

## Review

### FACTOR VII DEFICIENCY

The rare inherited disorders of coagulation are a fascinating group of diseases that have provided us with important insights into the structure and function of their respective deficient protein(s). Factor VII (FVII) deficiency is the commonest of the 'rare inherited disorders of coagulation' and this review summarizes current knowledge on the prevalence, diagnosis, management and the molecular pathology of factor VII deficiency.

### THE ROLE OF FACTOR VII IN THE INITIATION OF COAGULATION

Vascular injury results in the binding of FVII to Tissue Factor (TF), a sequence of events that initiates coagulation and ultimately generates a massive but highly focused burst of thrombin at the site of vascular damage. FVII bound to TF is activated to generate the active serine protease FVIIa and it is the TF–VIIa complex that, through limited proteolytic cleavage, activates factors X and IX (Wildgoose *et al.*, 1992a). The activated Factors Xa, VIIa, thrombin (IIa), IXa and XIIa have all been shown to activate FVII, but factor IXa in association with phospholipids appears to be most efficient at activating factor VII (Wildgoose & Kisiel, 1989; Butenas & Mann, 1996).

#### *Factor VII biochemistry*

Factor VII is a vitamin K-dependent glycoprotein and comprises 406 amino acids with a molecular weight of ~50 kDa. FVII circulates in plasma in two forms – the majority as a single-chain inactive zymogen with a concentration of 10 nmol/l (0.5 µg/ml) and a much smaller amount (~10–110 pmol/l) as the active two-chain form (Wildgoose *et al.*, 1992a). The conversion of the single-chain form of FVII to the two-chain form occurs by cleavage of a single peptide bond between Arginine 152 and Isoleucine 153, resulting in a light chain of 20 kDa (residues 1–152) and a heavy chain of 30 kDa (residues 153–406 – see Fig 1) and which contain the NH<sub>2</sub>- and COOH-terminal ends of the parent molecule respectively.

FVII contains 10 glutamic acid residues located towards the N-terminus of the molecule at residues 6, 7, 14, 16, 19, 20, 25, 26, 29 and 35. Post-translational modification of these residues to  $\gamma$ -carboxyglutamic acid (Gla) residues allows the binding of calcium, which causes a conforma-

tional change in the molecule, exposing novel epitopes that facilitate its subsequent binding to TF and phospholipid (Wildgoose *et al.*, 1992b).

FVII is a glycoprotein with O-linked glycosylation sites at Serine 52 and 60 and N-linked sites at Asn145 and 322. Glycosylation of FVII may be important for both its function and for its plasma half-life. The triad of amino acids Serine 344, Aspartate 242 and Histidine 193, which constitute the catalytic centre of FVIIa, are located on the heavy chain.

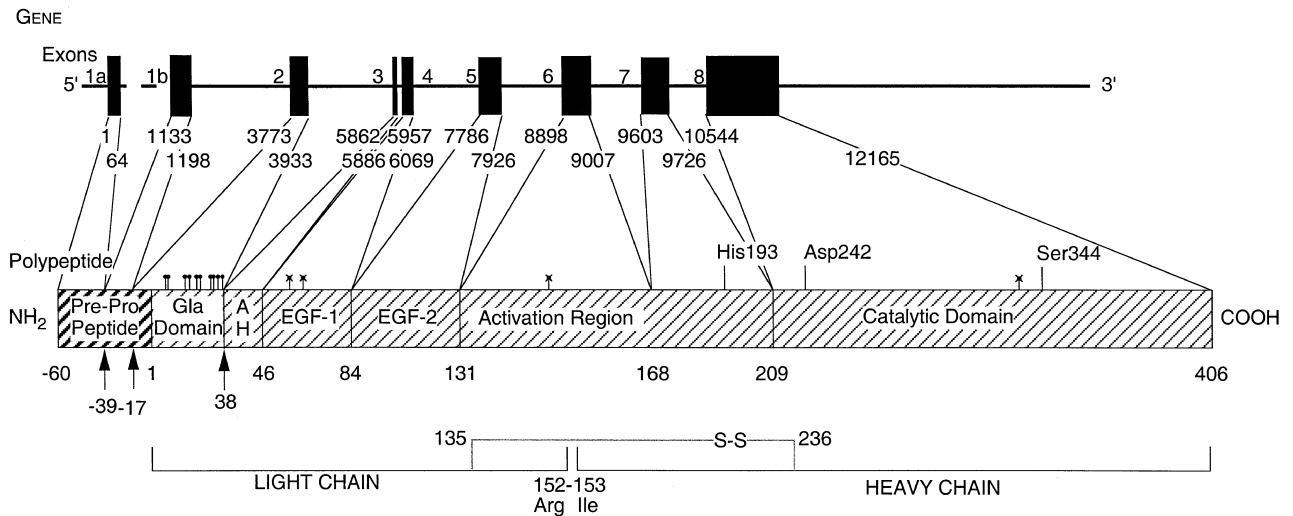
#### *Regulation of FVIIa activity*

The most important physiological inhibitor of the TF–VIIa complex is Tissue Factor Pathway Inhibitor (TFPI), a member of the Kunitz family of inhibitors (van der Logt *et al.*, 1991). Human TFPI is a 276-amino-acid molecule with a molecular weight of 32 kDa which circulates in plasma associated with lipoproteins, at a low concentration of 60–180 ng/ml. TFPI is synthesized by megakaryocytes and endothelial cells and its release from the surface of endothelial cells is increased both by heparin and various platelet agonists. TFPI forms an inactive quaternary complex comprising TF–VIIa, factor Xa and TFPI which rapidly inhibits the extrinsic pathway of coagulation. Antithrombin in the presence of heparin may also be involved in the regulation of FVIIa but its precise role remains controversial (Jesty *et al.*, 1996).

### FACTOR VII GENE

The factor VII gene (*F7*) maps to the long arm of chromosome 13 at 13q34, approximately 2.8 kb telomeric to the factor X gene (Miao *et al.*, 1992). The *F7* gene spans approximately 12 kb of DNA and consists of nine exons encoding a mature protein of 406 amino acids (Fig 1). FVII is synthesized with either a 38-amino-acid or a 60-amino-acid prepro-leader sequence containing a hydrophobic region (residues –36 to 24) which targets the protein for secretion and a pro sequence (residues –17 to –1) which is important for vitamin K-dependent gamma-carboxylation and which is highly conserved among other vitamin K-dependent proteins. The difference in the length of the prepro-leader sequence arises from alternative splicing of exon 1a/1b (Berkner *et al.*, 1986). Exon 1b (66 bp of DNA encoding amino acid residues –39 to –18) is absent in approximately 90% of factor VII mRNA transcripts (Berkner *et al.*, 1986). The pro-peptide and the Gla domain are encoded by exon 2. The pro-peptide is released from the mature protein by cleavage between Arginine –1 and Alanine +1. Exon 3 (residues 38–45) encodes the hydrophobic aromatic stack; exons 4 (residues 46–83) and 5

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**Fig 1.** Organization of the human factor VII gene and its encoded polypeptide. The upper part of the illustration represents the factor VII gene with the 9 exons shown by filled boxes. The position of the first and last nucleotides of each exon are shown (numbered according to O'Hara *et al.*, 1987). The lower part of the illustration shows the polypeptide structure and the various functional domains encoded by specific exons. Darker hatching indicates the prepro-peptide and lighter hatching the mature protein. Codons initiating each exon are shown (residue +1 is the first amino acid of the mature protein). Activation of factor VII occurs through cleavage at Arg152–Ile153 to generate a two-chain molecule. The light chain is encoded by residues +1 to 152 and the heavy chain by residues 153 to 406. The heavy and light chains of FVIIa are joined by a disulphide bond between Cys135 and Cys236. His193, Asp242 and Ser344 are the residues which constitute the catalytic triad. (♣) shows the position of the 10 Gla residues and (♣) the position of O- and N-linked glycosylation sites.

(residues 84–130) encode the two epidermal-like growth factor (EGF) domains, exons 6 (residues 131–167) and 7 (residues 168–208) encode the activation domain, and exon 8 (residues 209–406) encodes the catalytic domain and 1026 nucleotides of the 3' non-coding sequence including the poly(A) tail.

The *F7* gene contains a number of polymorphisms, five (and possibly six) of which have also been shown to influence FVII activity (Table I). Other polymorphisms within the *F7* gene have been reported but appear to be silent in terms of their effect upon protein function and/or secretion.

**Table I.** Factor VII gene polymorphisms.

Polymorphism type	Location	Alleles	Frequency
Decanucleotide [CCTATATCCT] insertion*	5' region (–323)	No insertion	0.77
		Insertion	0.23
Arg353Gln polymorphism*	Exon 8	Arg353 (10976 C)	0.80
		Gln353 (10976 T)	0.20
VNTR repeat (37 bp monomer repeat)*	Intron 7	A (7)	0.31
		B (6)	0.66
Intron 1a (G74A)*	Intron 1a	74 G	0.79
		74 A	0.21
Dimorphism (His 115)	Exon 5	7880 C	0.80
		7880 T	0.20
G/A dimorphism (Ser 333)	Exon 8	10916 G	0.99
		10916 A	0.01
G/T dimorphism*	5' region (–401)	–401 G	0.91
		–401 T	0.09
G/A dimorphism*	5' region (–402)	–402 G	0.71
		–402 A	0.29
G/A dimorphism	Intron 7	10523 G	0.82
		10523 A	0.18

\*Polymorphisms within the *F7* gene that may influence circulating FVII levels.

In contrast to many eukaryotic promoters and both factor X and factor IX, the FVII promoter lacks both a TATA and CAAT box. The major transcription initiation site for FVII is located 50 bp upstream from the initiation codon – Methionine +1. Analysis of the FVII promoter shows that maximal FVII activity resides within a 185-bp fragment and DNase I footprint analysis has identified protein binding sites at –51 to –32, –63 to –58, –108 to –84 and –233 to –215 (Pollak *et al.*, 1996). In addition, binding sites for both HNF-4 and Sp1 have been demonstrated and disruption of either of these sites results in a loss of promoter activity (Pollak *et al.*, 1996) and has been reported as a cause of factor VII deficiency (Arbini *et al.*, 1997; Carew *et al.*, 2000).

#### FACTOR VII LEVELS AND FACTOR VII GENE POLYMORPHISMS

Factor VII plasma levels are determined by both environmental and genetic factors with the latter accounting for up to one-third of the variation in plasma FVII levels (Bernardi *et al.*, 1996). Among environmental factors, dietary fat intake and the levels of plasma triglycerides are positively correlated with factor VII:C levels, but other factors such as age, obesity, diabetes (Heywood *et al.*, 1996) and, in women, the use of sex hormones can all affect FVII levels (Meade, 1988; Habiba *et al.*, 1996).

Five and possibly six polymorphisms within the human *F7* gene have been shown to affect both plasma FVII:C levels and, more recently, plasma FVIIa levels (Bernardi *et al.*, 1997). The first polymorphism to be reported within the *F7* gene that affects factor VII levels was the Arg353Gln polymorphism within exon 7, which arises from a G → A substitution at position 10976 (Green *et al.*, 1991). This substitution has a frequency in the UK population of approximately 0.2 and heterozygosity for this polymorphism is associated with an approximately 25% reduction in factor VII coagulant (or functional) activity (FVII:C) and factor VII antigen (FVII:Ag) levels. Individuals homozygous for this polymorphism have an approximately 50% reduction in circulating plasma factor VII. The Arg353Gln polymorphism is commonly found in association with a second polymorphism – the insertion of a 10-bp sequence (decanucleotide) within the 5' untranslated region of the factor VII gene at position –323 (Marchetti *et al.*, 1992). Until recently it was unclear which of these two polymorphisms was responsible for the alteration in plasma factor VII levels. However, studies of a group of Polish blood donors in which the Arg353Gln polymorphism is not in strong allelic association with the 10 bp promoter insertion has shown that both the Arg353Gln polymorphism and the 10 bp promoter polymorphism independently affect circulating factor VII plasma levels (Hunault *et al.*, 1997).

Two additional polymorphisms within the FVII promoter at positions –401 (G → T) and –402 (G → A) have also been shown to affect factor VII levels (Marchetti *et al.*, 1993; van't Hooft *et al.*, 1999).

A third polymorphism is located within intron 7 (IVS7) of the factor VII gene and is characterized by the presence of a variable number of a 37-base pair repeat sequence (March-

etti *et al.*, 1991, 1992). In the first repeat, which also contains the IVS7 donor splice site, sequence variations have also been identified. Quantitative mRNA analysis has shown that the higher numbers of repeats are associated with relatively higher mRNA expression and suggests that the IVS7 polymorphism contributes to plasma FVII levels (Pinotti *et al.*, 2000).

Finally, a recently identified G to A polymorphism within intron 1a of the FVII gene at position +74 has also been shown to affect FVII levels (Peyvandi *et al.*, 2000a), although it appears to be in strong linkage disequilibrium with both the 10-bp decanucleotide insertion and the Arg353Gln alleles.

#### FACTOR VII ASSAYS

The diagnosis of factor VII deficiency is usually suspected following the identification of a prolonged prothrombin time which corrects, unless an inhibitor is present, in a 50:50 mix with normal plasma. The activated partial thromboplastin time (APTT), thrombin time and fibrinogen concentration are usually normal. Specific assays of factor VII are undertaken to confirm the deficiency. It is important to exclude vitamin K deficiency or other acquired causes of a clotting disorder before the diagnosis of factor VII deficiency is made. Family studies may also be of value in establishing the diagnosis of factor VII deficiency.

##### *Functional FVII assays*

Functional factor VII activity (FVII:C) is frequently measured using a one-stage prothrombin time (PT)-based assay (Poggio *et al.*, 1991). However, the source of thromboplastin used in the assay can have a significant effect upon the FVII functional assays and for these reasons a series of different thromboplastins are often used when investigating patients with suspected factor VII deficiency (Poggio *et al.*, 1991). These potential problems are highlighted by Factor VII Padua, a variant molecule characterized by a prolonged rabbit brain prothrombin time, a normal Stypven cephalin clotting time and a normal Thrombotest. Factor VII activity is low when assayed using rabbit brain thromboplastin but is normal when assayed using ox brain thromboplastin. Factor VII antigen is normal and there is usually no bleeding history (Girolami *et al.*, 1978).

Because of limited conversion of FVII to FVIIa during functional FVII assays, FVII:C measures both the inactive zymogen, FVII and preformed FVIIa. FVII:C assays using bovine thromboplastin are more sensitive to preformed FVIIa (compared with zymogen FVII) than assays based on human or rabbit thromboplastins. Between 8% and 30% of the FVII:C activity of assays based on rabbit or bovine thromboplastin is attributable to preformed FVIIa, while the remaining 70–92% is due to zymogen FVII (Morrissey, 1996).

##### *Immunological FVII assays*

FVII:Ag is frequently measured using an enzyme-linked immunosorbent assay (ELISA) or immunoradiometric assay (IRMA) and either monoclonal or polyclonal antibodies

(Boyer *et al.*, 1986; Tirindelli *et al.*, 1987; Coppola *et al.*, 1992). Such assays can detect as little as 0.0001 µ/ml of factor VII (Coppola *et al.*, 1992).

#### Factor VIIa assays

In samples in which there has been no activation of FVII, assays for FVII:C and FVII:Ag should be equivalent. However, if there has been significant activation then these results will clearly differ. The direct assay of FVIIa was initially reported using a mutant Tissue Factor molecule in which the transmembrane and cytoplasmic domains were deleted (sTF1–219), resulting in a soluble Tissue Factor (sTF) which was selectively deficient in promoting the conversion of FVII to FVIIa but which retained cofactor activity toward factor VIIa in a one-stage clotting assay (Neuenschwander & Morrissey, 1992; Morrissey *et al.*, 1993). The basal FVIIa level in normal plasma measured using this technique is approximately 3.6 ng/ml (Morrissey *et al.*, 1993), about 1% of the total circulating FVII mass.

A second method for the assay for FVIIa levels in plasma has been reported that does not depend upon its functional activity but involves a specific immunoassay using a unique capture antibody with a high specificity for two-chain FVIIa (Philippou *et al.*, 1997). The activity-based assay for FVIIa and the ELISA show excellent agreement when measuring purified factor VII added to plasma (Philippou *et al.*, 1997). However, results from the two assays differ greatly when used to measure the basal endogenous levels of FVIIa in plasma. The ELISA estimates that normal plasma contains approximately 0.0125 ng/ml ( $\pm$  0.01 ng/ml) of FVIIa, approximately 100-fold lower than that measured by the activity sTF-based assay. The explanation for the discrepancy between the two assays is unclear although various mechanisms have been proposed. It is possible that the C-terminus of the light chain, towards which the capture antibody in the ELISA is directed, may undergo proteolysis in the circulation. This would render FVIIa unable to bind to the capture antibody but would have no effect upon the activity assay. An alternative hypothesis is that the assay specificity using the sTF activity assay is not absolute and TF-independent activation of FVII by FXa can occur (Neuenschwander & Morrissey, 1992). Activation of FVII by the sTF–FVIIa complex can also occur under certain reaction conditions (Fiore *et al.*, 1994). Clearly, if even a small proportion of the FVII is activated, the specificity of the assay would be reduced as the zymogen is present in great molar excess.

#### FACTOR VII DEFICIENCY

Inherited factor VII deficiency is the most common of the 'rare inherited coagulation disorders', with an estimated prevalence of between 1:300 000 and 1:500 000. Factor VII deficiency is inherited in an autosomal recessive manner and its frequency is significantly increased in countries where consanguineous marriage is practised (Table II). For reasons which are unclear, there is a relatively poor correlation between factor VII levels and the risk of bleeding, with some individuals having very low levels of

**Table II.** Prevalence of inherited bleeding disorders in differing populations (excluding von Willebrand's disease) (Peyvandi, 2000).

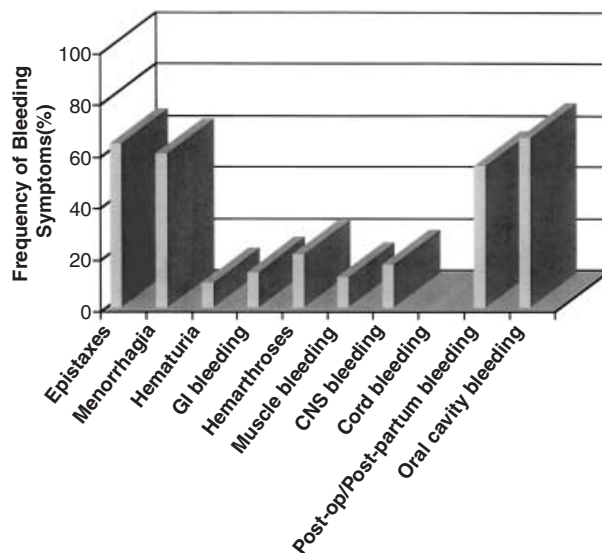
Deficiency*	Number of cases		
	Iran	Italy	UK
Fibrinogen	70	10	11
Prothrombin	15	7	1
Factor V	70	21	28
Factor V & VIII	80	29	18
Factor VII	300	58	62
Factor X	60	16	25
Factor XIII	80	31	26
Factor VIII	3000	3428	3554
Factor IX	900	626	762
Factor XI	20	60	150

\*Only patients with factor levels of 10 µ/dl or less are included (for fibrinogen deficiencies 0.1 g/l or less).

The populations of Iran, Italy and the UK are very similar at 64 m, 57.8 m and 60 m respectively.

factor VII exhibiting very few symptoms while others with much higher levels have a significant bleeding diathesis (Triplett *et al.*, 1985).

*Spectrum of bleeding problems in patients with FVII deficiency*  
In patients with severe haemophilia A or B, the disease is characterized by recurrent spontaneous bleeds into joints and muscles with little in the way of mucosal-type bleeding. In factor VII deficiency, the spectrum of bleeding problems is very variable (Fig 2) (Peyvandi *et al.*, 1997; Mariani *et al.*,



**Fig 2.** Spectrum of bleeding problems in patients with inherited deficiencies of factor VII (Peyvandi *et al.*, 1997). Factor VII:C < 10 µ/dl.

1998). In patients with mild–moderate–severe disease, epistaxes and gum bleeding, menorrhagia and other mucous membrane-type bleeding is common. Menorrhagia and chronic iron deficiency is common in women with factor VII deficiency. These mucosal-type bleeding patterns are similar to those seen in patients with inherited disorders of platelet function. The explanation for the mucosal-type bleeding in FVII deficiency is unclear, although a prolongation of the bleeding time has been reported (Bernardi *et al.* 1994).

In patients with severe factor VII deficiency, bleeding into the central nervous system is common and reported in between 15% and 60% of cases (Peyvandi *et al.* 1997; Mariani *et al.* 1998). Such cases often present shortly after birth and this presentation is associated with a high morbidity and mortality.

Joint bleeds have been reported in patients with factor VII deficiency (Peyvandi *et al.* 1997; Mariani *et al.* 1998) and, in one of these studies, the severity of haemarthroses and the long-term sequelae was similar to that seen in haemophilia (Mariani & Mazzucconi, 1983). However, haemarthroses are not a consistent finding and other studies have failed to identify this as a common problem in patients with severe FVII deficiency (Peyvandi *et al.* 1997).

#### *Factor VII knockout mouse*

The current theory of blood coagulation in man suggests that a failure to initiate coagulation through the binding of tissue factor to factor VII is likely to be incompatible with life. This theory is supported by the FVII knockout mouse which, although developing normally to term, dies shortly at/or after birth from major abdominal and intracranial haemorrhage (Rosen *et al.* 1997). Furthermore, of the mutations that have been reported within the human factor VII gene, there has until recently been a complete absence of any mutation that one could confidently predict would result in failure of factor VII production (see <http://europium.csc.mrc.ac.uk/usr/WWW/WebPages/FVII/database>). McVey *et al.* (1998) have reported a 5' splice site mutation within intron 4 of the FVII gene that leads to a deletion of exon 4 from FVII mRNA. Subsequent *in vitro* expression studies failed to demonstrate production of FVII protein and the authors concluded that the presence of this homozygous mutation in the index case led to a complete absence of FVII in the plasma. The child described by McVey *et al.* (1998) presented at the age of 10 d with massive intracerebral haemorrhage from which he subsequently died 2 d later. In addition, within the same family, a baby girl from a previous pregnancy died at the age of 1 month with cerebral haemorrhage and hydrocephaly. It seems probable that she had an identical genotype/phenotype to explain her symptoms. However, Peyvandi *et al.* (2000b) recently reported the case of a 5-year-old male of Chinese origin who was diagnosed with severe FVII deficiency at the age of 3 years when he presented with recurrent bleeding problems – haematuria, haemarthroses, muscle and soft tissue haematomas, and bleeding from the gastrointestinal tract. Many of these bleeding problems were spontaneous or occurred in relation to minor trauma. Investigations

showed a prolonged prothrombin time and markedly reduced plasma factor VII activity and antigen levels in plasma at >0.001 µ/ml consistent with severe factor VII deficiency. The child was treated initially with fresh-frozen plasma and subsequently with FVII concentrates and, to date, remains well. Mutational analysis identified a homozygous 2 bp deletion (nucleotides 27/28: –CT) within exon 1a of the FVII gene at codon –52/51, a region that encodes part of the prepro-peptide of FVII. Sequence analysis of both parents confirmed that they were both heterozygous for this 2 bp deletion mutation. This mutation results in a frameshift, the creation of a premature stop codon and the complete absence of any circulating factor VII. The explanation for the differences in the natural history of this case and that reported by McVey *et al.* (1998) is unclear, but the former case clearly indicates that a complete absence of FVII is compatible with life. The lack of correlation between *in vitro* FVII:C values and the clinical phenotype may reflect the finding that only trace amounts of FVIIa are required to initiate coagulation *in vivo*. *In vitro* assays fail to differentiate between a true 'null' mutation and one that yields very low but not zero FVII:C levels.

#### *FVII deficiency and thrombosis*

Thrombosis in association with factor VII deficiency has been reported (Gershwin & Gude, 1973; Ben Dridi *et al.* 1986; Escoffre *et al.* 1995; Kang *et al.* 1998) although the mechanism is unclear. One can speculate that, in some cases, the underlying mutation may be one that leads to different FVII:C results with differing thromboplastins and that the patient does not have true FVII deficiency but an unusual variant – similar to that seen with Factor VII Padua (Girolami *et al.* 1978). In such cases, the diagnosis of factor VII deficiency is misleading and other risk factors for thrombosis become more important. An alternative hypothesis to explain the association between factor VII deficiency and thrombosis is that the mutation giving rise to the deficient phenotype results in a factor VII molecule that fails to bind TFPI and, therefore, the TF–VIIa initiation pathway of coagulation remains active.

A reduction in FVII levels has also been reported in association with hyperhomocysteinaemia (Munnich *et al.* 1983; Palareti & Coccheri, 1989) and, in at least one study, normalized after patients were placed on a low methionine diet supplemented with betaine (Munnich *et al.* 1983). Abnormalities of the other vitamin K-dependent clotting factors have not been reported. The low FVII levels in hyperhomocysteinaemia has also been suggested as a possible mechanism to explain the rare cases of apparent factor VII deficiency associated with thrombosis (Munnich *et al.* 1983).

To date, mutational analysis in families with inherited FVII deficiency associated with thrombosis has not been reported and any proposed mechanism remains speculative.

Elevated levels of factor VII also appear to play an important role in atherothrombotic heart disease. Data from the Northwick Park Heart Study suggests that factor VII levels in the upper part of the normal distribution are an independent risk factor for the development of

cardiovascular disease (Meade *et al.*, 1986). Plasma FVII levels also increase with age, female sex and hyperlipidaemia, especially hypertriglyceridaemia (Simpson *et al.*, 1983). There are also a number of polymorphisms within the FVII gene that can influence FVII levels and these have been described in an earlier section.

Elevated factor VII levels as a risk factor for venous thromboembolic disease have been evaluated as part of the Leiden Thrombophilia Study (Koster *et al.*, 1994). The authors studied 199 unselected patients aged <70 years with a first objectively proved deep vein thrombosis (DVT) and without known malignancy. Although they were able to demonstrate a relationship between factor VII levels and various *F7* gene polymorphisms, they failed to show any correlation between factor VII levels and thrombotic risk.

#### INHERITED FACTOR VII DEFICIENCY

In the last 5 years there has been an explosion in the number of cases of FVII deficiency reported in the literature. The factor VII mutation web site (<http://europium.csc.mrc.ac.uk/usr/WWW/WebPages/FVII/database>) currently lists 120 mutations scattered throughout the factor VII gene and these are summarized in Table III. The majority of the mutations are located within the catalytic domain of factor VII, emphasizing the functional importance of this region. However, mutations are located throughout the gene, suggesting that all domains are important in maintaining the overall structure and function of factor VII. In common with many diseases, numerous examples of repeated mutations within the factor VII gene have been reported, but these are not shown in Table III. In a number of studies, haplotyping has shown that identical haplotypes co-segregate with identical mutations, suggesting a founder-effect rather than an independent origin for the mutation (Bernardi *et al.*, 1993; Wulff & Herrmann, 2000).

To date, only a relatively small number of factor VII mutations have been expressed *in vitro* and, therefore, the mechanism by which many mutations result in the disease

phenotype is unclear. Structural modelling has been used to try and predict the effects of specific mutations but such predictions remain speculative (Peyvandi *et al.*, 2000c). Peyvandi *et al.* (2000c) analysed the FVII gene in 27 patients with FVII deficiency from 21 unrelated families predominantly of Middle-Eastern extraction. A total of 19 different mutations were identified, of which 12 were novel and 7 had been previously reported. Nine of the 12 novel mutations were missense mutations located in the Gla domain (Ser23Pro), the second epidermal growth factor domain (Cys135Arg) and the catalytic serine protease domain (Arg247Cys, Arg277Cys, Ser282Arg, Pro303Thr, Ser363Ile, Trp364Cys, Trp364Phe), of which five were homozygous. Three novel splice mutations were identified in intron 1a (IVS1a+5), intron 2 (IVS2+1) and intron 6 (IVS6+1). Conformational analyses of crystal structures for FVIIa and the FVIIa–tissue factor complex provided likely explanations for the effect of the missense mutations on FVIIa secretion or function (Fig 3A and B). In particular, the majority of the missense mutations were located on the serine protease domain, mostly to the region between the catalytic triad and the contact surface with tissue factor, indicating that orientation of the serine protease domain relative to bound tissue factor in the complex is crucial for functional activity.

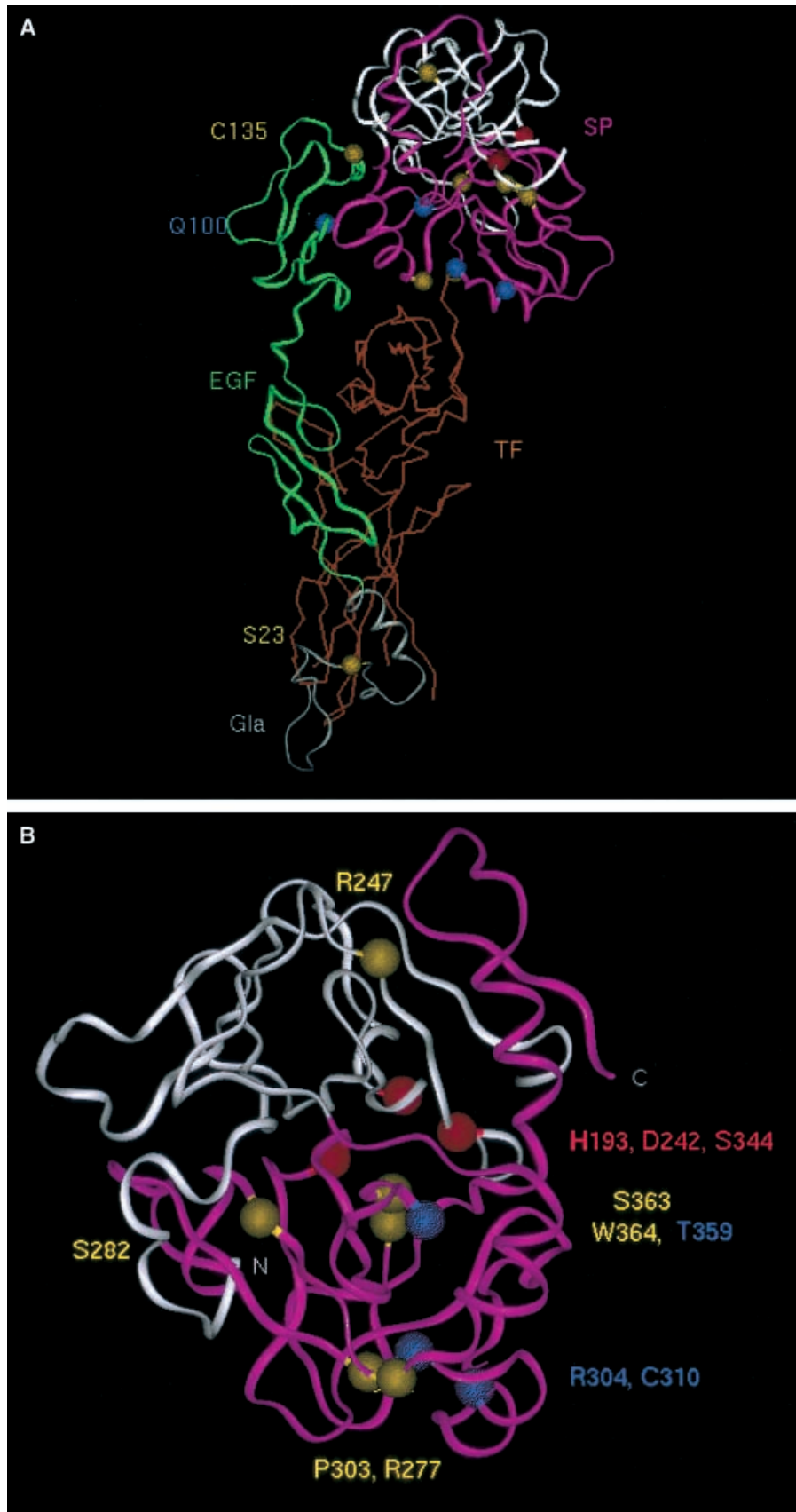
#### ACQUIRED FACTOR VII DEFICIENCY

Acquired deficiencies of vitamin K are seen in a wide variety of disorders but most are secondary to liver disease or vitamin K antagonists. Dicoumarol is a naturally occurring vitamin K antagonist and Warfarin (4-hydroxy-coumarin) the synthetic anticoagulant, although both have similar effects upon coagulation. Warfarin inhibits two vitamin K-dependent enzymes within the liver – a vitamin K-dependent reductase and a vitamin K-dependent quinone reductase. This prevents efficient recycling of vitamin K to its enzymatically active form and limits the activity of a vitamin K-dependent carboxylase. Other vitamin K antagonists include Acenocoumarol ('Sinthromin') and Phenprocoumon ('Marcoumar'). Warfarin is the most widely used oral anticoagulant in the USA and UK but Acenocoumarol

Table III. Summary of Factor VII gene mutations.

Mutation type	Number reported
Promoter mutations	6
Splice-type mutations	17
Nonsense mutations	6
Missense mutations	77
Insertions/deletions	14
<i>Location of mutation</i>	
Promoter region	6
Pre-pro-leader sequence	9
Gla domain	7
EGF-1 domain	9
EGF-2 domain	10
Activation region	8
Catalytic domain	61

Fig 3. (A) Ribbon view of the crystal structure of the complex between FVIIa and TF. The  $\alpha$ -carbon atoms of the novel mutations identified in the study of Peyvandi *et al.* (2000c) are shown in yellow and previously identified mutations in blue. The catalytic triad is represented by spheres and shown in red; the Gla domain in dark grey; the EGF-1 and EGF-2 domains in green; and the N-terminal and C-terminal serine protease (SP) subdomains in light grey and magenta respectively. The two fibronectin type III domains in tissue factor are identified in brown. (B) Ribbon view of the main-chain of the catalytic serine protease domain of FVIIa. The  $\alpha$ -carbon atoms of the catalytic triad H193, D242 and S344 are shown as red spheres at the interface between the two subdomains of the serine protease (SP) domains, shown in light grey (SP1) and magenta (SP2) respectively. Novel mutations identified in the study of Peyvandi *et al.* (2000c) are shown in yellow and previously identified mutations in blue.



and Phenprocoumon are popular in other countries. Phenindione is an indandione derivative rather than a 4-hydroxy derivative, although it appears to inhibit vitamin K reductases in a similar manner to the coumarins.

In patients with chronic liver disease, the levels of factor VII are often disproportionately lower than other vitamin K-dependent factors, e.g. prothrombin and factor X (Mammen, 1994). Other hepatic disorders, e.g. Dubin Johnson syndrome, Rotor syndrome and Gilberts syndrome may be associated with lowered factor VII levels (Roberts & Lefkowitz, 1998). The clotting abnormalities in liver disease are multifactorial and outwith the scope of this review.

A defect affecting all the vitamin K-dependent clotting factors has been reported and shown to be due to a mutation within the gamma-glutamyl carboxylase gene (Spronk *et al.*, 2000). These rare cases often respond to supplementation of the diet with oral vitamin K.

Rare cases of factor VII deficiency secondary to drugs other than oral anticoagulants, e.g. penicillins (Mehta *et al.*, 1992) and the cephalosporins (Kaiser *et al.*, 1991) have been reported. Acquired factor VII inhibitors have also been reported occurring either spontaneously (Meade, 1988; Ndimbie *et al.*, 1989; de Raucourt *et al.*, 1994; Kamikubo *et al.*, 2000) or in association with other diseases, e.g. myeloma (Elezovic *et al.*, 1989). Factor VII deficiency has been reported in association with an underlying malignancy (de Raucourt *et al.*, 1994), in patients with sepsis (Biron *et al.*, 1997), in association with the use of anti-thymocyte globulin (Fischer *et al.*, 1985), in patients receiving interleukin 2 (IL-2) therapy (Birchfield *et al.*, 1992) and in patients with aplastic anaemia (Weisdorf *et al.*, 1989).

## MANAGEMENT OF FACTOR VII DEFICIENCY

### *Inherited factor VII deficiency*

Inherited factor VII is a rare disorder and there are no generally agreed guidelines for the management of this disorder. For children and previously untreated adults with moderate-severe FVII deficiency, recombinant factor VIIa (rVIIa) is probably the treatment of choice.

Plasma FVII has a short *in vivo* half-life of approximately 5 h although this may be shorter during a bleeding episode (Lindley *et al.*, 1994). In contrast to deficiencies of factor VIII and IX in which a therapeutic level of 100 µ/dl is often required, efficient haemostasis even during surgery can be achieved with levels of FVII in the range of 10–15 µ/dl (Triplett, 1977; Zimmermann *et al.*, 1979; Kelleher *et al.*, 1986; Saint-Raymond *et al.*, 1989).

Current therapeutic options to treat inherited FVII deficiency include fibrinolytic inhibitors, plasma, intermediate purity factor IX concentrates (prothrombin complex concentrates), factor VII concentrate and recombinant VIIa (rVIIa).

### *Plasma*

The content of factor VII in normal plasma is, by definition 100 U/dl. Although plasma has been widely used in the management of factor VII deficiency, there is little informa-

tion available on its efficacy. Plasma has been successfully used to manage patients undergoing various surgical operations either by itself or in combination with FVII concentrate (Greene & McMillan, 1982; Doran *et al.*, 1994). However, in cases in which prolonged administration is required and because of the short half-life of FVII, it is often difficult to administer sufficient plasma quickly enough and problems with fluid overload may be encountered (Briet & Onvlee, 1987). If plasma is used to treat FVII deficiency it is sensible to use a virally inactivated plasma to minimize the risk of transfusion-transmitted disease.

### *Factor IX concentrates and prothrombin complex concentrates*

Intermediate purity factor IX concentrates (prothrombin complex concentrates), i.e. concentrates that are not produced by either monoclonal or recombinant techniques, contain variable amounts of factor VII and have been successfully used to manage patients with factor VII deficiency (White *et al.*, 1979; Sumi *et al.*, 1985). The amount of factor VII in each of these concentrates is very variable but is usually indicated by the manufacturer and this can then be used to calculate the amount required for replacement therapy. Many of these intermediate purity factor IX concentrates contain activated forms of factors VII, IX and X and should be used with caution, as there are reports of both venous and arterial thromboses associated with their use (Gershwin & Gude, 1973; Nakagawa *et al.*, 1990; Schulman *et al.*, 1991; Escoffre *et al.*, 1995). For these reasons, it is also unwise to use these concentrates in the presence of liver disease, in cases of major trauma, or in neonates whose livers are relatively immature.

### *Factor VII concentrates*

A number of plasma-derived factor VII concentrates have been developed and have been successfully used to manage patients with inherited factor VII deficiency with spontaneous bleeding episodes or undergoing a wide variety of surgical procedures (Mariani *et al.*, 1978; Dike *et al.*, 1980; Gagliardi *et al.*, 1983; Sumi *et al.*, 1985; Eikenboom *et al.*, 1992; Robertson *et al.*, 1992; Rivard *et al.*, 1994). Factor VII concentrates have also been successfully used for prophylaxis against bleeds in children with severe factor VII deficiency and suggested guidelines for the administration of factor VII for long-term prophylaxis are in the range of 10–50 U/kg one to three times a week. Although this seems illogical when one considers the short half-life of factor VII, in practice it seems successful (Cohen *et al.*, 1995). For surgery, doses ranging from 8 to 40 U/kg every 4–6 h have been successfully used (Schrickler & Neidhardt, 1981; Greene & McMillan, 1982; Gagliardi *et al.*, 1983; Sumi *et al.*, 1985; Ferster *et al.*, 1993). Careful monitoring of patients receiving FVII concentrates is essential to prevent excessively high peaks and troughs. For major surgery, trough factor VII levels should not fall below 20 U/dl.

### *Recombinant factor VIIa (rVIIa)*

A number of studies have shown that patients with FVII deficiency can be safely managed using rVIIa. Recombinant

FVIIa probably has a shorter half-life than that of plasma FVII (Thomsen *et al*, 1993; Lindley *et al*, 1994), particularly in children in whom an increased clearance has been demonstrated (Lusher *et al*, 1998; Schulman, 1998) and in pregnancy (Jimenez-Yuste *et al*, 2000). In such situations, more frequent dosing or a continuous infusion may be required to maintain haemostatically active FVII levels.

Mariani *et al* (1999) reported their experience with rVIIa in treating 17 patients with 27 spontaneous bleeding episodes who, in addition, also underwent 7 major and 13 minor surgical procedures (Mariani *et al*, 1999). The majority of the patients were severely deficient patients with FVII levels of <1%. Fifteen haemarthroses were treated with only a single dose of rVIIa (14–30 µg/kg) and in only one case was rVIIa ineffective. Seven major surgical procedures were performed in severely affected patients under cover of rVIIa and no bleeding occurred either during or after surgery. In the case of surgery, rVIIa was given at 2–3 h intervals for the first 24 h followed by longer intervals (3–8 h) for the remaining post-operative period. All the surgical patients also received tranexamic acid along with the rVIIa. In one patient, the drug was ineffective following the development of an anti-FVII antibody.

Several other groups have reported the successful use of rVIIa with a variety of clinical problems (Ingerslev *et al*, 1997; Scharrer, 1999; White *et al*, 2000; Wong *et al*, 2000). In general, a dose of 20–25 µg/kg administered every 4–6 h appears to be successful in treating the majority of patients with FVII deficiency who are either bleeding or who require treatment prior to and following surgery. The duration of treatment is very much dependent upon the indication for treatment but, at least in some cases, a single dose may be effective. In patients receiving 20 µg/kg of rVIIa every 6 h, this leads to peak FVII levels of 4.3–8.3 U/ml and trough levels of 0.3 U/ml. Inhibitors following treatment with rVIIa have been reported (Bauer, 1996).

Continuous infusion of rVIIa has been used in at least one patient with moderate factor VII deficiency (VII:C 3.7 U/dl) to provide haemostatic cover for an elective caesarean section (Jimenez-Yuste *et al*, 2000). Pharmacokinetic studies performed prior to the infusion revealed a very high clearance rate (0.208 l/h/kg) and a short half-life (0.884 h), which the authors attributed to the pregnancy. The patient received an initial bolus of rVIIa of 13.3 µg/kg followed by a continuous infusion initially at a rate of 3.3 µg/kg/h for 48 h and then at 1.66 µg/kg/h for a further 48 h. Plasma factor VII and VIIa levels were maintained between 1 and 1.5 U/ml and no bleeding problems were observed.

#### *Acquired factor VII deficiency*

Acquired factor VII deficiency, together with deficiencies of the other vitamin K-dependent clotting factors, is most commonly seen in patients receiving coumarin anticoagulant (commonly warfarin) therapy. In major life-threatening bleeds, prompt reversal of these anticoagulants can be achieved using prothrombin complex concentrates (Makris

& Watson, 2001). Fresh-frozen plasma has also been used to treat patients with bleeding secondary to oral anticoagulant therapy, although there are concerns that the International Normalized Ratio (INR) is not sensitive to changes in factor IX that may be important in determining the outcome of a bleeding episode (Makris & Watson, 2001). A comprehensive review of the management of over-anticoagulation secondary to coumarins has recently been published (Makris & Watson, 2001).

In patients with factor VII deficiency secondary to liver disease, the use of prothrombin complex concentrates is contraindicated because of the risks of thrombosis. In such patients, fresh-frozen plasma, desmopressin, vitamin K and platelets may be used to treat the coagulopathy. Recombinant factor VIIa (rVIIa) has been used successfully to treat the coagulopathy secondary to advanced cirrhosis (Bernstein, 2000) and may be a therapeutic option in patients who fail to respond to other treatment modalities.

Isolated factor VII deficiency in association with other disorders is rare. In general, only case reports exist in the literature and there is no consensus as to how such patients should be managed. Treatment or removal of the underlying cause, if this can be identified, is clearly important. In patients who are actively bleeding, the use of factor VII concentrates or rVIIa may be of value. Similarly, in such rare cases the use of intravenous immunoglobulin and immunosuppressive agents may be of value. Finally, fibrinolytic inhibitors, e.g. Tranexamic acid and fibrin glue, may be helpful in promoting and securing haemostasis.

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