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ARTICLE

Analysis of the genotypes and phenotypes of 37 unrelated patients with inherited factor VII deficiency

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Severe inherited factor VII (FVII) deficiency is a rare autosomal recessive disorder with a poor relationship between FVII coagulant activity and bleeding tendency. Both clinical expression and mutational spectrum are highly variable. We have screened for mutations the FVII gene of 37 unrelated patients with a FVII coagulant activity less than 5% of normal pooled plasmas. The nine exons with boundaries and the 5′ flanking region of the FVII gene were explored using a combination of denaturing gradient gel electrophoresis and direct DNA sequencing. This strategy allowed us to characterise 68 out of the 74 predicted FVII mutated alleles. They corresponded to a large panel of 40 different mutations. Among these, 18 were not already reported. Genotypes of the severely affected patients comprised, on both alleles, deleterious mutations which appeared to be related to a total absence of activated FVII. We suggest that this absence of functional FVII can explain the severe clinical expression. Whether a small release of FVII is sufficient to initiate the coagulation cascade and to prevent the expression of a severe phenotype, requires further investigations. *European Journal of Human Genetics* (2001) **9**, 105–112.

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Introduction

Human coagulation factor VII (FVII) is a vitamin-K-dependent glycoprotein that consists of a 406 amino-acid modular protein with a N-terminal-gamma-carboxyglutamic acid rich (Gla) domain, two epidermal growth factor-like (EGF) domains and a C-terminal serine proteinase domain. After cleavage of an Arg-Ile peptide bond at residues 152/153, FVII is converted to the active form, factor VIIa (FVIIa) comprising two chains connected by a disulphide bridge between Cys 135 and Cys 262. FVIIa on its own has virtually no catalytic activity but triggers the coagulation at sites of injury by binding to the cell surface receptor tissue factor (TF) in the presence of calcium ions, ultimately resulting in thrombin generation and the formation of a fibrin clot.[1]

Inherited FVII deficiency is a rare autosomal recessive disorder with an estimated incidence of 1 in 500 000. The bleeding tendency in affected patients is highly variable and correlates poorly with plasma FVII activity levels.[2] The clinical features vary considerably, from easy bruising, epistaxis, modest or severe postoperative bleeding, to life-threatening intracranial haemorrhages.[1–3] On the other hand, several asymptomatic patients with a FVII coagulant activity level less than 1% of normal have been reported.[1] In addition, up to 70 different mutations underlying the FVII deficiency phenotype have already been described in the

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human FVII mutation database (http://europium.csc.mrc.ac.uk/usr). Study of the heterogeneous molecular basis of FVII deficiency offers the opportunity to investigate the mechanisms through which genotype is able to modulate the residual FVII activity and to produce a large spectrum of clinical phenotypes. For this purpose, we have collected clinical features and characterised the FVII genotypes of a large cohort of patients displaying FVII coagulant activity less than 5% of normal pool plasmas. This study is one of the largest ever reported on severe FVII deficiency.[4–6]

Materials and methods

Patients

The panel consisted of 37 unrelated patients (22 females and 15 males) with inherited FVII deficiency attending 16 French centres specialising in the follow-up of haemorrhagic disorders. Patients who entered the study were selected on the basis of a factor VII coagulant activity level less than 5% of normal (Table 1). All patients gave written informed consent in accordance with the French law. A family history of consanguinity was known for six patients.

Clinical and biological data

Clinical features were recorded by the physician in charge of each patient in a standardised questionnaire. Collected data focused on the diagnostic circumstances and on the bleeding tendency. The type, severity and frequency of the haemorrhagic symptoms were noted. The FVII coagulant activity (FVIIc) was assayed in each centre by a one-stage method based on the prothrombin time using thromboplastin reagents and FVII deficient plasma as a substrate. Factor VII antigen (FVIIAg) was determined using the Asserachrom FVII:Ag Kit (Diagnostica Stago, Asniere, France) according to the manufacturer's instructions (Table 1).

Blood collection

Blood samples were obtained from the 16 centres and mailed to the laboratory at room temperature within 24 h, for DNA extraction.

DNA isolation and conditions for polymerase chain reaction

Genomic DNA was isolated according to standard methods. The coding portions, intron/exon boundaries and the 5′ flanking region (5′FR) of the FVII gene were amplified from genomic DNA by polymerase chain reaction (PCR). Primer sets were designed according to the published FVII gene sequence[7] and are listed in Table 2.

Mutation detection by denaturing gradient gel electrophoresis (DGGE)

DGGE was used to screen for sequence alterations, PCR fragments containing the portions of interest of FVII gene. MELT 87 and SQHTX computer programs,[8] which allow simulation of the melting behaviour of any DNA sequence, were used to optimise the DGGE conditions. To ensure that the DNA portion of interest was within the lowest melting temperature domain, a GC rich region was added at one end of the fragment to be screened as described.[8] As the melting map of exons 1a, 2, 3, 4, 6 and 7 did not easily allow the use of DGGE, these PCR fragments were directly sequenced. DGGE was performed for the mutation screening of exons 1b, 5 and 8. Three sets of primers were used to independently amplify the three melting domains of exon 8, generating fragments 8A, 8B and 8C. Migration gels consisted of 6.5% acrylamide in a linear 50–90% denaturing gradient and were run as previously described[9] (Table 2). Samples displaying abnormal DGGE profiles were sequenced.

Mutation detection by direct sequencing

PCR amplified fragments for coding regions 1a, 2, 3, 4, 6, 7, exon-intron boundaries, 5′FR and samples with an abnormal DGGE pattern were directly sequenced using the Big dye Terminator sequencing kit (Perkin Elmer, Norwalk, CT, USA) on an Applied 310 sequencing apparatus (PE, Applied Biosystems,Warrington, UK).

Mutation screening strategy

In this study, we have made an exhaustive genetic analysis of all FVII regions of interest so as not to miss double-mutations or polymorphisms that could be involved in the phenotypic variability. For each patient, the sequences of the nine exons and corresponding boundaries of FVII gene were analysed. Each nucleotide sequence alteration was confirmed on at least two independent PCR products by either restriction enzyme digestion, when the mutation modified a restriction site, or another independent sequencing analysis. If no mutation was found after the first screening-step, exons 1b, 5 and 8 were tested for heteroduplex formation under the same DGGE conditions, and the 5′FR was sequenced. If one or two mutations were still missing, exons 1b, 5 and 8 were then entirely sequenced.

Results

Clinical and biological phenotypes

The 37 patients investigated in this study were classified into three clinical groups (Table 1). Group A comprised asymptomatic patients with no bleeding history (n=15). Group B included patients who experienced menorrhagia, epistaxis or bruising with no need for substitutive therapy (n=13). Group C comprised patients with a history of severe bleeding (haematoma, haemarthrosis, intracranial haemorrhages) (n=9). The severe disease group comprised only females, whereas the mild disease groups included 15 males and 13 females. No thromboembolic episode was related.

Table 1 Identified phenotypes and genotypes of the 37 FVII deficient patients

				Biological phenotypes (%)		*Genotypes and haplotypes*		
Lab number	*Sex*	*Non French origin*	*Clinical group*	*FVII c*	*FVII Ag*	*Allele 1*	*Allele 2*	*FVII haplotypes*
F7-11	F		A	<1	64	**Gly331→Ser**	Gln100→Arg	(A1 H1 h6 I1 M1) X2
F7-16	M	N Afr	A	<5[a]	25	**Ala191→Thr**	**Ala191→Thr**	(A1 H1 h6 I1 M1) X2
F7-22	M		A	2[a]	72	Arg304→Gln	Arg304→Gln	(A1 H1 h6 I1 M1) X2
F7-24	M	N Afr	A	5[a]	44	Arg304→Gln	Ala244→Val	A1A1 H1H1 h6h7 I1I2 M1M2
F7-34	F		A	<5[a]	55	Arg304→Gln	Cys135→Arg	A1A1 H1H1 h6h7 I1I1 M1M1
F7-39	M		A	3[a]	10	Ala244→Val	**Gly179→Arg**	A2A2 H1H2 h7h7 I2I2 M2M2
F7-29	M	N Afr	A	1	9	Met298→Ile	Cys310→Phe	(A1 H1 h6 I1 M1) X2
F7-12	F	N Afr	A	2[a]	na	Arg304→Trp	Arg304→Trp	(A1 H1 h6 I1 M1) X2
F7-33	F		A	<1	na	**10543 del gcgagcacgacctca**	Cys310→Phe	(A1 H1 h6 I1 M1) X2
F7-38	F		A	<5[a]	na	Arg304→Gln	Arg304→Gln	(A1 H1 h7 I1 M1) X2
F7-40	M		A	<1[a]	na	Cys310→Phe	Cys310→Phe	(A1 H1 h6 I1 M1) X2
F7-17	M	N Afr	A		37	Met298→Ile	wt	A1A1 H1H1 h6h7 I1I1 M1M1
F7-31	F		A	2	19	**Cys102→Tyr**	wt	A1A1 H1H1 h6h7 I1I1 M1M1
F7-37	M	A		5[a]	7	Ala244→Val	wt	(A1 H2 h7 I2 M2) x2
F7-15	M	N Afr	A	1	<2	wt	wt	(A1 H1 h7 I1 M1) x2
F7-41	F		B	4[a]	48	11125 delC and Ala294→Val	Ala294→Val	(A2 H2 h7 I2 M2) x2
F7-2	F		B	<1	8	Gln100→Arg	Gly97→Cys	A1A1 H1H2 h6h7 I1I2 M1M2
F7-9	M	N Afr	B	<1	47	**Met327→Ile**	**Met327→Ile**	(A1 H1 h6 I1 M1) X2
F7-25	F	N Afr	B	5	76	Met298→Ile	Met298→Ile	(A1 H1 h6 I1 M1) X2
F7-1	M	N Afr	B	<1	6	**Arg379→Gly**	**Arg379→Gly**	(A2 H2 h7 I2 M2) x2
F7-4	F		B	2	62	64+5G→A	**Gly179→Arg**	A1A1 H1H1 h6h7 I1I1 M1M1
F7-5	M		B	<1	17	3933+1G→A	**3762G→A**	(A1 H1 h6 I1 M1) x2
F7-6	M		B	3	23	Arg304→Gln	**Gly180→Arg**	A1A2 H1H2 h6h7 I1I2 M1M2
F7-8	M		B	2[a]	15	**10743 delG**	Gly96→Ser	(A1 H1 h6 I1 M1) X2
F7-10	F		B	2[a]	na	11125 delC and Ala294→Val	**Leu13→Gln**	(A1 H2 h7 I2 M2) x2
F7-27	M	Swiss	B	2[a]	na	Arg277→Cys	**Arg28→Gly**	A1A2 H1H2 h7h7 I1I2 M1M2
F7-42	F		B	5[a]	na	Met298→Ile	**Trp364→Stop**	(A1 H1 h6 I1 M1) X2
F7-19	F		B	2	na	**Met1→Val**	wt	A1A1 H1H2 h6h7 I1I2 M1M2
F7-13	F		C	2	7	6070+1G→A	Cys135→Arg	(A1 H1 h6 I1 M1) x2
F7-20	F		C	<1	25	**5886+5G→A**	Glu16→Lys	A1A2 H1H1 h6h7 I1I1 M1M1
F7-26	F		C	<1	2	**Asn57→Ile**	**Asn57→Ile**	(A1 H1 h6 I1 M1) x2
F7-30	F		C	<1	65	Phe328→Ser	**Asp343→Asn**	(A1 H1 h6 I1 M1) X2
F7-32	F		C	<1[a]	na	11125 delC and Ala294→Val	Arg152→Gln	A1A1 H1H2 h7h7 I1I2 M1M2
F7-14	F		C	1	<2	Thr359→Met	Thr359→Met	(A1 H1 h6 I1 M1) X2
F7-18	F		C	<1[a]	na	**Gln49→Stop**	Gln100→Arg	(A1 H1 h6 I1 M1) X2
F7-23	F	Asian	C	2	2	His348→Gln	His348→Gln	(A1 H1 h6 I1 M1) X2
F7-35	F		C	2[a]	na	−61T→G	−55C→T	A1A1 H1H1 h6h7 I1I1 M1M1

The FVIIc column corresponds to the FVII coagulant activity levels. The recombinant human TF (Instrumentation laboratory, Lexington, USA) was used for the coagulant assays except for the samples denoted with a superior ([a]), which were tested with Thromboplastins from human or rabbit origins. The corresponding FVII haplotype is associated with each mutation when possible. A1: allele 73G, A2: allele 73A, H1: allele His 115 CAC, H2: allele His 115 CAT, h6: allele including 6 × 37 bp repeats at IVS7, h7: allele including 7 × 37 bp repeats at IVS7, I1: allele 10523G, I2: allele 10523A, M1: allele Arg 353 CGG, M2: allele Gln 353 CAG.[10–13] The mutations which are not reported in the FVII mutation database 2000, are indicated in bold. The numbering is according to O'Hara *et al*.[7] 'wt' indicates a wild type sequence in the screened area. 'na', data not available. 'N Afr' indicates origins from North Africa.

FVII antigen levels could be determined in 26 patients (Table 1). Four patients were classified as 'cross-reacting material negative' (CRM−) as they had very low or absent antigen levels (2% or less). Seventeen patients had reduced antigen levels (CRM^{red}). Five patients displayed normal antigen levels (>60%) (CRM+), maybe due to the presence in the plasma of functionally altered FVII molecules.[2] As can be seen in Table 1, none of these antigen-groups was related to the clinical distribution. For example, the clinically severely affected patient F7-30 had normal FVII antigen levels (65%) whereas an asymptomatic subject, F7-15, can have very low FVII antigen levels (<2%).

FVII mutations screening

DGGE analysis allowed us to characterise 46 out of 74 mutated alleles. Figure 1A,B shows typical electrophoretic patterns of different mutations within exons 5 and 8B respectively. All the PCR products displaying an abnormal DGGE pattern revealed a sequence alteration. The other exons were explored by direct sequencing and showed 22 additional gene defects. After this first screening-step, one or two mutations were still missing in five patients (F7-15, F7-17, F7-19, F7-31 and F7-37). Direct sequencing of the 5'FR did not allow the detection of another sequence alteration. We then re-examined the three exons previously explored by

Table 2 Oligonucleotides used to amplify FVII exons, PCR and denaturing gradient gel electrophoresis conditions

Exon	*5′ oligomer (5′-3′ sequence)*	*3′ oligomer (5′-3′ sequence)*	*Product size (bp)*	*AT (°C)*	*DGGE running time*
5FR	tatttacatccacacccaag	tgcctgttgacattccccat	400	56	
1a	ccatccctctgtcacccttg	atttgcccactgcccttcca	210	58	
1b	[a]agtaggggtgtggcgtgag	tgggaggggaaggaggtgat	289	64	5 h
2	aggatgggcgaacggggtgg	ccgcagccaaagagacgcag	444	60	
3 and 4	cgttgggtgctctggtgaag	ccctgaatgcccggcctaca	386	62	
5	[a]agaacaccactgctgaccca	tcccacccgtcttttgtcca	323	60	6 h 30 min[b]
6	cctgccttccaccacccctg	gctgacctgcccatttttcccct	369	66	
7	gagggcgagtcatcagagaa	tgagacacttgagagctgcg	421	62	
8A	gtgaggtggcaggtggtgga	cgttcgggcaggcagagggg[a]	222	64	4 h[b]
8B	[a]gcaccaccaaccacgacatc	ccttgctgccatccgagtag	303	62	10 h
8C	[a]gcagcagtcacggaaggtgg	tgccctcctctaccccatta	391	60	11 h

A typical 100 μl PCR reaction contained, 1 μg genomic DNA, 20 pmol of each oligonucleotide primer, 200 μM of each desoxynucleotide triphosphate (Pharmacia, Uppsala, Sweden), 1.5 units of Taq DNA polymerase and its buffer (Ampli TaqR, Perkin-Elmer Cetus, Norwalk, CT, USA). For the PCR reactions of both exons 1b and 7, the buffer was replaced by a mix containing 16.6 mM ammonium sulphate, 67 mM Tris-Hcl pH 8.8, 6.7 mM magnesium chloride, 67 μM Na_2EDTA, 170 μg bovine serum albumin per ml and 10 mM β-mercaptoethanol. [a]Indicates the primer-end with the additional GC clamp (cgcccgccgcgccccgcgcccgtcccgccgcccccgcccc). AT: annealing temperature. [b]Indicates that a 1 h-run prior to loading was applied.

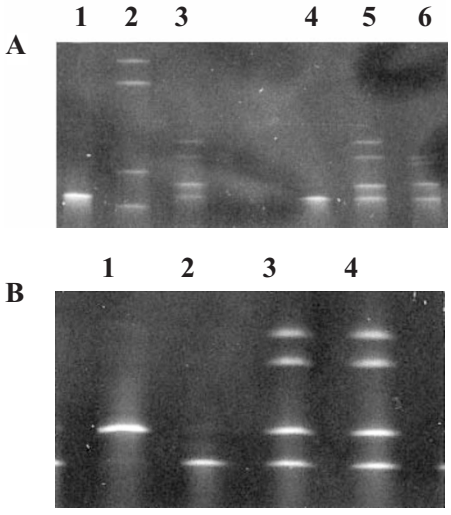

Figure 1 Denaturing gradient gel electrophoresis patterns of FVII mutations. (**A**) exon 5 DGGE pattern. Lane 1: Normal pattern (patient F7-34), lane 2: heterozygote for two mutations (Gln100→Arg and Gly97→Cys) and for the 115His dimorphism (patient F7-2), lane 3: heterozygote for the 115His dimorphism (patient F7-27), lane 4: normal pattern (patient F7-33), lane 5: heterozygote for the 115His dimorphism (patient F7-32), lane 6: heterozygote for the Cys102→Tyr mutation (patient F7-31). (**B**) exon 8B DGGE pattern. Lane 1: homozygote for the Arg304→Gln mutation (patient F7-22), lane 2: normal control, lanes 3 and 4: heterozygotes for the Arg304→Gln mutation (patients F7-24 and F7-34 respectively).

DGGE for these patients. However, direct sequencing of exons 1b, 5 and 8 failed to detect any sequence alteration.

FVII mutation spectrum of 37 unrelated patients with severe inherited FVII deficiency

Among the 68 identified mutated alleles, 40 different potential gene defects were detected. Twenty-two out of these 40 sequence alterations were mutations already described, while 18 had not been previously reported[1,4,6] (Table 1). More than a half of these sequence abnormalities (23/40) were located within both exons 2 and 8, whereas no mutation was found in exons 1b and 3. Only two mutations, affecting residues Cys and Arg at positions 135 and 152 respectively, were detected in exon 6 (Figure 2). The overwhelming majority of the identified lesions, except a short 15 base-pairs deletion, were single base substitutions.

Genotype distribution of FVII gene mutations (Table 1)

Twenty-five out of 37 patients were compound heterozygous for two FVII mutations, whereas 11 were found to be homozygotes. One patient still has an undetermined genotype. Among the 11 homozygous patients, six had a known family history of consanguinity (patients F7-1, -12, -14, -16, -23, -38). A seventh patient (F7-22) is also probably homozygous for the Arg304→Gln, a relatively frequent missense mutation. His parents could bear the same Arg304→Gln missense mutation at the heterozygous state by accident. As the parents' data were not available, the remaining four homozygous patients (F7-9, -25, -26, -40) can be either real homozygotes or compound heterozygotes for the identified mutation and a large deletion on the other allele. F7-10, F7-32 and F7-41 patients showed an unusual genotype including three different mutations (Table 1). The 11125 delC frameshift mutation has been shown to be frequently associated in *cis* with the Ala294→Val missense mutation in the Polish.[5] The DNA from these patient's families was not available to further characterise their genotype. However, we can assume that both 11125delC and Ala294→Val mutations probably lie on the same chromosome in these patients, whereas the third mutation represented the second mutated allele.

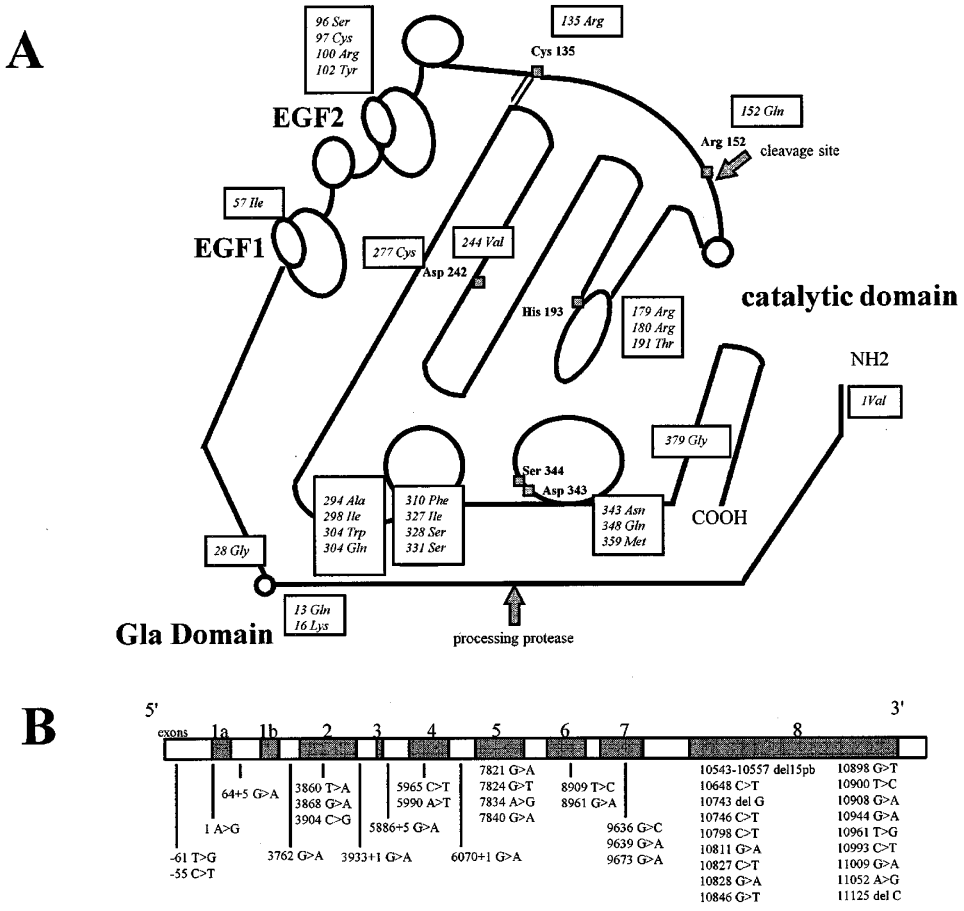

Figure 2 Mapping of the 40 FVII mutations that were identified in this study. (**A**) Mapping of the FVII missense mutations on the FVII molecule. The 28 FVII missense mutations are noted in italics. Critical residues of the FVII activation pathway and the typical residues of the catalytic triad are noted in bold type. (**B**) Location from the 5′ to the 3′ end of the FVII gene of the 40 different sequence alterations.

Polymorphisms and haplotypes associated with FVII mutations

The extensive analysis of the coding region and splice sites of FVII gene allowed us to detect eight sequence variations with apparently no disease causative effect. Six were already published common polymorphisms[10–13] whereas two were still undescribed. A Gln35 dimorphism was characterised in exon 2 at the heterozygous state in patient F7-33 and at the homozygous state in patient F7-40. Both patients F7-33 and F7-40 shared the same Cys310→Phe missense mutation. A C to T substitution at nucleotide 3954 in intron 2 was identified once at the homozygous state in patient F7-23 who is of Asian origins. This polymorphism has not been found in a 50 chromosomes control group. The most common polymorphisms were used to construct FVII haplotypes associated with each mutation when possible (Table 1).

Discussion

We have screened for mutations the entire coding regions and boundaries of FVII gene in 37 unrelated patients with inherited FVII deficiency. Among the 74 predicted FVII mutated alleles, 68 were characterised and corresponded to 32 complete FVII genotypes.

Strategy for the identification of FVII mutations

DGGE was chosen as the first screening step because of its reliability and of the large number of patients who can be simultaneously investigated. No mutation evaded detection in our study as far as we could check. DGGE could not be applied to the entire coding sequence because the base composition of FVII gene is GC rich with a 60% G+C content.[7] This characteristic is common to other vitamin K dependent factors such as factor X or Protein C.[7] This explains why gradients with high percentages of denaturant as well as long running times were necessary to accurately identify FVII mutations using DGGE. However, DGGE screening allowed the detection of 46 out of the 68 FVII mutated alleles (68%), corresponding to the complete genotypes of one third of the patients (13/37). DGGE is useful when studying large series of patients and should be first applied for the frequently mutated exons 8 and 5.

Furthermore, DGGE can be also performed as an individual screening tool, using a sequential loading on the same running gel.

FVII gene mutations spectrum in FVII deficiency

The overwhelming majority of the FVII mutations are single nucleotide variations according to the literature data. Short deletions (2–30 nucleotides) are rare on FVII gene. One was identified in this study, and another has been previously described.[1,14] Both are closely located, at the beginning of exon 8 which is a known region for nucleotide sequence repetitions. The single point mutations are distributed throughout the FVII gene suggesting that almost every domain of the molecule is essential for maintaining its overall structure and specific function. Only six gene defects could not be detected, despite an extensive screening. These missing mutations might occur within unexplored intronic regions and activate a competitive cryptic splice site. Further studies on illegitimate RNA transcripts would be useful to characterise the genetic mechanisms underlying the FVII deficiency, for these patients. Another possibility might be the existence of a large deletion or a gross gene rearrangement which would evade detection by DGGE or direct sequencing. Finally, the modification of an alternative locus which may control FVII levels has been suggested.[15]

Phenotype to genotype relationship in clinically severe FVII deficiency

Both FVII clotting activity and FVII antigen are known to be poorly correlated to the bleeding tendency in inherited FVII deficiency.[16] On the other hand, in many reports, severe mutations preventing synthesis of any amount of FVII protein have been shown to be associated with life-threatening haemorrhages.[16–18] This is in agreement with FVII gene knock out experiments in mice. These mice frequently die during the first day after birth because of fatal haemorrhages.[19] In our study, most of the genotypes of patients with severe bleeding diathesis, appeared to comprise on both alleles mutations preventing the production of a functional FVII molecule. Actually, in the clinically severe group C, 15 out of the 16 mutated allele can be considered as deleterious mutations on the basis of different mechanisms.

Alteration of the FVII activation pathway Three previously described missense mutations and one novel sequence-alteration occurred at residues implicated in the activation pathway of FVII. The Arg152→Gln occurs at the site that must be cleaved to generate activated FVII.[20] The rate of the 328Ser mutant activation by activated factor X was shown to be markedly reduced.[21] The 135Arg mutant[4] destroys the unique disulphide bridge, connecting the light and heavy chains of activated FVII. The novel Asp343→Asn mutation, involves a critical and highly conserved residue. After proteolytic activation of the factor VII zymogen, the β-carboxyl group of asparatic acid residue 343, adjacent to the active serine residue 344, forms the trypsin typical salt bridge with the newly exposed NH2-terminal alpha amino group of Ile 153. This salt bridge stabilises the active conformational state of the serine protease domain.[22] Thus, the residue Asp343 appears to be essential for the catalytic activity of the complex FVIIa/TF.

Secretion defect Both previously described 359Met and 348Gln mutants are sequestered in the protein secretory pathway.[23–24] In the same way, the FVII Arg100 mutant displays an abnormal conformation leading to a major, secretion defect.[25] The novel Asn57→Ile mutation lies within the N-terminal EGF like domain at an evolutionary conserved residue. A Asn57→Asp mutant has been reported by Leonard *et al*.[26] Because of the loss of an important intra-molecular hydrogen bond between Asn57 and Cys81, these mutations could alter the folding of the first EGF domain. In the Asn57→Asp model, this substitution resulted in a marked decrease in FVII mutant protein secretion with the loss of both procoagulant activity and TF binding.[26]

Generation of a truncated or elongated protein The frameshift 11125delC mutation has been reported at the homozygous state, in *cis* with the Ala294→Val substitution, in several subjects with moderately severe to severe bleeding diathesis.[5] The 6070+1 G→A transition occurs at the invariable dinucleotide splice site of intron 4.[17] Concurrently the newly described substitution of a C by a T at position 5965 generates a premature termination codon TAG at position 49 in the first EGF domain. The truncation of the EGF2 and catalytic domains, even if some amount of the mutant protein is still translated, would lead to an inactive polypeptide. The 5886+5 G→A transition occurred at the nucleotide position +5 at the 5′ splice site of intron 3. The frequency of a G at position +5 of the consensus splice site scored 0.84 whereas an A at the same position scored 0.05.[27] As an A is extremely rare at this position, the G to A transition may lead to the skipping of the corresponding exon or the activation of a cryptic splice site in the vicinity. A similar mutation in the FVII intron 7 (9726+5 G→A) has been found to disrupt the usual 5′ donor splice site.[28]

Expression defect Two mutations have been shown to alter FVII expression *in vitro*. Both −61 T→G and −55 C→T mutations occurred at the binding site of the transcription factor: hepatic nuclear factor 4 (HNF4).[29] The −61 T→G mutation completely abrogated gene expression in reporter gene assays,[18] whereas the −55 C→T mutation exhibited only 10% of expression level *in vitro*.[30]

Finally, the sixteenth Glu16→Lys mutated allele associated with a clinically severe phenotype, has an hypothetical severity. This mutation has a FIX counterpart (Glu17→Lys) causing severe haemophilia B,[31] but the phenotypic con-

sequences of the FVII 16Lys variant have to be further explored.

Thus in the severe group, both mutated alleles of eight out of nine patients could be related to severe FVII mutations, suggesting that the severe phenotypes could be related to the total absence of FVIIa. Conversely, this is not the rule in both mild or asymptomatic groups. In these groups, one severe mutation can be found occasionally but not on both alleles. In these cases, the other allele, that we could call 'mild', may allow the release of a minute amount of FVII protein that could be sufficient to trigger the haemostatic cascade. Conventional clotting assays may not be sensitive enough to detect the residual FVII activity. Whether a small release of FVII could be sufficient to prevent the expression of severe phenotypes needs further analyses. FVII variants from both A and B groups are currently under investigation to throw light on the structure-function relationships of FVII and on new prognostic parameters for bleeding tendency in severe inherited FVII deficiency.

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