Clinical and molecular characterization of nine Chinese patients affected by hypofibrinogenemia or dysfibrinogenemia

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Congential fibrinogen deficiency is a rare bleeding disorder caused by various mutations in three fibrinogen genes. It can be subdivided into four categories: afibrinogenemia, hypofibrinogenemia, hypodysfibrinogenemia and dysfibrinogenemia. This study was to elucidate the molecular defects in nine unrelated Chinese patients with hypofibrinogenemia or dysfibrinogenemia. Three fibrinogen genes were amplified by PCR and screened for variants. The identified variants were analyzed by bioinformatics prediction and molecular modeling analysis. Genetic screening disclosed seven different missense mutations, four of which were novel. All of the mutations were expected to impair the protein function/structure as assessed by bioinformatics prediction. This study has increased our knowledge of the mutational spectrum underlying fibrinogen deficiency. Blood Coagul Fibrinolysis 29:000–000

Introduction

Fibrinogen is a glycoprotein of 340 kDa comprised of two sets of three nonidentical polypeptide chains (\(\alpha\), \(\beta\), \(\gamma\)) [1]. Each chain is encoded by \(FGA\), \(FGB\) and \(FGG\) genes, respectively, at the chromosome 4q [2]. After synthesizing in hepatocytes, the six chains assembled and shaped into a symmetrical trinodular structure, with a central E domain connected to two distal D domains [3]. Fibrinogen, known as coagulation factor I, exerts its function in both blood coagulation and fibrinolysis [4,5]. Given the double role of fibrinogen in the coagulation process, fibrinogen deficiency may result in either hemorrhagic or thrombotic tendencies [6,7].

Congential fibrinogen deficiency can be classified as type I (afibrinogenemia or hypofibrinogenemia) or type II disorders (dysfibrinogenemia or hypodysfibrinogenemia) [8]. Type I defects are defined by the complete absence or markedly reduced fibrinogen levels, whereas type II suggest the presence of a dysfunctional protein in the circulation. At present, approximately 180 different missense mutations within fibrinogen gene cluster have been described in the literature. Among them, 40% were hypofibrinogenemia, whereas 50% had dysfibrinogenemia. In this study, we investigated nine unrelated Chinese patients suffering from hypofibrinogenemia or dysfibrinogenemia. Sequence analysis identified seven different missense mutations, four hitherto unknown and three previously reported.

Materials and methods

Coagulations tests

Peripheral blood samples from these patients and their family members were collected into standard 0.129-mmol/l trisodium citrate tubes. Plate poor plasma was then separated by centrifugation at 3000 r.p.m. for 15 min and was used for coagulation tests. Routine coagulation tests, including the activated partial thromboplastin time, prothrombin time (PT), thrombin time (TT) and functional fibrinogen level, were performed by STAR analyzer (Stago, Asnieres, France). The immunologic fibrinogen level was measured with ELISA.

Genetic analysis

The genomic DNA of all the participants was extracted from peripheral blood with DNA blood extracting kits following standard procedure (Tiangen, Beijing, China). All coding exons as well as flanking intronic regions of fibrinogen gene were amplified by PCR. Primers used for amplification were designed from the published sequence (GenBank accession number: M64982, M64983, M10014). PCR was carried out in a 50-\(\mu\)l reaction volume in an Applied Biosystem Thermal Cycler 2720 (ABI, Oakland, California, USA). Amplified regions were sequenced at Sunsoon BIO-Technology Corporation (Shanghai, China).

In-silico analysis of mutations

The conservation of the affected amino acids was checked by Clustal X software with sequences from...
Molecular modeling of novel mutations

The high-resolution radiograph structure of the fragment D domain (PDB entry 1FZA) was used as a wild-type template for the design of the various mutations we identified. Molecular graphics images were produced using SwissProt Deep-View software.

Results

Patients

Nine unrelated Chinese patients affected by congenital hypofibrinogenemia or dysfibrinogenemia were investigated in this study. This group consisted of two males and eight females patients with an age range 9–48 years. In all these patients, other hemostatic parameters were normal. Circulating and lupus anticoagulant were negative. There was no evidence of abnormalities in the liver and kidney. The phenotype and genotype data were summarized in Table 1. This study was approved by the ethics committee of the First Affiliated Hospital of Wenzhou Medical University (approval number CR2013-003). Written informed consent was obtained from all the participants.

P1 is a 19-year-old boy who came to clinical attention because of cervical lymphadenitis. His prolonged TT and PT were found in routine check-up. Then hypofibrinogenemia reflected by the concordantly reduced functional and immunologic fibrinogen levels (0.63 and 0.77 g/l, respectively) was diagnosed. He did not suffer from any unusual bleeding or thrombosis, whereas his mother had a history of easy bruising.

P2 is a 13-year-old boy who was admitted to the hospital for undergoing circumcision. Although the patient showed no bleeding or thrombotic events, the coagulation screening revealed that the PT and TT were prolonged and both functional and immunologic fibrinogen levels were proportional decrease (0.51 and 0.50 g/l, respectively). The operation was carried out well under fresh frozen plasma supplement. None of his family members had a history of abnormal bleeding or thrombotic tendency.

P3 is a 37-year-old woman who suffered from recurrent shoulder pain. An MRI exam showed the right shoulder with a nodule on the humerus head and joint effusion. At that time, she was pointed out to have prolonged PT and TT. Further investigation confirmed a decreased level of both functional and immunologic fibrinogen (0.64 and 0.79 g/l, respectively), consistent with a diagnosis of hypofibrinogenemia. Then she was treated by arthroscopic decompression and arcomioplasty. The surgery was uneventful after given the fresh frozen plasma. A bleeding tendency with menorrhagia and easy bruising was reported by the patient. No history of bleeding or thrombosis was manifest in other family members.

P4 is a 17-year-old girl who hospitalized for breast ade- nofibroma. Preoperative screening demonstrated low fibrinogen concentrations (0.71 and 0.79 g/l, respectively), with prolonged PT and TT. After given the fibrinogen, the operation was done successfully. She presented usually with spontaneous gum bleeding. Her half brother also had bleeding episodes of easy bruising. But no bleeding disorder was detected in their parents.

P5 is a 9-year-old asymptomatic girl. Hypofibrinogenemia was discovered incidentally before a tonsillectomy. Her functional and antigen fibrinogen levels were both significantly reduced, 0.77 and 0.90 g/l, respectively. The operation went smoothly under fresh frozen plasma transfusions. The parents also denied suffering any bleeding tendency and their fibrinogen levels were normal.

Table 1  Genotype and phenotype of six probands with Fg deficiency

<table>
<thead>
<tr>
<th>Patient</th>
<th>Fg : C (g/l)</th>
<th>Fg : Ag (g/l)</th>
<th>Clinical phenotype</th>
<th>Nucleotide</th>
<th>Amino acid</th>
<th>Bioinformatics prediction score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PROVEAN</td>
</tr>
<tr>
<td>1</td>
<td>0.63</td>
<td>0.77</td>
<td>Asymptomatic</td>
<td>g.5792G&gt;T</td>
<td>Tp208Leu</td>
<td>−10.891 1 0.99999</td>
</tr>
<tr>
<td>2</td>
<td>0.51</td>
<td>0.50</td>
<td>Asymptomatic</td>
<td>g.5792G&gt;T</td>
<td>Tp208Leu</td>
<td>−10.891 1 0.99999</td>
</tr>
<tr>
<td>3</td>
<td>0.64</td>
<td>0.79</td>
<td>Menorrhagia; easy bruising</td>
<td>g.5864A&gt;C</td>
<td>Lys232Thr</td>
<td>−3.796 0.998 0.99999</td>
</tr>
<tr>
<td>4</td>
<td>0.71</td>
<td>0.79</td>
<td>Spontaneous gum bleeding</td>
<td>g.7482C&gt;G</td>
<td>Thr277Arg</td>
<td>−3.089 0.891 0.99999</td>
</tr>
<tr>
<td>5</td>
<td>0.73</td>
<td>0.80</td>
<td>Asymptomatic</td>
<td>g.7598G&gt;C</td>
<td>Asp216His</td>
<td>−6.116 0.998 0.99999</td>
</tr>
<tr>
<td>6</td>
<td>0.76</td>
<td>2.26</td>
<td>Threatened abortion; menstrual bleeding</td>
<td>g.1213G&gt;C</td>
<td>Arg19Ser</td>
<td>−3.925 0.999 Polyphorphism</td>
</tr>
<tr>
<td>7</td>
<td>0.54</td>
<td>2.13</td>
<td>Postpartum bleeding</td>
<td>g.7476C&gt;T</td>
<td>Arg275Cys</td>
<td>−4.683 1 0.99999</td>
</tr>
<tr>
<td>8</td>
<td>0.52</td>
<td>2.86</td>
<td>Posttraumatic bleeding</td>
<td>g.7476C&gt;T</td>
<td>Arg275Cys</td>
<td>−4.683 1 0.99999</td>
</tr>
<tr>
<td>9</td>
<td>0.87</td>
<td>2.35</td>
<td>Postpartum bleeding; menorrhagia</td>
<td>g.7476 G&gt;A</td>
<td>Arg275His</td>
<td>−2.504 0.999 0.99999</td>
</tr>
</tbody>
</table>

PROVEAN: variants with a score equal to or below −2.5 are considered ‘deleterious’; above −2.5 are considered ‘neutral’. PolyPhen-2: The score is measured on a scale of 0–1, with higher scores indicating more damaging. MutationTaster: a probability close to 1 indicates a high security of prediction. *Indicates novel mutations.
P6 is a 25-year-old woman who came to medical attention because of threatened abortion. Coagulation study revealed all clotting factors were normal except for the decreased fibrinogen. The functional and antigen fibrinogen levels in the patient were 0.76 and 2.26 g/l, respectively. Based on these data, dysfibrinogenemia was suspected. She had only experienced heavy menstrual bleeding, without history of thrombosis. On the contrary, we did not examine other family members.

P7 is a 46-year-old woman whose right eye was injured by the pincer. Laboratory tests showed low functional but normal antigen fibrinogen levels in her plasma, with prolonged PT and TT, suggesting a dysfibrinogenemia. She was given fresh frozen plasma prior to the vitrectomy, which proceeded uneventfully. The patient only had a past history of postpartum hemorrhage. Her family history was negative for bleeding or thrombotic tendencies.

P8 is a 48-year-old woman complaint of pelvic mass. A follow-up B-scan ultrasonography examination demonstrated hysteromyoma. Preoperative screening revealed prolonged PT and TT, with a low functional but normal immunologic fibrinogen level, leading to the diagnosis of dysfibrinogenemia. She had undergone successful surgical procedures under fresh frozen plasma cover. She reported no spontaneous but posttraumatic bleeding. On the contrary, no other family members were available for investigation.

P9 is a 26-year-old woman who was admitted to hospital for health check, when her functional fibrinogen level was found to be decreased, whereas immunologic fibrinogen level was normal. Judging from fibrinogen activity and antigen levels, we suspected that the patient had dysfibrinogenemia. She had developed severe postpartum bleeding and menorrhagia. On the contrary, no other family members were available for the study.

Mutational screening
DNA sequencing of P1 revealed a heterozygous g.5792 G>T mutation in exon 7 of the FGG, leading to a novel γTrp208Leu substitution in the γD domain. The patient’s mother, aunt and cousin were also bearing this mutation. DNA sequencing of P2 demonstrated the same missense mutation as patient 1. His father and paternal grandmother were heterozygous carriers of this mutation. DNA sequencing of P3 revealed a heterozygous g.5864 A>G mutation in exon 7 of FGG causing a novel γLys232Thr substitution in the γD domain. The candidate mutation was also identified in her mother, son, brother and nephew. DNA sequencing of P4 revealed a heterozygous g.7482C>G mutation in exon 8 of FGG, predicting a novel γThr277Arg substitution in the γD domain. Further sequencing established that her father and half brother were also heterozygous for the mutation. DNA sequencing of P5 revealed a heterozygous g.7598 G>C mutation in exon 8 of FGG, predicting a novel...
Fibrinogen gene mutations in nine families.
γAsp316His substitution in the γD domain. However, her parents, who had normal laboratory results, were negative for this mutation.

DNA sequencing of P6 revealed a heterozygous g.1213G>C mutation in exon 2 of FGA, predicting a αArg19Ser substitution in the αE domain. DNA sequencing of P7 revealed a heterozygous g.7475G>T mutation in exon 8 of FGG, predicting a γArg275Cys substitution in the γD domain. This mutation was also found in her son but was absent in her two daughters. On the contrary, DNA samples from his parents were not available for analysis, as they pass away. DNA sequencing of P8 showed the same mutation as patient 7. DNA sequencing of P9 revealed a heterozygous g.7476 G>A mutation in exon 8 of FGG, predicting a γArg275His substitution in the γD domain.

In the nine analyzed patients above, we disclosed a total of seven different missense mutations, all present at a heterozygous state. In addition to three previously described mutations (αArg19Ser, γArg275Cys and γArg275His), four novel causative genetic variations were detected. The majority of them have been found in unique families; only the γTrp208Leu and γArg275Cys have been found in more than one (unrelated) family. All of them were absent in 150 healthy and unrelated controls (Figs. 1 and 2).

Bioinformatics prediction
All affected residue (γTrp208, γLys232, γArg275, γThr277, γAsp316, αArg19Ser) are strictly conserved in all known γ chain sequences from 10 species. The identified mutations were all predicted to be disease-causing by the three online bioinformatics tools: PROVEAN, PolyPhen2 and MutationTaster.

Discussion
Congenital fibrinogen deficiency can be divided into four categories: afibrinogenemia, hypofibrinogenemia, hypodysfibrinogenemia and dysfibrinogenemia. The prevalence of afibrinogenemia is estimated at about 1 to 1 per million, whereas that of hypofibrinogenemia is difficult to establish because of the large number of asymptomatic cases. According to the latest figures, around 92 different genetic defects responsible for hypofibrinogenemia have been so far identified. A total of 68 of these are missense mutations (eight in Aα chain, 21 in Bβ and 39 in γ chain). Dysfibrinogenemia is generally inherited in an autosomal dominant manner, and so it is more frequent than hypofibrinogenemia [9]. As many as 110 types of mutations have been identified, missense mutations account for over 90% [10].

In this article, we studied nine patients with hypofibrinogenemia or dysfibrinogenemia from unrelated Chinese families and identified seven different point mutations, including four novel mutations (γTrp208Leu, γLys232Thr, γThr277Arg, γAsp316His) and three previously reported mutations (αArg19Ser, γArg275Cys and γArg275His). Particularly, all of the affected residues are highly conserved across all aligned species, pointing out these residues might be essential for maintaining the tertiary structure of the protein. According to the modeling analysis and functional prediction in silico, four novel mutations are considered damaging and would be expected to induce protein instability. As for clinical manifestation, probands with γLys232Thr and γThr277Arg mutations had experienced mild bleeding, whereas probands with γTrp208Leu and γAsp316His mutations were asymptomatic. However, because of the young age of our patient, we cannot exclude the possibility that clinical phenotypes will be developed in the future.

Concerning the other mutations identified in this study, αArg19Ser, γArg275Cys and γArg275His, they all result in dysfibrinogenemia and are already reported in the literature. The αArg19Ser, discovered in P6 who had a history of threatened abortion and heavy menstrual bleeding, was known to inhibit fibrin polymerization [11]. Only one homozygous patient for this mutation has been previously discovered in 1964, who suffered from severe bleeding. The γArg275Cys and γArg275His, as the most common cause of dysfibrinogenemia [12], was identified in three unrelated patients. The documented clinical symptoms of these dysfibrinogenemia usually present as thrombosis, sometimes as hemorrhage. In contrast, the three patients in our case were all reported to have episodes of posttraumatic bleeding.

In conclusion, we have identified seven missense mutations, comprising four novel mutations (γTrp208Leu, γLys232Thr, γThr277Arg, γAsp316His) and three previously described mutations (αArg19Ser, γArg275Cys and γArg275His), causing hypofibrinogenemia or dysfibrinogenemia. The study of these mutations, specifically these novel mutations, highlights some regions and residues that are essential for fibrinogen structure or function.

Acknowledgements
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Conflicts of interest
The authors stated that they had no interests which might be perceived as posing a conflict or bias.

References
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