Severe coagulation factor V deficiency caused by 2 novel frameshift mutations: 2952delT in exon 13 and 5493insG in exon 16 of factor 5 gene

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A male infant with severe bleeding tendency had undetectable factor V activity. Sequence analysis of the proband’s DNA revealed one base deletion in exon 13 (2952delT) and one base insertion in exon 16 (5493insG) in heterozygous form. Both mutations introduced a frameshift and a premature stop at codons 930 and 1776, respectively. The proband’s father and mother were heterozygous for 2952delT and for 5493insG, respectively. Both mutations would result in the synthesis of truncated proteins lacking complete light chain or its C-terminal part. In the patient’s plasma, no factor V light chain was detected by enzyme-linked immunosorbent assay. The N-terminal portion of factor V containing the heavy chain, and the connecting B domain was severely reduced but detectable (1.7%). A small amount of truncated factor V–specific protein with a molecular weight ratio of 236 kd could be immunoprecipitated from the plasma and detected by Western blotting. This protein, factor VDebrecen, corresponds to the translated product of exon 16 mutant allele. (Blood. 2002;99:702-705)

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described with a final platelet count of 1000 G/L (10^9 platelets per liter), and lysed with 1% Triton X-100.

**Factor V activity and antigen assays**

Factor V coagulant activity in plasma was measured by a one-stage assay based on prothrombin time. Standard human plasma (Dade Behring, Marburg, Germany) was used for calibration. Factor V activity in platelet lysate samples prepared as described above was determined by the same assay system, but in this case the assay was calibrated against pooled normal platelet lysate. Factor V, factor V HC, and factor V LC antigen levels were determined by sandwich enzyme–linked immunosorbent assay (ELISA). Sheep antihuman factor V polyclonal antibody (The Binding Site, Birmingham, United Kingdom), monoclonal antibody directed to epitope on the 150-kd activation peptide in the connecting B domain (clone B10) (Chemicon, Temecula, CA), and monoclonal antibody directed to epitope on C2 domain of factor V LC (clone HV1) (Sigma) were used as capture antibodies. Rabbit antihuman factor V antiserum (Diagnostica Stago, Asnières, France) was used as second antibody and was followed by peroxidase-labeled goat antirabbit immunoglobulin (Ig)–G (Dako, Glostrup, Denmark). The assays were calibrated against standard human plasma (Dade Behring), and factor V antigen levels were expressed as a percentage of the normal average.

**Immunoprecipitation, sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and immunoblotting**

Factor V was isolated from the plasma by immunoprecipitation with the use of sheep polyclonal anti–factor V antibody (The Binding Site) biotinylated at carbohydrate residues and streptavidin agarse (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom). Immunoprecipitates and whole plasma samples were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and by Western blotting. The following antibodies were used as primary antibody: biotinylated sheep polyclonal antibody against factor V.
(see above); rabbit anti–factor V antiserum (Assera V, Diagnostica Stago); and mouse monoclonal antibody against the B domain of factor V (Chemicon). The immunoreaction with the biotinylated antibody was visualized by avidin H and biotinylated peroxidase complex (Vectastain ABC kit) (Vector, Burlingame, CA). Nonbiotinylated anti–factor V rabbit antibody and anti–factor V B-domain mouse antibody were followed by peroxidase-labeled goat antirabbit IgG and rabbit antimi

Polymerase chain reaction amplification and sequencing

Genomic DNA was isolated from buffy coats by QIAamp Blood Mini Kit (QiAGEN, Hilden, Germany). The following oligonucleotides used in polymerase chain reaction (PCR) amplification and sequencing were designed from factor V gene sequence18: e1F, 5′-cccaagctctactgagtc-3′; e1R, 5′-caggctcatctaattcag-3′; e2F, 5′-tagttgggttattcatc-3′; e2R, 5′-gatctgctgcccaatac-3′; e5F, 5′-tctgtcttcattgagtg-3′; e7F, 5′-ttgatcagatattctacc-3′; e8F, 5′-attgaaagattttaattct-3′; e8R, 5′-cattgaaattatatcagtc-3′; e17F, 5′-cagaaagcttccctat-3′; e17R, 5′-atcagatagaaatatg-3′; e21F, 5′-gtaatggagttgcagtc-3′; e21R, 5′-tcgagatcagatattctacc-3′; e22F, 5′-ttcattctctttcatgtct-3′; e22R, 5′-tttctctttcataattttcagtaaatgg-3′; e24F, 5′-ctgctgcccaatacactct-3′; e24R, 5′-gcagactctctgctgcccaatac-3′; e25F, 5′-ttctttattttgctttcag-3′; e25R, 5′-attcagatagaaatatg-3′; Exon 13 was amplified in 4 overlapping frag-

Results and discussion

The proband had highly prolonged prothrombin time (58.1 seconds; control, 8.7-11.5 seconds) and activated partial thromboplastin time (198.8 seconds; control, 29.5-42.7 seconds). Plasma clotting factor activities were within the reference (control) range with the exception of factor V. Factor V activity was undetectable in the proband’s plasma (Figure 1A) and platelet lysate. The mother, the father, and one grandmother had moderately decreased factor V activity corresponding to a platelet lysate. The mother, the father, and one grandmother had antigen values around 50%. No factor V was detected in the patient’s plasma by Western blotting (Figure 2B, lane 2); however, when factor V antigen was detected, it was concentrated 100-fold by immunoprecipitation, a faint band with an M, of 236 kd reacted with monoclonal anti–factor V B-domain antibody (Figure 2B, lane 4) or polyclonal anti–factor V antibodies (results with the latter antibodies are not shown). No intact factor V could be seen, and a further 4-fold increase in the amount of immunoprecipitate obtained from the patient’s plasma did not change the situation (not shown). The predicted protein resulting from the exon 13 mutation would lack part of the B domain and the complete LC. The exon 16 mutant protein would lack a significant part of the LC, and this 1775 amino acid–long polypeptide would have an M, of 200 kd. Considering that it contains the heavily glycosylated B domain, it is very likely that the 236-kd protein that we now designate factor VDebrecen represents this larger truncated protein. The absence of smaller truncated protein and the highly reduced amount of factor VDebrecen could be due to reduced synthesis of mutant messenger RNAs12 to the instability and intracellular degradation of mutant proteins, and to the accelerated plasma clearance of truncated factor V.19

Complete factor V deficiency is lethal in knockout mice; however, they can be rescued by a very low level (less than 0.1%) of transgene factor V expression.20,21 Although we were unable to detect intact factor V in the patient’s plasma, it cannot be excluded that the patient expresses a very low level of factor V as a result of ribosomal slippage or somatic reversion. Such a low level of factor V might be undetectable on the Western blot, even by the highly sensitive chemiluminescent technique. Alternatively, intact factor V, owing to its extreme protease sensitivity, could have been degraded during the immunoprecipitation procedure. A further possibility is that the truncated protein possesses some residual procoagulant activity22 that is sufficient to rescue the patient from fatal consequences.

References


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