboplastins, were affected by severe symptoms (GI in 10, hemarthrosis in 8 and CNS in 1).

These observations lead us to propose a clinical classification based on the results reported in Figure 1. Based on quality and number of symptoms, patients were classified as reported in Table 2.

Features of classified patients and their differences are reported in Table 3. Patient allocation to severity classes was not dependent on age at enrollment ($p = 0.2$ at logistic regression [or-

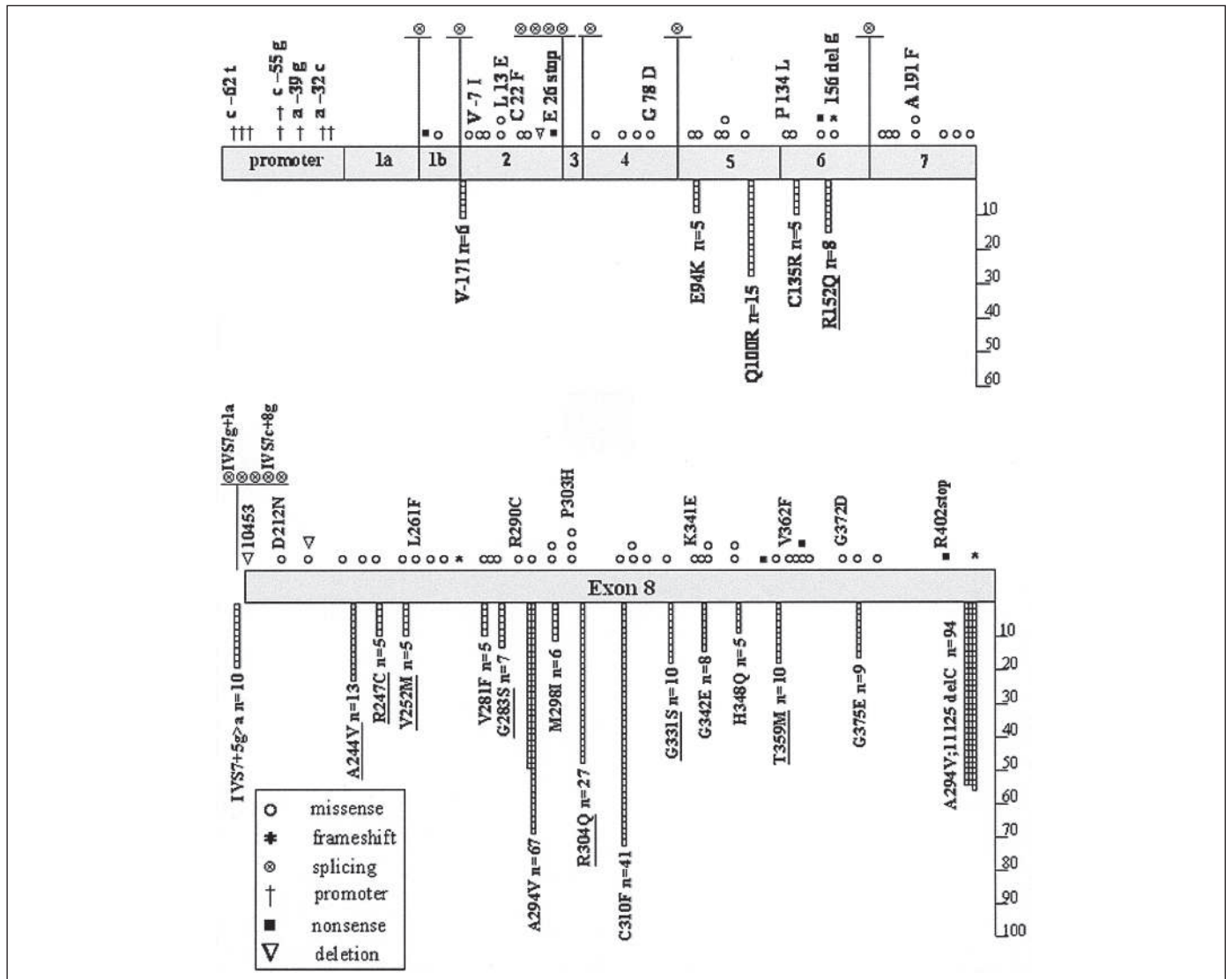


Figure 2: Mutational spectrum. Gray, horizontal bars denote promoter and cDNA segments. Novel mutations are indicated above the bars. Identity and absolute frequencies of mutations detected in more than 5 alleles are reported under the bars; underlined: mutations occurring in CpG sites.

Table 3: Relation of clinical phenotypes with FVIIc and zygosity.

Phenotype (n) Male/female	FVIIc (%) Median (IQR)	Zygosity* (n; %)
		<div style="display: flex; justify-content: space-between; font-size: 0.8em;"> ■ homozygotes ▒ double heterozygotes □ hererozygotes </div>
Severe (90) 45/45	1.4 (1–3.8)	
Moderate (83) 23/60	3.3 (1–21.7)	
Mild (140) 71/69	14 (3–31)	

* χ^2 for distribution of genotypes among classes of severity: 34.9 ($p < 0.0001$). Moderate and mild bleeders also differed ($p = 0.002$). All severity groups showed significantly (Kruskal-Wallis test $p < 0.0001$ $\alpha = 5\%$) different FVIIc median levels.

dinal response model], with age [at enrollment] as independent variable).

Genotype-phenotype relationship

Mutations of the FVII gene were characterized in 313 subjects. Three additional subjects with FVIIc levels of 50% and no causative mutation detected were homozygous for common polymorphisms already known to be associated with decreased FVII levels. In 12 further subjects, no mutation was found. In total, we detected 103 different mutations. In Figure 2 we report the gene distribution and prevalence of changes, including the 22 mutations detected among 10% of patients, which, to the best of our knowledge, have not been previously reported to the FVII mutation database (<http://europium.csc.mrc.ac.uk>) or described in other recent publications. Missense mutations were the most frequent and occurred in 248 subjects (73%), whereas splicing-site, nonsense and small deletion (<17 nucleotides) causing frameshift mutations were found in 25, 12 and 10 individuals, respectively. A frequent allele bearing a double change, the missense Ala294Val mutation and a single nucleotide deletion, 11125delC, occurred in 68 subjects. Twenty-one mutations each occurred in at least 5 patients and, amongst these, 8 were located in CpG sequences (Fig. 2), known to be ‘hot spot’ mutation sites. Among the genotyped subjects, 98 (30.9%), 91 (28.7%) and 128 (40.4%) were homozygous, double heterozygous and heterozygous for FVII mutations, respectively. All the severe patients but one resulted either homozygotes or double heterozygotes

Table 4: Genotype, clinical presentation and FVII levels in homozygotes.

Mutation	N	FVIIAg% Median # (Range)	Phenotype (FVIIc %)			
			Asymptomatic	Mild	Moderate	Severe
A294V; 11125delC	24	1.9 (1–4)	2 (1, 3)	7 (1–1.6)	11 (<1–3)	4 (1–1.9)
A294V	8	48 (37–98)	4 (6–25)	2 (4, 8)	1 (6)	1 (3)
R304Q	8	118 (72–240)	3 (2–24)	4 (<1–37)	1 (22)	
C310F	6	98.5 (80–129)	1 (<1)	2 (<1, 4)	1 (1)	2 (<1, <1)
T359M	4	1.2 (1–2)		3 (<1–5)		1 (1.9)
Q100R	4	16 (10–18)			1 (<1)	3 (<1–<1)
IVS7+5g>a	4	1 (1–1)		1 (2)		3 (1–1.9)
G331S	3	N.a.			3 (<1–<1)	
E94L	2	N.a.	2 (13,21)			
A244V	2	4, 7	1 (7)	1 (3,4)		
IVS2+g3933c	2	13, 13				2 (2, 2)
R277C	2	12°		1 (6)		1 (<1)
G283S	2	N.a.				2 (2.5, 7)
H348Q	2	N.a.			1 (2)	1 (1)
S363I	2	80, 130			2 (<1, <1)	
W364C	2	77, 132				2 (<1, 6)

Mutations found in at least 2 subjects are reported. Mutations found in asymptomatic homozygotes only are not listed. N, Number of homozygotes. FVIIc levels (range) are indicated in parenthesis. Abbreviations: N.a.=not available. °Values reported only for n=4. °Only one value available.

for mutations (Table 3). A similar proportion of homozygotes and double heterozygotes was observed among the affected patients (Table 3) with a similar age at disease presentation (data not shown). The same was true for the other classes of severity.

Carriership of the M2 allele (353Gln), known to be associated with low FVIIc levels (14, 20) was similar in symptomatic (73%) and asymptomatic (69%) patients with FVII deficiency, and particularly among asymptomatic (70%) and symptomatic (79%) heterozygotes.

The influence of the M2 allele on FVIIc levels was apparent only in asymptomatic subjects: FVIIc median levels in homozygotes or doubly heterozygotes were 6% (M2-carriers) and 11.4% (non carriers, $p < 0.05$). Similarly, asymptomatic heterozygotes had levels of 37% (M2-carriers) and 47% ($p < 0.001$).

Table 5: FXa generation in homozygotes for the A294V; 11125delC mutation.

A294V;11125delC homozygotes	Degree of severity	FVIIc (% PNP)	FXa generation (% PNP)
A	Moderate	<1	0.2 ± 0.1
B	Mild	1.2	0.2 ± 0.1
C	Severe	1.8	0.2 ± 0.1
D	Mild	1.0	0.1 ± 0.1
Recombinant A294V;11125delC-FVII	/	/	0.3 ± 0.2*

*% of wild type-FVII. Abbreviations: FXa = factor Xa; FVII = factor VII; FVIIc = factor VII clotting activity; PNP = pooled normal plasma.

Multivariate analysis (sex, zygosity status, FVIIc and FVII antigen and all their two ways interaction), carried out in the 313 symptomatic subjects revealed the following independent variables to be significantly associated with bleeding severity: FVIIc (coefficient -0.0474 , $p=0.03$), and FVII gene zygosity (homozygotes and double heterozygotes vs. heterozygotes, coefficient 1.16 , $p=0.018$).

Whereas double heterozygotes ($n=91$, 14 asymptomatic and 77 symptomatic) were characterized by an extreme mutational heterogeneity and by rare combinations, which hampered an informative genotype-phenotype analysis, homozygotes were useful for this purpose.

Our study provides information on 98 subjects (15 asymptomatic and 83 symptomatic) homozygous for 36 different mutations. Twenty-seven patients were classified as mild, 26 as moderate, and 30 as severe bleeders. The phenotypic features of homozygotes grouped for identical FVII gene mutations that were present in at least 2 patients are provided in Table 4. Frequent mutations (Ala294Val, Ala294Val;11125delC, Cys310Phe) were associated with all clinical classes of severity and neither FVIIc levels (Table 4) nor gender or age provided an explanation for this heterogeneity. Some heterogeneity in clotting activity is explained by the use of different thromboplastins, for instance in Arg304Gln homozygotes (Table 4), tested with human and/or rabbit thromboplastins. However, plasma from patients homozygous for the Ala294Val;11125delC, Ala294Val and Cys310Phe mutations, the most frequent changes (Table 4), were all tested with human (placenta) or recombinant thromboplastins.

When an extrinsic FXa generation assay was performed using plasma samples drawn from 4 patients carrying the same Ala294Val;11125delC mutation and belonging to different severity classes (Table 5), no differences were found. The very low but detectable level of FXa generation observed in plasma was confirmed by the expression of recombinant Ala294Val;11125delC-FVII, obtained through mutagenesis: this recombinant mutant showed a very reduced residual activity level (0.3% of Wt-FVII).

Discussion

While several case series of severe FVII deficiency have been previously reported, this paper is the first to analyze a virtually complete clinical spectrum of this polymorphic disease.

We have also characterized the underlying FVII gene lesions in a large number of cases and found a remarkable number of previously unreported mutations. Throughout our analysis, genotype and zygosity information have been used as a working tool to extract distinct clinical phenotype information.

Multivariate analysis indicates that clotting and clinical phenotypes of double heterozygotes and homozygotes are virtually indistinguishable and that the vast majority of them (85%) are symptomatic.

Our analysis revealed that the most common bleeding symptoms in FVII deficient patients are mild (i.e. hemorrhages of the mucous membranes and skin) (Table 1) and that epistaxis is by far the most frequent presenting symptom. Life-threatening bleeds (CNS or GI, 20% of patients) are distinguished from the

other bleeds by their early presentation (Fig. 1) and are associated with lower FVIIc levels; in detail, they occur most frequently (65% and 70% of the cases) during the first 6 months of life. In newborn patients (< 1 month), symptoms, ranked for frequency, were CNS, GI, cephal-hematoma and umbilical bleeding.

Hemarthrosis in FVII-deficient patients is clinically indistinguishable from that occurring in classical hemophilia, although much rarer and not gender-related. We found hemarthrosis to be most prevalent when children started to crawl or walk: 70% of FVII-deficient patients presenting with joint bleeds were less than 5 years of age. The very low FVIIc levels (median 1%, range 0.9–3%) found in patients with recurrent joint bleeds further support the importance of this symptom as a marker of severity.

Autosomally inherited diseases offer the opportunity to study the effect of gender on the clinical phenotype. We found a higher prevalence of females among symptomatic subjects (Table 1, Fig. 1) and in particular among moderate bleeders (Table 3): much of the excess of bleeding tendency can be attributed to menorrhagia, the most frequent symptom in this gender and, though to a lesser extent, to gum bleeding and easy bruising (Table 1). In FVII deficient females with both genes affected, the prevalence of menorrhagia was 60%, a value similar to that reported for female patients with von Willebrand Disease type III (69%, [21]; 65%, [22]) or factor XI deficiency (67%, [22]). This suggests that FVII deficiency should be considered as a determinant of menorrhagia, especially in the context of a negative pelvic examination finding. Therefore, when invasive procedures are planned in women with excessive genital blood losses, a hemostatic screening should be carried out (an isolated prothrombin time is likely to detect the deficiency).

Classification of patients according to clinical severity would allow for grouping of patients with similar bleeding risk so that therapeutic strategies can be assessed and improved. While in hemophilia classification of severity can be reliably determined by FVIII or FIX levels, our analysis indicates that FVII levels are significant determinants of the degree of severity but cannot be used to create useful cut-off values for clinical purposes. Indeed, a cut-off value (FVIIc 7.44%) was recently (23) proposed, based on 11 severe patients. The use of this cut-off in our sample size would be poorly informative.

Because of this, we have set up an empiric classification based on the early presentation of the life-threatening and crippling symptoms (Table 2). This approach provides a qualitative distinction between ‘severe’ patients and the other patients, who were further classified by the number of “less severe” symptoms. Median FVIIc values, significantly different between mild and moderate bleeders (Table 3), and patient’s zygosity further support our classification. The earliness of presentation in severely affected patients provides an important argument in favour of an early management of the disease, prophylaxis included.

We found that FVII gene polymorphisms, known to lower FVII levels in normals (14), contribute significantly to the levels in the asymptomatic subjects but not in the symptomatic ones. Although these polymorphisms could contribute to clinical heterogeneity, we were unable to detect their influence in clinical phenotypes, which suggests a major clinical impact be played by the severe gene lesions and environmental factors.

The variability in bleeding phenotypes among homozygotes for identical mutations emphasizes the clinical heterogeneity of this disorder. To better understand the genotype/phenotype relationship we performed a more careful evaluation of the clotting activity through a FXa generation assay using a fluorogenic substrate; this sensitive method provided evidence for a substantial degree of functional homogeneity among the Ala294Val; 11125delC homozygotes, despite their differences in clinical phenotypes. Neither plasma assays nor age and gender were able

to explain clinical differences in subjects selected for the presence of identical, homozygous mutations in FVII gene (Tabs 3 and 4). The array of phenotypes in patients homozygous for the same mutation and even for FVII haplotypes (FH and FB, unpublished results), still remains unexplained. These observations point toward the existence of environmental factors and/or of extra-genic (non-FVII gene) components with the capacity of modifying the function of the clotting process and the expressivity of FVII deficiency.

Appendix

The following investigators participate in the Study Group (alphabetical order): K. Auberger-Kurnik, München, Germany; G. Auerswald, Bremen, Germany; V. Aumann, D. Franke, Magdeburg, Germany; C. Barro, Grenoble, France; A. Batorova, Bratislava, Slovak Republic; F. Baudo, Milano, Italy; K. Bergmann, Eisenach, Germany; F. Bernardi, D. Etro, Ferrara, Italy; E. Berntorp, J. Astermark, Malmö, Sweden; A. Chuansumrit, Bangkok, Thailand; N. de Bosch, A. Saez, Caracas, Venezuela; P. De Moerloose, Geneve, Switzerland; D. Di Michele, New York, NY; R. D' Oiron, Paris, France; S. Eber, Zurich, Switzerland; B. Eifrig, F. Bergmann, G. Marx, N. Muenchow, Hamburg, Germany; R. Eisert, M. Barthels, Hannover, Germany; K. Fukutake, Tokyo, Japan; A. Ghirarduzzi, Reggio Emilia, Italy; M. Grundeis, Chemnitz, Germany; C R M Hay, Manchester, United Kingdom; D. C. Heaton, Christchurch, New Zealand; Ch. Heinrichs, Berlin, Germany; M. Heinrichs, Bergen, Germany; F. Hermann, K. Wulff, Greifswald, Germany; P. Iannaccaro, Catanzaro, Italy; J. Ingerslev, Skejby, Denmark; H. Irsfeld, Düsseldorf, Germany; H. Konrad, Rostock, Germany; W. Kreuz, Frankfurt am Main,

Germany; H. Lenk, H. Scheel, Leipzig, Germany; R. M. Loreth, Kaiserslautern, Germany; H. Losonczy, Pecs, Hungary; B. Maak, Saalfeld, Germany; G. Mariani, S. Siragusa, Palermo, Italy; E. Meili, Zurich, Switzerland; P. A. Ockelford, Auckland, New Zealand; L. Papayan, St. Petersburg, Russia; S. Pipe, Ann Arbor, USA; F. Peyvandi, Milan, Italy; H. Pollmann, J. Horst, Münster, Germany; J. Pschirrer, Willmering, Germany; A. Rocino, Napoli, Italy; C. Rothschild, Paris, France; J.F. Schenk, E. Wenzel, Homburg, Germany; R. Schobess, Halle, Germany; M. Serban, Timisoara, Romania; S. Shulman, Stockholm, Sweden; JF. Schved, M. Giansily-Blaizot, Montpellier, France; A. Street, Prahan, Australia; A. H. Sutor, Freiburg, Germany; G. Syrbe, Stadtroda, Germany; A. Tagliaferri, Parma, Italy; A. Till, Adelaide, Australia; Th. Valerius, Erlanger, Germany; G. Vogel, E. Bratanoff, Erfurt, Germany; D. Vollmann, Aschersleben, Germany; I. Warriier, Detroit, USA; N. Weinstock, Karlsruhe, Germany; J. Wendisch, U. Platzbecker, Dresden, Germany.

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