Novel fibrinogen (Bβ401Gly→Val) presents as dys- or hypodysfibrinogenaemia due to alterations in sialic acid content

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Dear Sirs,

Fibrinogen, an acute phase protein, is synthesized in the liver from individual α, β and γ chains (1). A signal peptide directs the nascent chains into the lumen of the endoplasmic reticulum and following cleavage of the leader sequence, the mature protein is assembled in a step wise manner from α-γ and β-β intermediates (2). After acquiring an additional Bβ or αα chain respectively, the resulting αα-Bβ-γ half-molecules dimerise and the (αα-Bβ-γ)2 assembly proceeds to the Golgi for pruning and extension of the N-linked oligosaccharides. Subsequent cleavage of the αα chain propeptide is followed by secretion into the circulation.

With biantennary oligosaccharide chains attached to each of the Bβ and γ chains there can be a maximum of 8 moles of sialic acid per fibrinogen molecule (3). However, the terminal sites are not fully saturated and the amount of sialic acid attached is dependant on the activity of CMP-neuraminic acid transferase and other enzymes in the trans-Golgi network of the hepatocyte. Both the level of sialylation, and hence the functionality of fibrinogen, can be influenced by liver disease such as hepatoma (4), or alcoholic liver disease (5). Conversely fibrinogen mutation itself can cause liver disease and hypersialylation; in these cases the substitution causes hepatic storage disease, which can lead to hepatitis. Known: Brescia (γ284Gly→Arg), Agualidía (γ375Arg→Trp), Angers (γ346→350AGVYG) and Al du Pont (γ314Thr→Pro), and suspected (γ335Trp→Arg) inclusion body substitutions are associated with increased sialic acid levels, decreased antigenic fibrinogen and prolonged thrombin clotting times (TCT) (6–10). Whilst no inclusion body mutations have been identified to date in the Bβ gene, there is no reason to believe there will not be in the future as the two chains have similar sequences and crystal structures.

Here we report a novel mutation (Bβ401Gly→Val) substitution that presents as both a functional and quantitative defect (hypodysfibrinogenaemia) in one individual, but as only a quantitative defect in other carriers.

The subject of this investigation, a 79-year-old woman with a bleeding diathesis arising from a surgical challenge, was diagnosed as having familial hypofibrinogenaemia in 1978. She contracted hepatitis C at that time, probably from a cryoprecipitate transfusion. Current results (►Table 1) show a low functional (0.7 mg/ml) and physical fibrinogen concentration (0.9 mg/ml) together with a prolonged TCT (43 s). The PT was extended (17.1 s; normal range 12–15) and the INR elevated at 1.4 (normal range 0.8–1.1). When examined later, one of her daughters and one of her granddaughters were also found to have low fibrinogen levels (1.6 mg/ml), but this was accompanied in their case by a normal TCT.

Amplification and direct sequencing of the coding regions and intronic boundaries of the fibrinogen genes established that the subject, together with her daughter (2. II) and granddaughter (3. II), were heterozygous for a novel c.1292C>T mutation in exon eight of FGB. This GGA→GTG transversion results in a substitution of Gly→Val at codon 401 of the mature Bβ chain and we have named the variant fibrinogen Kogarah. In addition, the subject was also heterozygous for the less common Bβ235Leu polymorphism, as was her haematologically normal daughter (2. I).

Reducing SDS PAGE of ammonium sulphate-purified fibrinogen (11) from the proposita showed normal αα and γ chains but a slightly faster migrating Bβ chain compared to adjacent controls (►Fig. 1). The normal daughter (2. I), on the other hand, who was also heterozygous for the Bβ235Leu polymorphism showed the broadened Bβ band characteristic of equal expression of both Bβ chains (12, 13).

Table 1: Coagulation parameters and FGB genotypes. *FpB is a physical fibrinogen concentration from B peptide quantitation.

<table>
<thead>
<tr>
<th>Individual</th>
<th>FpB* (mg/ml)</th>
<th>Class (mg/ml)</th>
<th>TCT (s)</th>
<th>Bβ401</th>
<th>Bβ235</th>
<th>Sialic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.I subject</td>
<td>0.9</td>
<td>0.7</td>
<td>43</td>
<td>Gly</td>
<td>Leu</td>
<td>6.0</td>
</tr>
<tr>
<td>2.I daughter</td>
<td>1.7</td>
<td>2.0</td>
<td>23</td>
<td>Gly</td>
<td>Gly</td>
<td>5.0</td>
</tr>
<tr>
<td>3.I granddaughter</td>
<td>2.9</td>
<td>2.9</td>
<td>21</td>
<td>Gly</td>
<td>Gly</td>
<td>5.1</td>
</tr>
<tr>
<td>2.II daughter</td>
<td>1.6</td>
<td>26</td>
<td>Gly</td>
<td>Val</td>
<td>Gly</td>
<td>5.2</td>
</tr>
<tr>
<td>3.II granddaughter</td>
<td>1.6</td>
<td>27</td>
<td>Gly</td>
<td>Val</td>
<td>Pro</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Ref. range 1.5–4.0 18–28 Gly Pro mol/mol

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Figure 1: Analysis of purified fibrinogen chains. Upper panel: SDS PAGE; lanes 1, 3, 4 and 7 control fibrinogen samples; lane 2, proposita; lane 5, daughter (2.I); lane 6, granddaughter (3.I). The polymorphic β235 chains are represented as βL and βP; the proposita appears to be homozygous for the less common βP allele due to non expression of her βPro chain. Lower panel: Transformed electrospray mass spectra of fibrinogen γ and Bβ chains from designated family members with different genotypes. The proposita (1.I) has lack of expression of the common Bβ235P chain in the proposita suggested it contained the novel Bβ401Gly→Val mutation and that the substitution caused the low fibrinogen concentration. This was confirmed by the independent segregation of the Bβ401Gly→Val mutation to the daughter with hypofibrinogenaemia (2.II) and the Bβ235Leu to the normal daughter (2.I).

Residue Bβ401 is located in the globular C-terminal region of the chain and underpins the five-stranded sheet (14). It occurs at a bend and is conserved as glycine in all fibrinogen chains across all sequenced species, but has evolved in a limited way to alanine in the fibrinogen-like proteins. Mutation of flanking residues, G400D in the Bβ chain (15) and W335R in the γ chain (10), have also been associated with low fibrinogen; a- and hypofibrinogenaemia, respectively, highlighting the importance of the conserved GGWW sequence of which it is part.

In summary, (i) the phylogenetic conservation of Gly401, (ii) the phenotypic consequences of adjacent mutations, (iii) the radical change in bulk and hydrophobicity of the side chain, (iv) the absence of expression of the variant Bβ chain and, (iv) the segregation of the mutation within the family clearly establishes the mutation as the cause of the hypofibrinogenaemia. In addition, the lack of expression of a Bβ401Val chain was confirmed by mass spectrometry of isolated Bβ chains, which showed no significant mass difference between affected (3.II, 54,217 Da) and unaffected (3.I, 54,224 Da) granddaughters. These are both homozygous for Bβ235P.
and if the Bβ401Val variant chain was expressed then 3.II would be expected to have a mass increase of 42.2 Da (10).

The findings do not, however, explain the differences in fibrinogen functionality between carriers; the proposita had a significantly extended TCT of 43 s while both of the other two G401V carriers had normal TCTs of 26 and 27 s. We surmised this might be due to post translational modifications of the circulating protein. To explore this further, fibrinogen chains were isolated by reverse phase HPLC and the peaks analysed by ESI MS (11, 12) (Fig. 1). As expected the γ and Bβ chains from each individual showed a pair of peaks differing by 292 Da and representing the mono- and disialo-isofoms of the glycoproteins. The subject (1.I) showed a significantly higher proportion of the disialo-isofoms of the γ and Bβ chains than other family members with the G401V mutation or P235L polymorphism alone; she had a total of 6.0 residues of sialic acid per molecule while the others had values ranging from 3.0 to 5.2 residues per molecule (Table 1). It has been well established that the electrostatic repulsion caused by the additional negative charge delays fibrin polymerisation and extends TCTs (4). It therefore seemed probable that the unexpressed abnormally conformed Bβ chains are successfully turned over by the endoplasmic reticulum associated degradation pathways (15) preventing the development of inclusion body mediated liver disease.

In conclusion it is unlikely that the 401Gly→Val mutation is associated with inclusion bodies, but it does result in the complete exclusion of the effected chain from circulating fibrinogen. This results in hypofibrinogenaemia but not dysfibrinogenaemia; this is caused by post translational modifications consequent to unrelated liver disease.

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Conflict of interest
None declared.

References