A Novel Fibrinogen Mutation p.BßAla68Asp Causes an Inherited Dysfibrinogenemia

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Abstract

Objective Our study aimed to analyze the phenotype and genotype of a pedigree with inherited dysfibrinogenemia, and preliminarily elucidate the probable pathogenesis.

Methods The one-stage clotting method was used to test the fibrinogen activity (FIB:C), whereas immunoturbidimetry was performed to quantify the fibrinogen antigen (FIB:Ag). Furthermore, DNA sequence analysis was conducted to confirm the site of mutation. Conservation analysis and protein model analysis were performed using online bioinformatics software.

Results The FIB:C and FIB:Ag of the proband were 1.28 and 2.20 g/L, respectively. Gene analysis revealed a heterozygous c.293C>A (p.BßAla68Asp) mutation in FGB. Bioinformatics and modeling analysis suggested that the missense mutation could potentially have a deleterious effect on fibrinogen.

Conclusion The BßAla68Asp mutation in exon 2 of FGB may account for the reduced FIB:C levels observed in the pedigree. To our knowledge, this point mutation is the first report in the world.

Keywords ► FGB ► novel mutation ► dysfibrinogenemia ► genetic analysis

Introduction

Fibrinogen is a 340-kDa glycoprotein, composed of two sets of three polypeptide chains (Aα, Bβ, and γ) that are joined by multiple disulfide bonds.1 The Aα, Bβ, and γ-chains are encoded by three related genes (FGA, FGB, FGG), respectively, which are located at the long arm of chromosome 4 (4q32.1).2 These genes are composed of 6, 8, and 10 exons, respectively.3 Fibrinogen exhibits a typical trinodal structure, with a central E-domain and two peripheral D domains.3 The D domains consist of the COOH-terminal regions of the Bβ and γ chains, whereas the central E-domain is formed by amnio-terminal regions of three chains.4 The COOH-terminal region of the Aα chain is positioned close to the central E-domain.4 Owing to its critical role in the process of hemostasis, wherein active thrombin catalyzes the conversion of soluble fibrinogen into insoluble fibrin, fibrinogen is commonly referred to as coagulation factor I.5 Furthermore, fibrinogen also plays an essential role in inflammation, wound healing, and other biological functions.1

Congenital fibrinogen deficiency is a rare disease that can be classified into two types of disorders: quantitative and qualitative. Quantitative disorders comprise afibrinogenemia (complete absence of functional and antigenic fibrinogen) and hypofibrinogenemia (proportional decrease of functional and antigenic fibrinogen).5 Qualitative disorders...
include dysfibrinogenemia (normal antigenic fibrinogen and decreased functional fibrinogen levels) and hypodysfibrinogenemia (discrepant decrease of functional and antigenic fibrinogen levels). The molecular abnormalities of congenital dysfibrinogenemia are frequently located in exon 2 of FGA and exon 8 of FGG. In this study, we have detected a heterozygous mutation in the FGB gene that resulted in the substitution of BßAla68Asp amino acid. To investigate the underlying pathogenic mechanism, we employed various methods including coagulation test, DNA sequence analysis, and bioinformatics analysis.

Materials and Methods

Materials
The proband is a 48-year-old woman, who was admitted to our hospital due to persistent symptoms of regurgitation and gastric distension for 6 months. She had a regular menstrual cycle with normal volume and no history of hemorrhage or thrombosis. Routine tests were conducted before undergoing gastroscopy and colonoscopy exams, which revealed normal results except for the coagulation test indicating prolonged thrombin time (TT) and low fibrinogen activity (FIB:C). The fibrinogen antigen (FIB:Ag), activated partial thromboplastin time (APTT), and prothrombin time (PT) were all within normal range. None of the nine members of the three-generation family (►Fig. 1A) had any history of abnormal bleeding or thrombosis. The present study selected 150 healthy individuals aged between 20 and 54 years as control groups to exclude the polymorphism. The control group’s standard consisted of healthy individuals who exhibited no abnormality in hepatic and renal function and had no history of bleeding or thrombotic diseases. Our study was approved by the Ethics Committee of the First Hospital Affiliated of Wenzhou Medical University (China).

Coagulation Tests
After obtaining informed consent, the peripheral blood samples were extracted from the family. Routine coagulation tests such as PT, APTT, TT, and FIB:C were tested by a clotting assay. D-dimer (D-D) and fibrin degradation products (FDPs) were measured using immunoturbidimetry on the STAGO-STAR analyzer (Diagnostica Stago, Asnières-Sur-Seine, France), while the level of FIB:Ag was determined using immunoturbidimetry on the Beckman Coulter AU5800 Chemistry Analyzer (Beckman Coulter, California, United States). All operations were performed according to the manufacturers’ protocols.

DNA Sequence Analysis
Following informed consent, genomic DNA was extracted from blood samples of family members following the manufacturer’s protocol (Tiangen, Beijing, China). Twenty-six pairs of primers for all fibrinogen genes (FGA, FGB, and FGG), including all exons and flanking regions, were designed based on the published sequence (GenBank accession...
numbers: M64982, M64983, and M10004). The polymerase chain reaction (PCR) was amplified under standard conditions on Applied Biosystem Thermal Cycler 2720 (ABI, Foster City, California, United States). The PCR products were purified and sequenced by Sunny Biotechnology Corporation (Shanghai, China). The sequencing results were compared with the sequence published by the NCBI gene bank and mutation sites were identified using the software Chromas.

**Thrombin-Catalyzed Fibrin Polymerization**

First, the concentration of plasma fibrinogen in both the family members and the control groups was diluted to 0.5 g/L through the process of blood coagulation analysis dilution (Barbital sodium-buffered saline solution, Diagnostica Stago). Subsequently, 140 μL of the diluted samples and 10 μL of human calcium thrombin (Diagnostica Stago) were added into a 96-well plate to maintain the concentration at 1 NIH U/mL. The control groups were the first to be assessed, and the increased in turbidity at 350 nm was continuously monitored every second for 60 seconds using the multi-well plate reader (Thermo Scientific Varioskan Flash).

**Conservation Analysis and Bioinformatics Analysis**

Conservation analysis was conducted using multisequence alignment software ClustalX-2.1-win (Science Foundation Ireland) with six homologous species as listed in ► Fig. 1B (HomoloGene, http://www.ncbi.nlm.nih.gov/homologene).

To assess potential adverse effects on fibrinogen function, four online bioinformatics software (MutationTaster, PolyPhen-2, PROVEAN, and SIFT) were utilized. Besides, to examine the molecular structure of the mutation, a mutant protein model was constructed using Swiss-Pdb Viewer 4.0.1 and Protein Interactions Calculator programs (PIC, http://pic.mbu.iisc.ernet.in) based on the three-dimensional (3D) structure in the Protein Data Bank (PDB; http://www.rcsb.org/pdb/home/home.do, PDB ID: 3GHG).

**Results**

**Coagulation Tests**

The phenotypic characterizations of the proband and her family members are shown in ► Table 1. The APTT, PT, D-D, and FDP of the proband were within the normal range, although her TT value was slightly elevated at 22.5 seconds (normal range: 14.0–20.0 seconds). The TT levels of her father, sister, younger brother, and daughter showed varying degrees of increased levels. In addition, the proband’s FIB:Ag level was within the normal range, while her FIB:C level was decreased to 1.28 g/L. Her father, sister, younger brother, and sister also exhibited decreased FIB:C/FIB:Ag levels, with values less than 0.7. On the other hand, the results of her brother, spouse, younger sister, and son presented normal results.

**DNA Sequence Analysis**

According to the results of sequence analysis, the presence of a heterozygous c.293C > A point mutation in exon 2 of FGB has led to the emergence of the p.B8Ala68Asp missense

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Phenotypic and genetic analysis of the pedigree of family members</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family</td>
<td>PT (s)</td>
</tr>
<tr>
<td>Proband (II3)</td>
<td>14.2</td>
</tr>
<tr>
<td>Father (I1)</td>
<td>13.9</td>
</tr>
<tr>
<td>Brother (II1)</td>
<td>14.1</td>
</tr>
<tr>
<td>Sister (II2)</td>
<td>12.6</td>
</tr>
<tr>
<td>Spouse (II4)</td>
<td>13.5</td>
</tr>
<tr>
<td>Younger brother (II5)</td>
<td>12.0</td>
</tr>
<tr>
<td>Younger sister (II6)</td>
<td>13.2</td>
</tr>
<tr>
<td>Daughter (III1)</td>
<td>13.5</td>
</tr>
<tr>
<td>Son (III2)</td>
<td>12.2</td>
</tr>
<tr>
<td>Reference range</td>
<td>11.5–14.6</td>
</tr>
</tbody>
</table>
Table 2 Bioinformatics prediction results of Ala68Asp mutation

<table>
<thead>
<tr>
<th>Software</th>
<th>Prediction results</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>MutationTaster</td>
<td>Disease causing</td>
<td>1.000</td>
</tr>
<tr>
<td>PolyPhen-2</td>
<td>Possible damaging</td>
<td>0.859</td>
</tr>
<tr>
<td>PROVEAN</td>
<td>Tolerable</td>
<td>-1.740</td>
</tr>
<tr>
<td>SIFT</td>
<td>Tolerable</td>
<td>0.056</td>
</tr>
</tbody>
</table>

Notes: The meanings of scores are as followed: (1) MutationTaster→a result closer to 1.000 indicates higher confidence in the prediction. (2) PolyPhen-2→on a scale from 0.000 to 1.000, the closer to 0, the less harmful, and the closer to 1, the more harmful. (3) PROVEAN→the predefined threshold of score is −2.5, the score ≤ −2.5 (deleterious), > −2.5 (neutral). (4) SIFT→range from 0 to 1; the score less than 0.05 means damaging; others show tolerated.

Discussion

The clinical presentation of congenital fibrinogen deficiency is diverse and includes various symptoms such as asymptomatic cases, bleeding, thrombosis, recurrent miscarriages in women, and so on.8 In contrast to the bleeding tendency observed in afibrinogenemia, approximately 55% of patients with dysfibrinogenemia remain asymptomatic, while 25% exhibit a mild bleeding tendency, and nearly 20% are at risk of developing arteriovenous thrombosis.9 The mechanisms for the risk of thrombosis in patients with dysfibrinogenemia include increased thrombin levels due to defective binding with fibrinogen (fibrinogens Malmo, Naples, New York I, Pamplona II, and Poitiers), altered structure, and stability of fibrinogen, as well as the impaired function of the tissue plasminogen activator-mediated fibrinolysis (fibrinogens Argenteuil, Chapel Hill III, Date, New York I, Nijmegen, Pamplona II, and Paris V).10,11 Previous research has demonstrated that specific fibrinogen fragments in the E region, namely, Aα27–50 and Bβ15–42 portions, as well as the B6Aα68 site participate in interaction with thrombin. Thrombophilic dysfibrinogenemia is characterized by defective non-substrate thrombin binding, particularly in fibrinogen New York I (des Bβ9–72) and fibrinogen Naples (B6Aα68Thr). In the study conducted by Al-Mondhiry et al, a 54-year-old female possessing the fibrinogen New York I presented with a 23-year history of repeated life-threatening thrombosis.12 Fibrinogen New York I lacks a
major portion of the binding between fibrinogen and thrombin, which is associated with recurrent deep venous thrombosis (DVT) and fatal pulmonary embolism.13

The mutations in FGB are less common since β-chain is regarded as a rate-limiting factor in the production of fibrinogen hexamer within the liver. Missense mutations in FGB are primarily observed in the highly conserved C-terminal domain of the β-chain.14 In the present investigation, we examined a pedigree affected with congenital fibrinogen deficiency. The proband carried a heterozygous mutation c.293C > A in exon 2 of the FGB gene, leading to the BßAla68Asp amino acid substitution. The proband presented normal FIB:Ag but low FIB:C, thus was diagnosed with dysﬁbrinogenemia. Thrombin-catalyzed fibrin polymerization showed a decreasing trend for impaired fibrinogen aggregation. According to the bioinformatics analysis, the BßAla68Asp mutation was identified as a “disease-causing” mutation. Furthermore, the model analysis revealed an additional hydrogen bond due to the missense mutation. Hence, it is speculated that the BßAla68Asp heterozygous mutation could negatively affect both the structure and function of fibrinogen.

Ceznerová et al15 have reported that the BßAla68 is located in the sequence of BßHis67-Pro70, which forms a β-turn with high conservation. Replacement of BßAla68 with BßAsp68 introduced a natural hydrogen bond which altered the partial conformation of fibrinogen. Multiple alignments had indicated that BßAla68 is conserved among various species, indicating its importance for fibrinogen structure. According to the findings of Koopman et al, the mutation of BßAla68Thr (ﬁbrinogen Naples) reduced the interaction of thrombin to fibrinogen and impaired the release of ﬁbrinopeptides catalyzed by thrombin.13,16,17 In the central E domain, BßAla68 leads to the hydrophobic interior of the interface. The mutation of BßAla68Thr led to the replacement of the nonpolar group with a polar one, leading to steric problems and disrupting the fibrinogen–thrombin binding. In addition, Mullin et al18 have discovered that the BßAla68Thr mutation led to the subtle conformation changes, affecting the enzymatic phase of the polymerization process and resulting in decreased lateral aggregation.

The study by Koopman et al16 presented a case of consanguineous marriage, wherein three homozygotes for the BßAla68Thr mutation had a severe medical history that involved both arterial and venous thrombosis. Two of the individuals suffered from strokes at the young age of 21 and 25 years, primarily due to thrombotic occlusion of the artery, and the third individual developed deep vein thrombosis. Similarly, Yoshida et al19 reported a 17-year-old Japanese male with homozygous BßAla68Thr mutation, who suffered from superior sagittal sinus thrombosis, bilateral pulmonary embolisms, and DVT in the bilateral lower extremities. These findings indicate that the homozygous BßAla68Thr mutation could be a significant contributor to thrombophilia. One possible mechanism could be the regulation of active thrombin in the plasma through thrombin binding to the fibrinogen, which helps sequester thrombin and decrease the feedback reaction that enhances thrombin production and

has been referred to as “antithrombin I.”20 However, reduced thrombin binding to fibrinogen could lead to an increase in active thrombin levels, which could eventually cause excessive coagulation or even thrombosis.8,16

In a study by Ceznerová, a 17-year-old female presented with mild posttraumatic cutaneous bleeding.15 After conducting DNA sequencing, a heterozygous mutation was found in position c.292G > T in exon 2 of the FGB gene, which resulted in the amino acid substitution BßAla68Ser. This mutation was named fibrinogen Svetec. Although the patient’s brother also showed mild bleeding, he did not have the BßAla68Ser mutation, leading Ceznerová to believe that their bleeding tendency may not be linked to the said mutation.15

The BßAla68Asp mutation has a similar effect on the amino acid site as the mutation found in fibrinogen Naples (BßAla68Thr), where both threonine and aspartic acid are polar. This suggests that both alterations may result in similar mechanisms for thrombosis and decreased polymerization. Homozygous individuals with the BßAla68Asp mutation may experience thrombosis due to impaired thrombin binding to fibrinogen, while heterozygous carriers may exhibit asymptomatic.13 However, heterozygous carriers with the BßAla68Asp mutation may increase the risk of thrombosis when combined with other thrombogenic factors such as malignancy, obesity, trauma, and pregnancy, among others.

In conclusion, we discovered a novel heterozygous mutation in exon 2 of the FGB gene (p.BßAla68Asp) caused the dysﬁbrinogenemia. We hope to provide insights into the early detection and treatment of thrombosis associated with FGB gene mutations.

What is known about this topic?

Congenital fibrinogen deﬁciency is a rare disease that presents with varying clinical manifestations. Of those diagnosed with dysﬁbrinogenemia, more than half (55%) are always asymptomatic, whereas approximately 25% exhibits a mild bleeding tendency, with the remaining approximately 20% having a risk for thrombosis. The BßAla68Thr mutation is one of the most common mutations associated with thrombosis due to the defective thrombin binding to fibrinogen.

What does this paper add?

We probed into a pedigree with congenital fibrinogen deﬁciency, found a novel BßAla68Asp missense mutation, and preliminarily analyzed the possible pathogenesis. Based on our research, the BßAla68Asp mutation possibly inﬂuences the interaction of thrombin between fibrinogens, leading to higher levels of active thrombin in plasma. The homozygous mutation maybe associated with thrombosis, while heterozygous carriers might be asymptomatic. Additionally, the mutation may decline fibrinogen lateral aggregation.

Ethical Approval

Our study was approved by the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University (China).
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Conflicts of Interest
The authors declare that they have no conflict of interest.

Acknowledgments
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