Coagulation factor V (FV) deficiency is a rare coagulopathy associated with moderate to severe bleeding symptoms. A total of 34 mutations, all located in the FV gene (F5), have been described in patients with severe FV deficiency, only eight of whom being of Asian descent. Sequencing of F5 in five unrelated Indian patients identified three novel small deletions in exon 13, all present in the homozygous state (g.50936-50937delAA or AG and g.51660delA, both occurring in two different patients, and g.52162delC). Besides widening the knowledge on the mutational spectrum of FV deficiency in Asian populations, these data will also be useful for purposes of prenatal diagnosis.

Table 1. Clinical and genetic characteristics of the analyzed FV-deficient Indian patients.

<table>
<thead>
<tr>
<th>Patient*</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Present age (y)</td>
<td>28</td>
<td>30</td>
<td>n.a.</td>
<td>30</td>
<td>16</td>
</tr>
<tr>
<td>Consanguinity</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes*</td>
<td>No</td>
</tr>
<tr>
<td>Main symptoms</td>
<td>Gum, nose, and joint bleeds</td>
<td>n.a.</td>
<td>Gum and nose bleeds</td>
<td>Hematomas</td>
<td>Gum and nose bleeds</td>
</tr>
<tr>
<td>FVAg (%)</td>
<td>2.4</td>
<td>2.4</td>
<td>2.9</td>
<td>2.9</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Mutation†</td>
<td>Genomic (g.50936-50937delAA or AG)</td>
<td>g.50936-50937delAA</td>
<td>g.51660delA</td>
<td>g.51660delA</td>
<td>g.52162delC</td>
</tr>
<tr>
<td>cDNA††</td>
<td>c.2662-2663delAA</td>
<td>c.2662-2663delAA</td>
<td>c.3386delA</td>
<td>c.3386delA</td>
<td>c.3888delC</td>
</tr>
<tr>
<td>Mature protein</td>
<td>F538pter</td>
<td>F538pter</td>
<td>F51142pter</td>
<td>F51142ter</td>
<td>F51259pter</td>
</tr>
</tbody>
</table>

* Informed consent was obtained from all examined patients; the study was approved by the Institutional Review Board of the University of Milan. **Unknown degree of consanguinity: *FV* antigen levels (FVAg) were determined using an in-house developed sandwich enzyme immunoassay (EIA), based on a sheep anti-human polyclonal antibody (Affinity Biologicals, Hamilton, ON, Canada). FV levels were expressed as percentage of control plasma pooled from 40 normal individuals, set at 100% (normal range: 64-159%; sensitivity of the test 1%). † Primer pairs for mutation detection were designed from flanking intronic sequences (GenBank, accession number Z95972) and their sequences are available on request. †† Numbering according to GenBank accession number Z95972, inverted and complemented. ††† Numbering according to GenBank accession number M19867. n.a. not available; g: genomic sequence; c: cDNA sequence; F5: frameshift.

Coagulation factor V (FV) is a 330-kDa single-chain plasmatic pro-co-factor, with an A1-A2-B-A3-C1-C2 domain structure. FV is converted into its active form (FVa) by α-thrombin or activated factor X (FXa) through proteolytic removal of the large B domain. Once activated, FVa acts as an essential co-factor for the FXa-catalyzed activation of prothrombin, whereas it is rapidly inactivated by activated protein C, thus limiting thrombin generation. The FV gene (F5) has been mapped to chromosome 1q24.2. Defects in this gene result in either an autosomal dominant form of thrombophilia (activated protein C resistance) or in a hemorrhagic diathesis with an autosomal recessive mode of inheritance [severe or moderately severe FV deficiency; Online Mendelian Inheritance in Man (OMIM) +227400].

The estimated prevalence of severe FV deficiency in the general population is one in one million; the disorder is less rare in populations in which consanguineous marriages are traditionally frequent, such as those from Muslim countries and Southern India.6,5 FV-deficient patients have a lifelong hemorrhagic diathesis of variable severity, due to the complete absence or extremely low levels of both functional and immunoreactive plasma FV.5,6 Molecular analysis of F5 in affected individuals has led to the identification of 34 causative mutations.7,9 Mutations introducing premature termination codons (PTCs) account for two thirds of the total; most of them are located in the large exon 13 (six deletions and four nonsense mutations). So far, only ten molecular defects, including the recurrent Tyr1702Cys mutation, have been described in patients of Asian origin (three probands were from China, while one each was from Japan, Korea, Iran, Taiwan, and from a not-specified area of South Asia).7,9 This sparse information makes it impossible to demonstrate a correlation between genetic defects and the ethnicity or the geographical distribution of the patients, thus hampering the possibility to design a population-specific strategy for genetic/prenatal diagnosis of the disorder. To address this issue, a cohort of five unrelated patients with FV deficiency from Northern India was analyzed.

All patients, whose main clinical details are summarized in Table 1, had very low plasma antigen FV levels and did not suffer from any additional concomitant coagulation disorder. No bleeding history was reported in any of the probands’ relatives. Genomic DNA of each patient was extracted from blood samples according to standard procedures. All 25 F5 exons, including exon-intron boundaries and about 300 bp of the promoter region, were amplified by polymerase chain reaction (PCR) under standard conditions from genomic DNA. Sequencing, which was carried out as described elsewhere, revealed three small deletions in exon 13, each present in the homozygous state (Table 1). In particular, (i) in patients A and B, a deletion of two nucleotides was found at genomical positions 50,936-50,937 (g.50936-50937delAA or AG), giving rise to a frameshift (introducing three aberrant residues) that is predicted to encode for a severely truncated polypeptide chain because of the presence of a PTC.

The predicted mutant mature FV molecule would be composed of only 834 amino acids (F583pter). Since the nucleotide position 50,937 is polymorphic (A or G; rs4524), it is impossible to establish unambiguously whether the deletion involves an AA or an AG dinucleotide; (ii) a single bp deletion, involving the nucleotide at genomic position 51,660 (g.51660delA) was identified in both patients C and D. This frameshift mutation would lead to a premature stop at codon 1142 (F51142ter), preceded by an abnormal and long stretch of 72 amino acids resulting from the translation of the putative mutant FV mRNA in a different reading frame; (iii) in patient E, a single C deletion (within a double C) was found at nucleotide position 52,162 (g.52162delC). This mutation would predict the synthesis of a truncated protein (F51259ter), which shows a novel aberrant sequence of 21 residues at the C-terminus as a consequence of the frameshift.

In all cases, these three newly identified molecular defects lead to the introduction of a PTC, making them a
possible trigger for the nonsense-mediated mRNA decay pathway (NMD). Indeed, when investigated at the mRNA level, PTC-causing mutations in F5 were always demonstrated to induce the selective degradation of the corresponding transcript; should the three PTC-carrying transcripts escape the NMD surveillance system, the encoded proteins would lack part of the B domain and the complete light chain (corresponding to the A3-C1-C2 domains) (Figure 1A). In order to check for the existence of a common ancestor, haplotype analysis for polymorphic markers covering the whole F5 (18 biallelic and one microsatellite in intron 11) was performed in patients carrying the same mutation (g.50936-50937delAA or AG in patients A and B; g.51660delA in patients C and D). All patients were homozygous for each marker (Figure 1B).

In particular, patients A and B shared the same haplotype, suggesting that they had a common ancestor. The same conclusions could be drawn for patients C and D, who both carry the same haplotype, differing by five markers (spread all over F5, from exon 2 to intron 16) from the previous one. However, these results are also compatible with the presence of a heterozygous large deletion, leading to the PCR amplification of only one F5 allele. Unfortunately, the most straightforward approach to test the patients for hemizygosity (i.e. by genotyping their parents) could not be adopted, due to the unavailability of DNA samples from the patients’ relatives. Knowledge on the existence of founder effects for specific mutations can be useful to lower costs and speed up procedures for the molecular diagnosis of the disease and, whenever morally warranted by the severity of symptoms, for its prevention through prenatal diagnosis. This is especially true for those countries in which the
disorder is highly prevalent (high level of inbreeding) and resources (facilities and budget) are not abundant.

In this frame, considering that FV deficiency is characterized by a high level of allelic heterogeneity, the identification of two homozygous mutations in four Indian families with FV deficiency, might suggest the presence of prevalent mutations in this population. Nonetheless, the identification of founder effects requires the genetic characterization of large cohorts of patients, an important task for future genetic studies in Asian populations.

Rosanna Asselta,* Claudia Dall’Osso,* Stefano Duga,* Marta Spreafico,* Renata Saxena, # Maria Luisa Tenchini*
*Department of Biology and Genetics for Medical Sciences, University of Milan; 
°A. Bianchi Bonomi, Hemophilia and Thrombosis Center, University of Milan and Department of Medicine and Medical Specialties, IRCCS Maggiore Hospital, Mangiagalli and Regina Elena Foundation, Milan, Italy; 
°Department of Hematology, All Indian Institute of Medical Science, New Delhi, India

Funding: R.A. is a recipient of a Bayer Hemophilia Early Career Investigator Award 2005. The financial support of Telethon – Italy (Grant n. GGP050326) and of PRIN (Programmi di Ricerca Scientifica di Rilevante Interesse Nazionale, Grant n. 2005058307_002) is gratefully acknowledged.

Acknowledgments: The authors thank Paolo Motta and Roberta Maretta for excellent technical assistance.

Key words: coagulation factor V, factor V deficiency, gene analysis, mutation.

Correspondence: Maria Luisa Tenchini, Department of Biology and Genetics for Medical Sciences, Via Venti, 3/5 -20133 Milan, Italy. Phone: international +39.02.50315864. E-mail: marialuisa.tenchini@unimi.it

References


3. UCSC Genome Browser, http://genome.ucsc.edu/


