

## Molecular basis of hereditary factor VII deficiency in India: five novel mutations including a double missense mutation (Ala191Glu; Trp364Cys) in 11 unrelated patients

**We have studied the molecular basis of factor (F) VII deficiency in 11 unrelated Indian patients. Mutations were identified in all 11 and included 5 missense, 2 nonsense and a frame shift mutation. Five of these were novel. These mutations were considered to be causative of disease because of their nature, evolutionary conservation and molecular modeling. This is the first report of mutations in patients with FVII deficiency from southern India.**

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Factor VII (FVII) (OMIM: 227500) deficiency is a rare (1:500,000) autosomal recessive disorder of blood coagulation caused by heterogeneous mutations (~140) in FVII gene.<sup>1</sup> We describe the molecular abnormalities in the FVII

gene of south Indian patients with FVII deficiency and their genotype-phenotype correlations.

The clinical and phenotypic data of the 11 unrelated patients studied are detailed in Table 1. Genomic DNA was screened for mutations in FVII gene by PCR (Table 2), conformation sensitive gel electrophoresis (CSGE)<sup>2</sup> and DNA sequencing (ABI 310 genetic analyzer, Applied Biosystems, Foster city, CA, USA). The potential effects of missense substitutions were modeled by SwissPdb Viewer based on the three-dimensional structure (PDB: 1dan) for the wild-type FVII: tissue factor complex.<sup>3</sup>

We identified 8 different mutations and 6 (g.-323A1/A2, g.-122T→C, g.73G→A, g.7880C→T, g.10523G→A, g.10976G→A) previously reported polymorphisms<sup>1</sup> in the 11 patients. The mutations included 5 missense, 2 nonsense and 1 frame shift of which five were novel (Table 1).

A novel p.Leu-55fs identified in two patients (33 and 114, FVII: C <1%) predicts a premature termination codon (PTC) at residue -15 in the propeptide region. This mutant protein may not be generated due to nonsense-mediated decay of the mRNA carrying the PTC. Similar null mutations (p.Leu-52fs) resulting in the complete absence of FVII in plasma but not incompatible with life have been previously described.<sup>4</sup>

**Table 1.** Clinical, hematologic and genetic data of patients with hereditary factor VII deficiency.

UPN	AOI <sup>a</sup> /Sex	Cor <sup>b</sup>	Sites of bleeding	FVII:Cc	Residue <sup>d</sup>	Location	Nucleotide <sup>d</sup>	Codon change	Aminoacid change	Domain	Reported Novel <sup>e</sup>	Restriction site altered <sup>f</sup>	Haplotype <sup>g</sup>
13	1/Male	Yes	Umbilical stump gastrointestinal, dental extraction	<1%	227	exon 8	10596	CAG→TAG (homozygous)	Gln→X	Catalytic	Novel	HhaI-	A1A1-GG-GG-GG
33	29/Male	Yes	Epistaxis, hemetemesis	<1%	-55	exon 1a	16delC		Leu→fs	Prepropeptide	Novel	StuI+	A1A1-GG-GG-GG
46	33/Female	No	Epistaxis, menorrhagia	21%	-1	exon 2	3820	CGC→TGC (homozygous)	Arg→Cys	Prepropeptide	Novel	AscI-	A2A2-GG-GG-GG
60	13/Female	No	Umbilical stump, epistaxis, gastrointestinal, hemarthroses, gingival	<1%	191	exon 7	9673	GCG→GAG (homozygous)	Ala→Glu	Activation	Novel	SacII-	A1A1-GG-GG-GG
63	1/Male	Yes	Umbilical stump, epistaxis, gastrointestinal, gingival, hemarthroses, central nervous system	<1%	382	exon 8	11061	CAG→TAG (homozygous)	Gln→X	Catalytic	Novel	NA	A2A2-GG-GG-AA
101	16/Female	No	Gingival, hemarthroses, hematoma, dental extraction	<1%	227	exon 8	10596	CAG→TAG (homozygous)	Gln→X	Catalytic	Novel	HhaI-	A1A1-GG-GG-GG
105	35/Female	No	Gingival, hemarthroses	<1%	227	exon 8	10596	CAG→TAG (homozygous)	Gln→X	Catalytic	Novel	HhaI-	A2A2-AA-GG-GG
106	13/Female	Yes	Gingival, menorrhagia	<1%	191 364	exon 7 exon 8	9673 11009	GCG→GAG TGG→TGC homozygous for double mutation	Ala→Glu Trp→Cys	Activation Catalytic	Novel Reported	SacII- Sau96I-	A2A2-GG-AA-AA
114	11/Female	No	Gingival, hemarthroses, umbilical stump bleeds	<1%	-55	exon 1a	16delC		Leu→fs	Prepropeptide	Novel	StuI+	A1A1-GG-GG-GG
120	10/Male	Yes	Gingival, epistaxis	<1%	117	exon 5	7884	GGG→AGG (homozygous)	Gly→Arg	EGF2	Reported	NA	A2A2-AA-AA-AA
128	15/Female	Yes	Umbilical stump, epistaxis, gingival, hemarthroses, central nervous system	<1%	152	exon 6	8961	CGA→CAA (homozygous)	Arg→Gln	Activation	Reported	NA	A1A1-GG-GG-GG

<sup>a</sup>Age at first investigation; <sup>b</sup>consanguinity status; <sup>c</sup>factor VII coagulant (FVII: C) activity. <sup>d</sup>Nucleotide and amino acid numbering according to O'Hara et al, 1987 10 and NCBI nucleotide accession number J02933. <sup>e</sup>Novel mutations/polymorphisms, not reported previously in factor VII mutation database 1. <sup>f</sup>(+) sign indicates that a mutation creates a restriction site; (-) indicates that the mutation abolishes a restriction site for the restriction enzyme; NA- restriction enzyme not available. <sup>g</sup>Haplotype construction based on promoter -323 decanucleotide insertion (A2), no insertion (A1), intron 1a 73G→A, intron 7 10523G→A, exon 8 10976C→T (Arg353Gln) polymorphisms.

**Table 2.** Primers used for amplification and sequencing of factor VII gene.

Exon	Forward primer (5'-3')	Reverse primer (5'-3')	Region of FVII gene amplified*	Amplicon size (bp)	Annealing temperature (°C)
Promoter+1a	GCATGATTGCTATGGGACAA	CTGCCCTTCCACCAAGTTTA	g.383 - g.108	491	58
1b	GTGGGCTGTGAGGGACAGT	GCAGGGAACACCCTCCTT	g.1047-g.1328	282	58
2	GTGGGCGGTGGGGCGGTCTC	GCCCCACGGCCCTGGTTCA	g.3699-g.4000	302	64
3+4	TGGTGTGCTCAGTGCTTACC	CAATTTCCAACCTGGGCTGAG	g.5787-g.6211	425	58
5	CTTCCAGGCAGAACACCACT	ATCCCACTCACAATTGGTC	g.7679-g.8066	388	58
6	CTCAGAGGATGGGTGTTTCTG	TGCTAGGTGTGCTGACTTGG	g.8810-g.9139	330	58
7	AGGGCGAGTCATCAGAGAAA	AGTGGTACCCACCCAGCAC	g.9538-g.9923	386	64
8A	CTTGCCCCAGAAGGAGACT	TCTCCACCTTCCGTGACT	g.10422-g.10873	452	58
8B	CTGGAGCTCATGGTCTCA	TGCCCTCTCTACCCCATTA	g.10800-g.11240	441	58

\*Nucleotide numbering according to O'Hara et al.<sup>10</sup>

A common founder for p.Leu-55fs is likely as both patients had similar haplotype (Table1). A novel p.Arg-1Cys (Patient 46, FVII: C- 21%) occurs at a cleavage site (Arg-1 and Ala+1) for a processing protease thereby disrupting the removal of pre-prosequence from mature FVII during its biosynthesis.<sup>5</sup> A novel p.Ala191Glu in activation domain was identified in patients 60 and 106 (FVII: C<1%). Patient 106 had a second homozygous mutation (p.Trp364Cys) that had been previously reported.<sup>1</sup> The p.Ala191Glu and p.Trp364Cys mutations identified in patient 106 were in a double heterozygous state in her parents, confirming its double homozygosity in the proband. Ala191 is a conserved (11 out of 13 related serine proteases) hydrophobic amino acid buried in the hydrophobic core involving Trp187, Val188, Val189, Ser190Ala192, Cys194, and Phe195. Ala191 is close to His193 that is a part of the active site catalytic triad (Asp242 and Ser344). His193 is partly exposed (accessibility 3) in this hydrophobic stretch (Trp187-Phe195) of inaccessible (accessibility 0) amino acids.<sup>6</sup> The replacement of Ala191 by a moderately hydrophilic Glu191 can surface expose this residue and disturb the adjoining active site His193. Other novel mutations detected in this study were nonsense (p.Gln227X and p.Gln382X) mutations in catalytic domain that result in PTCs. Of these, the p.Gln227X was identified in three unrelated patients (13, 101, 105). Two of them (13 and 101) had a shared haplotype (Table1) and a common founder is likely.

Previously reported approaches for FVII gene mutation screening include denaturing gradient gel electrophoresis (DGGE) and direct sequencing.<sup>7,8</sup> Using our novel PCR-CSGE strategy, 7 out of 8 disease causing mutations were detected with a comparable sensitivity (88% vs. 91%) to DGGE. DGGE requires GC clamped primers and optimization of electrophoresis conditions for each of the PCR fragments as opposed to the universal electrophoresis conditions for CSGE. It is also possible to consider FVII gene direct sequencing, but for reasons of cost and wide applicability a simple mutation screening method such as CSGE prior to sequencing provides a powerful tool for genetic diagnosis. The genotype and phenotype relationship in FVII deficiency is variable.<sup>7</sup> A lack of correlation between in vitro FVII: C and the clinical phenotype were noticed in some of our patients. A mild-moderate phenotype (gum bleeding, hemetemesis, Patient 33) contrasted with severe bleeding symptoms (umbilical stump bleeds, hemarthroses, Patient 114) in spite of an identical p.Leu-55fs mutation. It would be of interest to determine their thrombin generating potential, as small amounts of FVIIa are sufficient to initiate coagulation and additional genetic or environmental factors may play a role in modulating FVII levels.<sup>9</sup> Of the 8 causative mutations identified in this study, 3 (p.Leu-55fs, p.Ala191Glu, p.Gln227X) were in 7 unrelated families.

Using haplotype analysis, we have shown that p.Leu-55fs and p.Gln227X in two patients each had a common founder. These data suggest that these common p.Leu-55fs, p.Ala191Glu, p.Gln227X mutations could be first analyzed by *StuI*, *SacII* and *HhaI* restriction fragment length polymorphism analysis for the genetic diagnosis of FVII deficiency in the Indian population.

This is the first report describing mutations in FVII gene from Southern India and the data show that mutations of this gene in Indian patients are as heterogeneous as in other populations.

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