Dear Sir,

Factor V (FV) is a high-molecular-weight glycoprotein (330 kd), synthesized mainly by hepatocytes and megakaryocytes as a single polypeptide chain, circulating in plasma as an inactive non-enzymatic cofactor (15%-20% of total FV is in the alpha granules of platelets) (1, 2). FV shares a structure characterized by an overall organization of A1-A2-B-A3-C1-C2 protein domains with the highly homologous factor VIII (FVIII) (3). The large B domain, which has no known homology with any other protein, is proteolytically removed during activation of both FV and FVIII. The 3 A domains of FV and FVIII share about 30% amino acid identity with each other and with the triplicate A domains of ceruloplasmin (CP) (4). FV deficiency is extremely uncommon, occurring either because of a homozygous inheritance or because of a combination of defective alleles. Its incidence is about 1 in 1 million (5). The FV gene (F5) was mapped to chromosome 1q23, spans more than 80 kb, and contains 25 exons (6).

Up to now, only a few mutations scattered through F5 have been identified (2, 7–9). Most mutations are nonsense, frameshift, or splicing mutations. Of 10 missense mutations identified, only 5 have been characterized by in vitro expression studies, which demonstrated an impairment of FV secretion associated with intracellular degradation of the mutant protein (7, 10, 11).

We report the molecular characterization by transient expression in COS-1 cells of the Gly392Cys missense mutation in F5 causing severe FV deficiency in a Chinese patient. This missense mutation has also been reported in an unrelated Chinese patient.

The proband was a 22-year-old Taiwanese man. He suffered from mild gum bleeding and easy bruising since childhood, and had been treated with fresh-frozen plasma for intraabdominal bleeding after an appendectomy at the age of 7. On this occasion, diagnosis of severe FV deficiency was made. He had not had a major bleeding episode since then. His parents were not related, and all came from southern Taiwan. No bleeding tendency had been reported for the other family members. Informed consent for this study was obtained from the proband and his family members.

FV coagulant activity was measured using a one-stage clotting assay (International Laboratories, Milan, Italy) and a FV-deficient plasma (International Laboratories). FV antigen was measured using an enzyme immunoassay (EIA) with a polyclonal antibody (Affinity Biologicals, Hamilton, ON, Canada) and a normal plasma pool as standard. As shown in Figure 1A, the proband had severely reduced levels: 2% activity and 3% of FV antigen compared with the standard. Except for his brother, all other family members had intermediate levels, consistent with heterozygous FV deficiency.

Genomic DNA was extracted from whole blood and the primers for PCR were designed according to the published genomic FV sequence (GenBank Z99572). The PCR products of all 25 exons and their intron-exon boundaries were sequenced on...
an ABI sequencer (Applied Biosystems, Foster City, CA). Direct sequencing of FV revealed the patient to be homozygous for G1348T in exon 8. G1348T was a missense mutation which caused amino acid substitution of Gly to Cys at position 392. The proband’s parents and two sisters were heterozygous for the Gly392Cys mutations.

Site-directed mutagenesis of pMT2/FV plasmid was performed to replace Gly392 with a Cys codon using the Quick-Change IIXL Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA). The pMT2/FV mammalian expression plasmid, containing the full-length FV complementary DNA (cDNA), was kindly provided by Dr R. J. Kaufman (Howard Hughes Medical Institute, University of Michigan Medical School, Ann Arbor). COS-1 cells were cultured and transfected using the Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA). FV antigen levels were measured 72 hours after transfection in conditioned media and cell lysates by EIA. The mutant protein was analyzed in 3 independent transfection experiments, each performed in duplicate. Transfection results are shown in Figure 1B. The mutation caused an approximately 7-fold reduction in FV antigen level in the corresponding conditioned media. In lysates of cells expressing the mutant allele, FV antigen levels were reduced to approximately 50% of those measured in cells expressing the wild-type allele.

The Gly392Cys mutation was first reported as a compound heterozygous mutation in an unrelated Chinese patient with severe FV deficiency (8), and four of the proband’s family were heterozygous for this mutation. Our patient is the first reported to be homozygous for Gly392Cys. Most FV mutations are private, having been detected in only one patient and his/her family members. Only Tyr1702Cys has been reported to be a recurrent mutation in the Italian FV-deficiency patients (12). Further study should be done to elucidate the role of the Gly392Cys mutation in Chinese FV deficient patients.

The glycine residue in the 392 position is conserved in all three A domains of FV and its homologous counterparts, FVIII, and CP (13). This mutation, which determines the exposure of a novel cysteine residue, has been proposed to cause protein instability by disrupting the A2 domain scaffold and by interfering with the correct disulfide bond formation between the other four cysteines on the A2 domain (14, 15). This results in the impairment of secretion or stability of the FV molecule.

Among the 10 missense mutations reported so far which result in severe or moderate severe FV deficiency, five mutations (Arg413Cys, Cys472Gly, Val1813Met, Pro2070Leu, and Arg2074Cys) have been characterized by in vitro expression studies, and all resulted in a secretion defect with a rapid intracellular degradation. While severely reduced FV levels were found in cell supernatants in all cases, intracellular levels of mutant FV molecules ranged from 16.9% to 77% of the wild type, suggesting differences in the degradation rates of the mutant FVs (11). The lowest FV intracellular level was associated with the Arg2074Cys mutation. They further explored the degradation pathway of this molecule using different inhibitors of protein degradation, and learned that the degradation of this molecule occurs primarily in a post-Golgi compartment (11). Further study with the different inhibitors of protein degradation will clarify where the present mutant degrades.

References