First molecular characterization of a patient with combined factor V and factor VII deficiency

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Combined factor V and VII deficiency is a rare bleeding disorder, with three cases reported in the literature (1–3). None of these cases were characterized at a molecular level. We now report a fourth case of factor V and VII deficiency, and for the first time characterize the factor V and VII mutations/polymorphisms in the proband and parents.

Patient and methods

Family history
The proband is a 5-year-old female who presented with microscopic hematuria and mild mucosal bleeding. Laboratory evaluation demonstrated a prolonged PT and a normal aPTT, and she was found to have 45% FV activity and 38% FVII activity. Her father has a history of delayed wound healing and mild mucosal bleeding. The father’s FV activity was high normal (151%), and his FVII activity was decreased (55%). Her mother has history of mild epistaxis, menorrhagia, and gingival bleeding. The mother’s FV activity was 42%, and her FVII activity was in the low normal range (65%) (Table 1).

Factor V and VII activity
Blood from the proband and parents was obtained with informed consent and IRB approval. Factor V and VII coagulant activity was determined in the proband and the parents by a clotting assay using a Behring BCS automated coagulation instrument.

Polymerase chain reaction amplification and sequencing
Genomic DNA was isolated from peripheral blood. The 25 exons of the F5 gene were amplified using intronic primers. Due to the large length of exon 13, it was divided into seven smaller overlapping fragments for amplification. The primers and the PCR conditions for all the exons can be found at http://193.60.222.13/. Annotation of FV nucleotide sequences are as per O’Hara et al. (5). Direct sequencing of the PCR products was performed using fluorescent dideoxy chain terminators (BigDye 3.1, Applied Biosystems, Foster City, CA, USA) on an ABI 310 sequencing instrument (Applied Biosystems).

Results and discussion

The genotyping results for the proband and parents are shown in Table 1. In addition, several known FV polymorphisms were found; three in exon 13 (R830K, H837R, K897E) and one in exon 16 (M1736V). The proband, father, and mother were heterozygous for all these polymorphisms, except R830K for which all were homozygous. An additional, novel FV polymorphism was found in heterozygous form in both the proband and father in exon 13 (P1438H). The father’s high FV activity levels suggest that this substitution of a proline for a histidine in the B-domain is of no functional consequence. In the FVII gene a novel mutation was also found heterozygously in the proband and father (G306A). This polymorphism is located in the promoter region and does not appear to reside in or near any known binding regions. Its significance is not known.

The proband has combined deficiencies of both FV and FVII, inheriting different FV and FVII mutations from each parent, and resulting in a complex genotype. For the FV deficiency, the G524del frameshift with premature termination on the maternally-inherited allele obviously contributes to the proband’s phenotype. Both proband and mother have FV activity levels consistent with a single FV premature termination codon (45% and 42%, respectively). This functional data and the father’s high FV activity levels suggest that the P1438H B-domain FV mutation, also inherited by the proband, is likely a polymorphism of no functional consequence. This is the first report to our knowledge for each of these mutations.

The FVII genotype is more complicated, and it is not uncommon for multiple polymorphisms to be involved in cases of FVII deficiency (6). The paternal allele contains three polymorphisms that, at least in the Italian population, have strong allelic association (7). These polymorphisms include a 10-bp insertion [CCTATATCCT] at –323 in the 5’ region of the promoter, a G to A substitution in intron 1a (73 g→a), and an R353Q polymorphism in exon 8. While their respective contributions toward FVII deficiency are controversial, the reductions in FVII activity average approximately 20%, although the variability among the
cases is high (8). Thus, it seems likely that this constellation of polymorphisms contributes to the proband’s FVII deficiency. Finally, we cannot rule out a contribution by the novel G306A mutation in the promoter region on the paternal allele, although the presence of the three known polymorphisms is sufficient to explain his decrease in FVII activity.

The maternal FVII allele contains only the R315W substitution in exon 8. There is one other case reported involving this mutation, a compound heterozygote involving R315W and FVII Padua (R304Q) (9). This case had a 26% FVII activity level and a mild clinical phenotype. In vitro expression studies of the 315W protein demonstrated decreased FVII activity. The mutation occurs in a serine protease domain involved directly and indirectly with tissue factor interactions. In the family reported here, the mother represents the first case wherein the sole FVII mutation is R315W (homozygous). Her FVII activity level of 65%, while within the normal range, appears to corroborate the previous in vitro finding of decreased FVII activity in the R315W allele. The finding that the proband inherited the maternal R315W allele and phenotypically has a more severe FVII deficiency than the father (38% vs. 55% in the father) is also consistent with a loss of function consequence for R315W.

In summary, this is the first molecular characterization of a patient with combined factor V and VII deficiency. We postulate that the proband’s FV deficiency is due solely to a maternally inherited frameshift mutation, while her FVII deficiency results from the combination of mutations inherited from both parents. These results are consistent with this rare syndrome being due to chance co-inheritance of multiple FV and FVII mutations rather than a single post-translational defect such as that seen in some cases of combined FV and FVIII deficiency.

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References