Accurate characterization of the IVS7 repeat polymorphism of FVII gene and identification of three novel allelic forms

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Dear Sir,

Factor VII (FVII) is a vitamin K-dependent clotting protein which plays an essential role in blood coagulation. After binding with tissue factor, activated FVII triggers the coagulation process by activating factor IX (FIX) and factor X (FX), ultimately resulting in the fibrin clot. Numerous polymorphisms have been described within the FVII gene. Four of them have been found to be of physiological relevance for FVII metabolism and account for about one-third of FVII level variation in the plasma (1).

These include i) and ii) two polymorphisms in strong linkage disequilibrium: the p.Arg413Gln (classically referred to as R353Q) variant (2) and the promoter haplotype [g.2957G>T at -401 of the ATG translation initiator codon; g.2035_2036ins(ctatatcct) (classically referred to as -323 10 base pair insertion); g.3236 T>C at -122 of the ATG translation initiator codon] (3-4), iii) another promoter polymorphism at g 2956 G>A at -402 of the ATG translation initiator codon (5) and iv) a polymorphism within the hypervariable region 4 of intron 7 (IVS7) of the FVII gene (6-7). Throughout the literature, IVS7 allelic forms are essentially defined as H5, H6, H7 or H8 depending on the number of repeats of the 37-bp monomers, (5, 6, 7, and 8 repeats, respectively). Therefore, we wondered whether the sequence of this “37-bp monomer” could differ between allelic forms. In order to better characterize the IVS7 repeat polymorphism of the FVII gene and to identify additional allelic forms, we analysed the coding portion and intron/exon boundaries of exon 7 of the FVII gene of one hundred unrelated subjects with inherited FVII deficiency and fifty healthy individuals from the South of France. All patients gave written informed consent in compliance with the French law. The primer set used for polymerase chain reaction (PCR) was designed according to the reference sequence of the FVII gene (GenBank accession #AY212252) (F7LR1: cggaggagggagctgctcagag, F7LR2: gaggcaggatgggcagagtcc). PCR amplified fragments were directly sequenced using the Big-dye Terminator version 1.1 Cycle Sequencing kit, on an Applied 310 sequencing apparatus (Applied Biosystems, Warrington, UK).

We used a unique number from 1 to 6, then the allele designated by the monomer nomenclature #AY212252 and NM_000131 respectively). As the residue numbering uses the Met as amino acid number one instead of the first amino acid in the mature protein, the previously published names, using the former nomenclatures, have been given in brackets.

Direct sequencing identified six separate 37-bp monomers (M1-M6) which differed from one another in only 1 to 2 nucleotide positions (Fig. 1A). Among these, the monomer M3 is overrepresented whereas the monomer M4 is extremely rare in our series. Numerous allelic forms of the IVS7 polymorphism exist, depending on the number of repeats of the 37-bp monomers, as already reported. However, the classical terminology of the allelic forms H6 and H7 actually includes various arrangements of the different 37-bp monomers. In order to clearly name these IVS7 allelic forms, the polymorphic monomers could be assigned a unique number from 1 to 6, then the allele designated according to the number of repeats and their order (Fig. 1B). Using this terminology, the H6(123356) and H7(1333356) allelic forms were the most common in our series with an allele frequency of 0.595 and 0.346, respectively. We also identified two novel allelic forms, H6(133356) and H7(13334356), which differed from the common H6(123356) and H7(1333356), respectively by two distinct nucleotide substitutions. Both H6(133356) and H7(13334356) allelic forms are rare. The H6(133356) form was found in the heterozygous state in only two patients and none of the controls (allele frequency: 0.006). One of them was a Caucasian female who carried a compound heterozygous FVII mutation genotype, c.[64+4C>T] (+) p.[Arg462Stop] (classically referred to as Arg402Stop). As her parents’ data were not available, we could not assign the H6(133356) form to a particular mutation. The other patient was of African descent. He also carried a compound heterozygous FVII genotype including a splice site mutation at the consensus GT sequence of the intron 7 c.805+1G>A and the frequent p.Arg364Gln (classically R304Q)
missense mutation. DNA analysis of his parents indicated that the H6(133356) allelic form was associated in cis with the c.805+1G>A mutation and with a synonymous change in the exon 7 g.13028C>T. Both sequence variations (c.805+1G>A and g.13028C>T) are reported here for the first time. The H7(1334356) form was also found in only two patients with mild FVII deficiency (allele frequency: 0.006). No other mutation was detected despite extensive DNA sequencing of either all coding portions, intron/exon boundaries or the 5' untranslated region of the FVII gene. As both patients were homozygous for the promoter g.2035_2036ins(cctatatcct) and for the p.Arg413Gln alleles, the H7(1334356) allelic form is supposed to be associated with.

Table 1: FVII genotypes and phenotypes of patients with new IVS7 allelic forms. FVII:Ag measurements were performed using the Asserachrom® FVII:Ag kit (Diagnostica Stago, Asnières sur Seine, France). The FVII promoter polymorphism g.2035_2036ins(cctatatcct) was defined as A1 and A2, depending on the decanucleotide deletion or insertion respectively. ND: not determined; *undetected despite DNA sequencing of the whole FVII gene coding sequence; A: indicates asymptomatic; M: indicates moderate bleeding phenotypes including epistaxes and bruises.

<table>
<thead>
<tr>
<th>Patients</th>
<th>FVII genotype</th>
<th>FVII phenotype</th>
<th>Clinical phenotype</th>
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<tbody>
<tr>
<td></td>
<td>IVS7</td>
<td>S'UTR</td>
<td>p.Arg413Gln</td>
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<tr>
<td>1</td>
<td>H6(133356) / H7(1333356)</td>
<td>A1/A2</td>
<td>Arg/Arg</td>
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<tr>
<td>2</td>
<td>H6(133356) / H7(1333356)</td>
<td>A1/A2</td>
<td>Arg/Arg</td>
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<tr>
<td>3</td>
<td>H7(1334356) / H7(1333356)</td>
<td>A2/A2</td>
<td>Gln/Gln</td>
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<tr>
<td>4</td>
<td>H7(1334356) / H7(1333356)</td>
<td>A2/A2</td>
<td>Gln/Gln</td>
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Fig. 1: A) Monomers included in the FVII IVS7 repeat polymorphism. The nucleotide variation between monomers is highlighted in bold. B) Various monomer rearrangements and allelic frequency.
in cis with these two polymorphic markers. FVII genotypes and phenotypes of the four abovementioned patients are reported in Table 1. In addition, our panel included six unrelated patients bearing the c.805+7A>G mutation, also reported as the 9726+7A>G transition. After direct sequencing, all six had the same IVS7 allelic form. The so-called 9726+7A>G transition could actually correspond to a particular rearrangement of the 37-bp monomers with a duplication of the M3 monomer (Fig. 1B). According to the proposed terminology, this form could be designed as H6(3333356). Although the H8 allelic forms are rare, two different forms designed as H8(13333556) and H8(13333336) could be identified depending on the monomer arrangement (Fig. 1B).

Repeat polymorphisms have been associated with a number of genetic disorders. Among these, the FVII IVS7 polymorphism is of particular interest because it spans an exon-intron boundary and was found to contribute to the plasmatic variance of FVII antigen and activity levels via differential mRNA splicing efficiency. A parallel decrease was observed in the IVS7 repeat number and in both the relative mRNA expression levels and FVII levels (8). Moreover, previous findings have suggested a relationship between myocardial infarction and certain IVS7 allelic forms (9–10). Nevertheless, in all of the above-mentioned studies, IVS7 genotypes were only defined by the number of 37-bp repeats. One can hypothesise that variations within the sequence of the 37-bp monomers could play an additional role in modulating FVII mRNA expression and FVII levels in plasma. We suggest that accurate identification of allelic forms of the FVII IVS7 polymorphism might be of interest in further clinical studies in order to investigate the physiological relevance of such polymorphisms in FVII level modulation.

References