Inherited factor V deficiency associated with a novel heterozygous missense mutation (p.G493R) in a patient with excessive surgical bleeding

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Blood coagulation factor V (FV) is a single-chain glycoprotein (Mr 330 kDa) synthesised in the liver and megakaryocytes. Its plasma concentration is 20 nM (7 μg/mL) and approximately 20% of total FV is found in platelet α-granules (1). FV has a mosaic-like structure, with a domain organisation (A1–A2-B-A3-C1-C2) that shows high similarity to factor VIII (FVIII) (2). FV, an essential cofactor of activated factor X, is activated by thrombin. Thrombin removes the B-domain and the remaining heavy chain and light chain are associated via a calcium ion (3, 4). Activated FV (FVa) enhances the rate of prothrombin activation by several orders of magnitude (3). The gene for FV (F5) has been localised to chromosome 1q21–25; it spans approximately 80 kilobases and consists of 25 exons and 24 introns (5).

Congenital FV deficiency (Online Mendelian Inheritance in Man, OMIM +227400) is a rare autosomal recessive bleeding disorder with a prevalence of 1:1 million in the general population (6). Patients with homozygous FV deficiency usually have FV activity below 10% and show moderate to heavy bleeding symptoms (7). Most heterozygotes are asymptomatic (6–8). The vast majority of the cases are type I FV deficient, however one patient with type II deficiency has also been published (9). Over 50 different mutations in F5 have been described so far in the literature. These mutations are listed in different databases (10). The majority of missense mutations are located in the A2 and C2 domains (11).

Four missense mutations were found in F5 of the proband (Table 1). The previously published, normal sequence variations, c.2863 A>G (p.K897E) and c.5380A>G (p.M1736V) were found in heterozygous form and the silent c.327A>G (p.Q51) mutation was present in homozygous form (11–15). A novel mutation c.1651G>A (p.G493R) was detected in heterozygous form. The same mutation was also found in one of the two daughters of the proband, who also showed considerably decreased FV activity and antigen levels. This mutation was absent in 100 healthy Hungarian individuals.

Discussion

Our results revealed a p.G493R mutation of FV in the proband and in one of her daughters (child 2). Expression of the mutant allele at mRNA level was not investigated, the low FV antigen...
Table 1: Factor activity, antigen concentration and genotypic results of the proband and her family members. FV activity and antigen concentration were determined in a one-stage clotting assay on a BCS analyser (Siemens/Dade-Behring, Marburg, Germany) and by an in-house sandwich ELISA using two polyclonal anti FV antibodies (Affinity Biologicals, Ancaster, Canada) respectively. Plasma FV activity of the patient was determined at two different occasions; its reference interval was 70–120%. Genomic DNA was isolated from peripheral blood and amplified in PCR reactions using primers described earlier (7, 20). Double stranded sequencing of the PCR products of all 25 exons and the exon-intron boundaries was performed on an ABI PRISM 3100 DNA sequencer (Perkin-Elmer Biosystems, Foster City, CA). Nucleotides were numbered according to Jenny et al (12).

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<thead>
<tr>
<th>Genotype</th>
<th>FV activity in plasma</th>
<th>FV antigen in plasma</th>
<th>FV antigen in platelets</th>
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<tr>
<td>c.327A&gt;G</td>
<td>c.1651G&gt;A</td>
<td>c.2863A&gt;G</td>
<td>c.5380A&gt;G</td>
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<tr>
<td>27% 37%</td>
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<td>59%</td>
<td>29%</td>
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<tr>
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<td>33%</td>
<td>29%</td>
<td></td>
</tr>
<tr>
<td>119%</td>
<td>Not determined</td>
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Only three patients with heterozygous FV mutation in association with haemorrhagic complications have been reported. The first patient with FV activity of 45% had microscopic haematuria and mild mucosal bleedings. He carried the nt524delG mutation in one allele of F5, however he was also heterozygous for a mutation in the FVII gene (18). The second patient, who was heterozygous for the p.E1608K mutation, suffered from spontaneous haematomas and metrorrhagia (19). Her FV activity and antigen levels were 38% and 50%, respectively. The third patient had haemorrhagic complications following tonsillectomy, but no spontaneous bleeding. His FV activity was 30% and he carried the p.Y1702C mutation, which is a common cause of FV deficiency in the Italian population (8). In the latter two cases no other causes of bleeding disorder were reported.

Our patient had no spontaneous bleeding, however her case suggests that upon surgical interventions, significant bleeding may occur in heterozygous FV deficient patients. It is to be noted that plasma FV activity of the proband and her daughter with the p.G493R mutation was consistently lower than 50%, which might contribute to the bleeding tendency. Further evaluation of FV p.G493R by expression studies would be helpful to confirm the proposed mechanism of the factor V deficiency in our patient. Although the bleeding risks associated with mild factor V deficiency are uncertain, our findings suggest that heterozygosity for some factor V mutations increases bleeding risks.

Figure 1: FV A2 domain. A) The averaged geometry obtained from the last 5 ns of the 20 ns molecular dynamics simulation of the A2 domain of wild-type (G493) protein. The Cys472 and Cys498-bridged loop, in which the p.G493R mutation is located, and the adjacent 390ILGPIIRAQVR400 region are depicted in green color. B) The region of interest in the FV A2 domain of wild-type (G493) protein. C) The region of interest in the FV A2 domain of mutant (R493) protein. The simulations were carried out by Gromacs 3.3. molecular dynamics package (21) using 2fs time step, OPLS-AA/L force field (22), TIP3P explicit solvent model (23), periodic boundary condition and particle mesh Ewald method (24) for long range electrostatics. The Cys residues involved in the loop formation and the mutated residue are shown by ball and stick representation.

The simulation revealed that the side chain of Arg does not fit into the (hydrophobic) cavity reserved for the small αH “side chain” of Gly. Its polar guanidinium tail deformed the adjacent, also highly conservative 390ILGPIIRAQVR400 β-sheet and opened a channel to the (polar) solvent environment. Although an expression study that directly links the mutation to FV deficiency was not performed, molecular modelling strongly suggested that the local conformational change resulted in the instability of that region and, finally, to a mis-folding of the A2 domain (Fig. 1).

Table: 788
References