

Analysis of Iranian patients allowed the identification of the first truncating mutation in the fibrinogen B β -chain gene causing afibrinogenemia

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Background and Objectives. Congenital afibrinogenemia is a rare coagulation disorder whose molecular basis is still poorly characterized. Most mutations have been identified in the fibrinogen A α - and γ -chain genes, whereas only two missense mutations have been reported in the B β -chain gene. The aim of this work was to widen knowledge about the mutational spectrum of this disease by analyzing the molecular bases of congenital afibrinogenemia in three unrelated Iranian patients.

Design and Methods. All patients showed unmeasurable levels of clottable fibrinogen in plasma. Mutational screening was performed by sequencing the whole coding region, including exon-intron boundaries and part of the promoter region of the three fibrinogen genes.

Results. Sequencing in one patient revealed the presence of a novel nonsense mutation (3282C \rightarrow T) in exon 2 of the fibrinogen B β -chain gene, causing a severe truncation of the corresponding polypeptide (R17X). In the remaining probands, two already known small deletions (4209delA and 4220delT), both located in exon 5 of the fibrinogen A α -chain gene, were identified, and their effect at the protein level explored by computer-assisted analysis.

Interpretation and Conclusions. The identification of the first truncating mutation in the fibrinogen B β -chain gene confirms the involvement of all three fibrinogen genes in the pathogenesis of congenital afibrinogenemia and widens the mutational spectrum of the disease. This knowledge is clinically essential in order to carry out prenatal diagnosis in families at risk.

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Key words: afibrinogenemia, fibrinogen, B β -chain gene, gene analysis, nonsense mutation.

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Congenital afibrinogenemia [Online Mendelian Inheritance in Man (OMIM) #202400] is a rare coagulation disorder with an autosomal recessive mode of inheritance and an estimated incidence of one case in 10⁶ people.¹ The disorder is less rare in populations in which consanguineous marriages are traditionally frequent such as those from Muslim countries and Southern India.¹ Afibrinogenemic patients display a lifelong hemorrhagic diathesis of variable severity, due to the complete absence or extremely low levels of both functional and immunoreactive plasma fibrinogen.²

Molecular analysis of the three fibrinogen genes (A α , B β , and γ) in affected individuals has led to the identification of 25 causative mutations. Most mutations are located in the fibrinogen A α -chain gene (17 molecular defects), while two have been found in the fibrinogen B β -chain gene and six in the fibrinogen γ -chain gene.^{3,4} All mutations lead to severe truncation of the corresponding polypeptide chain, the only exception being the two missense mutations identified in the fibrinogen B β -chain gene, both responsible for a secretion defect of the mutant protein.⁵

In this study, three afibrinogenemic Iranian patients were analyzed. Identification of the first truncating mutation in the fibrinogen B β -chain gene is reported. Two already known small deletions in the A α -chain gene were also identified, and their pathogenetic role is discussed.

Design and Methods

Informed consent to this study was obtained from patients and family members, after approval by the Ethics Committee.

Coagulation tests

Clottable plasma fibrinogen was measured by evaluating fibrin polymerization time (Laboratoire Stago, Asnières, France). Immunoreactive fibrino-

gen was measured by an enzyme immunoassay.⁶ The sensitivity of the functional assay and of the immunoassay was 5 and 0.02 mg/dL, respectively (normal range for both tests: 160–400 mg/dL).

DNA extraction and sequence analysis

Genomic DNA was extracted from blood samples according to standard procedures. All A α -, B β -, and γ -chain gene exons, including exon-intron boundaries and about 300 bp of each promoter region were polymerase chain reaction (PCR)-amplified from genomic DNA, using sense and anti-sense primers designed on the basis of known sequences of the fibrinogen cluster (GenBank M64982, M64983, M10014, U36478 and AF229198). Primer sequences (Life Technologies, Inchinnan, Paisley, UK) are available on request. DNA sequencing was carried out on both strands directly on purified PCR products using the BigDye Terminator Cycle Sequencing Kit and an automated multicapillary 3100 DNA sequencer (Applied Biosystems, Foster City, CA, USA). Mutation detection was performed by Fatura and Sequence Navigator software packages (Applied Biosystems).

Results

Case history and laboratory measurements

The three Iranian afibrinogenemic probands were all born from consanguineous matings.

Patient #1, a 17-year-old female, bled at birth from the umbilical cord. Subsequently, she suffered from gastrointestinal bleeding and from other relatively mild hemorrhagic symptoms (epistaxis, mouth bleeding and menorrhagia). Her plasma level of immunoreactive fibrinogen was 1.4 mg/dL, while her parents, both asymptomatic, showed half the normal level (Figure 1A).

Patient #2 is a 11-year old girl, who bled at birth from the umbilical cord. Later, she bled from the gastrointestinal tract and had additional hemorrhagic symptoms (mouth bleeding, hematuria and post-traumatic hematoma). Her plasma level of immunoreactive fibrinogen was 0.44 mg/dL. Her father and mother, both asymptomatic, had plasma fibrinogen levels in the lower part of the normal range (208 and 191 mg/dL, respectively).

The diagnosis of afibrinogenemia in the 17-year-old patient #3 was made at birth because of umbilical cord bleeding. The proband's plasma fibrinogen level was unmeasurable at the time of the diagnosis. Subsequently, he suffered from epistaxis, post-traumatic hemarthrosis and prolonged excessive bleeding after a dental extraction. While the parents were asymptomatic, out of the proband's four

brothers, two had been affected and had died (one from complications due to gastrointestinal bleeding, one from post-traumatic hemorrhage), one was diagnosed as afibrinogenemic and one was asymptomatic. Blood samples from the proband's brothers were not available for this study.

Sequence analysis

Sequencing of the fibrinogen gene cluster of each proband disclosed three point mutations, each present in the homozygous state.

In patient #1, a C \rightarrow T transition was found at position 3282 in exon 2 of the fibrinogen B β -chain gene (numbering according to GenBank M64983) (Figure 1B,C), giving rise to a severely truncated polypeptide because of the presence of a premature termination codon. The predicted mutant mature B β -chain is composed of only 16 amino acids (R17X) (Figure 1D). The proband's parents were heterozygous for this mutation (Figure 1B).

Two single bp deletions, both located in exon 5 of the fibrinogen A α -chain gene (4209delA, 4220delT; numbering according to GenBank M64982) were identified in probands #2 and 3, respectively. Both frameshift mutations would lead to the same premature stop at codon 419, preceded by abnormal stretches of 108 and 104 amino acids for 4209delA and 4220delT deletions, respectively. Each mutation was present in the heterozygous state in the respective parents.

No additional nucleotide variations were observed in any sequenced region of the fibrinogen cluster of the three probands.

Discussion

Populations with high rates of inbreeding, such as Islamic populations, prove to be very useful in studies on rare recessive coagulation disorders.^{1,2} Here three Iranian probands from consanguineous marriages were analyzed to extend knowledge on the spectrum of defined afibrinogenemia-causing mutations. Knowledge of the underlying mutations is essential in order to carry out prenatal diagnosis in kindreds at risk, such as that of patient #3. All patients suffered from severe hemorrhagic episodes, which started at birth with umbilical cord bleeding, the most frequent and severe symptom in afibrinogenemia.² Moreover, probands #1 and 2 bled from the gastrointestinal tract and one brother of proband 3 had died from the same type of hemorrhage. Interestingly, gastrointestinal bleeding is usually rare in afibrinogenemic individuals.²

Sequence analysis of the three fibrinogen genes enabled the identification of a novel nonsense 3282C \rightarrow T mutation. This is the first report of a null

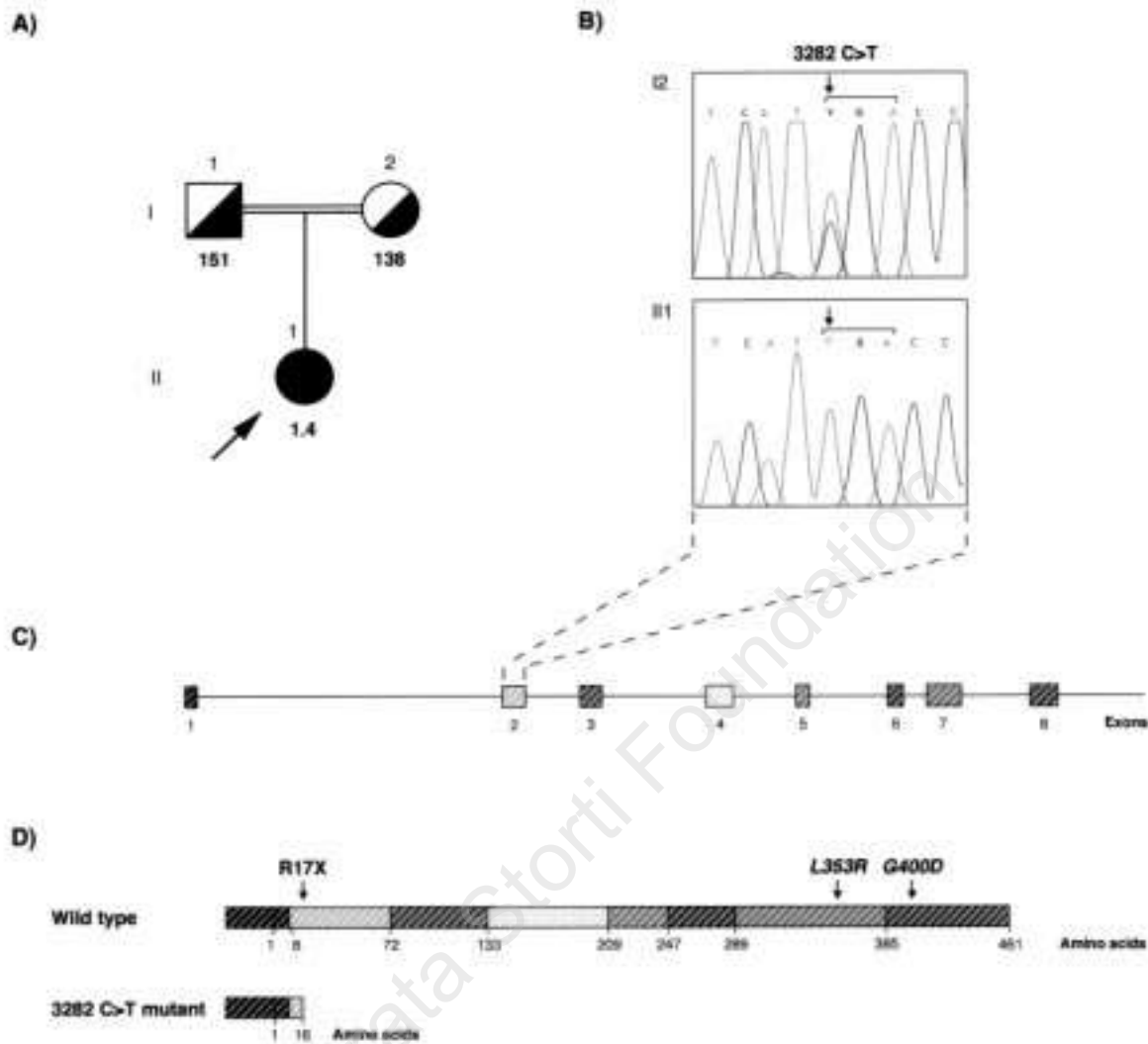


Figure 1. Novel nonsense mutation in the fibrinogen B β -chain gene identified in the family of the afibrinogenemic patient #1. **A)** Pedigree of the Iranian family of patient #1. Plasma immunoreactive fibrinogen level (mg/dL) is indicated below each symbol. The arrow indicates the proband. **B)** Electropherograms showing the mutation identified in patient #1. The C \rightarrow T transition, whose nucleotide position is indicated by an arrow, was present in the heterozygous state in both parents (upper panel), and in the homozygous state in the proband (lower panel). **C)** Schematic representation of the B β -chain gene showing the position of the identified mutation. Exons and introns are indicated by boxes and lines, respectively, and are drawn to scale. **D)** Predicted effect of the identified mutation at the protein level. Schematic representations are shown of the wild type B β -chain and of the mutant truncated polypeptide. Amino acid numbering omits the signal peptide. Regions of the B β -chain encoded by different exons are drawn to scale and shaded in different grays. The positions of previously described mutations affecting the B β -chain⁵ are also indicated (italics).

mutation in the B β -chain gene, causing a severe truncation of the corresponding polypeptide (R17X); the other two molecular defects identified so far in the same gene are missense mutations.⁵ The truncation involves almost the entire B β -chain protein and can be considered as the counterpart of truncating mutations causing afibrinogenemia described in the A α - and γ -chain genes.^{7,8} The C-

terminal half of the B β -chain does not affect fibrinogen secretion, as demonstrated by expression experiments. However truncations which restrict the B β -chain to 73 residues cause a severe reduction of fibrinogen secretion.⁹ The truncation identified in patient #1 represents the severest B β -chain truncation ever described and, as expected, it is associated with an almost complete absence

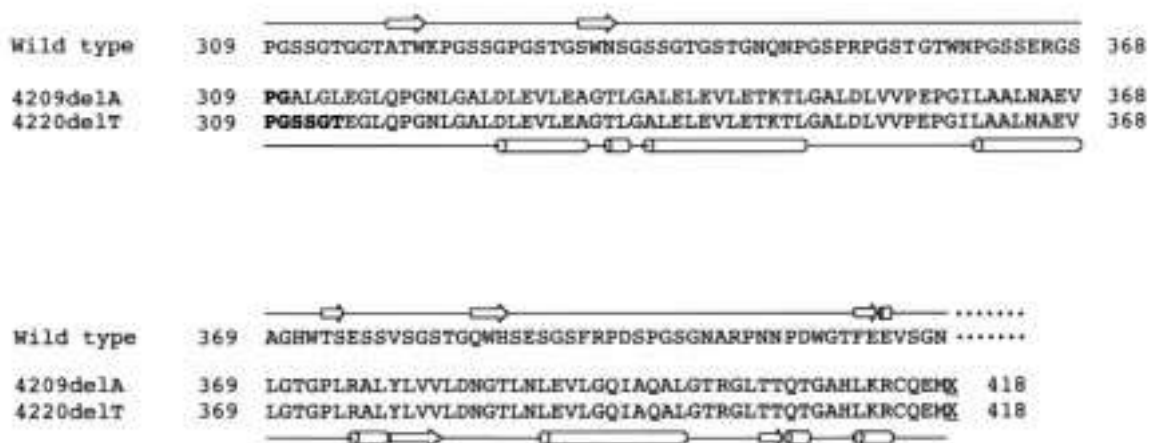


Figure 2. Predicted secondary structure of the C-terminal region of normal and mutant fibrinogen A α -chains. Multiple alignment between the wild type and 4209delA (proband 2) and 4220delT (proband 3) mutant A α -chains in the region between residues 309 and 418. Amino acid sequences upstream of each deletion are in bold. The underlined X denotes the stop codon. The predicted secondary structures produced using the nnPredict program (Protein Secondary Structure Prediction; <http://www.cmpharm.ucsf.edu/cgi-bin/nnpredict.pl>) are drawn above or below the corresponding sequence. Cylinders and arrows indicate α -helix and β -strand motifs, respectively. A straight line denotes a region of random coil.

of plasma fibrinogen. These data point to a possible correlation between genotype and clinical phenotype: the more extended the B β -truncation, the more severe the fibrinogen deficiency.

The two small deletions (4209delA, 4220delT) in the A α -chain gene are identical to previously reported mutations.¹⁰ These mutations are interesting in that the resulting frameshifts predict the synthesis of exceptionally long polypeptides (108 and 104 amino acids, respectively), which show the same sequence and are truncated at the same residue. These aberrant, leucine- and valine-rich amino acid stretches (Figure 2) may perturb the correct folding of the A α -chain, and hence prevent or reduce fibrinogen assembly and/or secretion. A computer-assisted analysis that we performed on the two aberrant C-terminal regions of A α -chain predicts the presence of several α -helices (Figure 2), whereas these motifs are absent in the corresponding part of the normal protein.¹¹

Although congenital afibrinogenemia is a rare disease, its frequency is currently increasing in several countries as a consequence of migratory flows from Muslim countries. Detailed knowledge of the mutational spectrum of this disorder is crucial in order to perform prenatal diagnosis in families at risk. This study describes the identification of the molecular defects responsible for afibrinogenemia in three Iranian patients. In non-European popula-

tions, the major locus involved in the pathogenesis of this disease has so far been considered to be the γ -chain gene, whereas in Europeans A α -chain gene mutations are more frequent.³ Our results point to a probable equal importance of the three genes, at least in non-European populations.

Contributions and Acknowledgments

All the authors participated in the conception and design of the present study, in the analysis and interpretation of data, and in revising the manuscript. RA, SS and SD were responsible for PCR amplifications, sequence analysis, interpretation of results, computer analysis and drafting the manuscript. FP and PMM enrolled patients in the study, collected the clinical histories and evaluated the clinical phenotypes. MM and MLT were involved in the conception of the study, discussion of the results and correction of the manuscript. MLT supervised the entire study.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

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PEER REVIEW OUTCOMES

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Marguerite Neerman-Arbez, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Professor Neerman-Arbez and the Editors. Manuscript received May 7, 2002; accepted June 19, 2002.

What is already known on this topic

Congenital afibrinogenemia is caused by mutations in the fibrinogen gene cluster (FGG, FGA or FGB) on human chromosome 4. Since the identification of the first mutation for this disorder in 1999, i.e. an 11 kb deletion of FGA, more than 25 distinct mutations have been characterized, the majority of which are null mutations in FGA.

What this study adds

This study reports a novel truncating mutation in the FGB gene, identified in an Iranian patient, which is predicted to lead to a severely truncated fibrinogen β chain retaining only the signal peptide and the following 16 amino acids

Potential implications for clinical practice

Every new mutation identified increases our understanding of the molecular basis of congenital afibrinogenemia. This growing knowledge is important in order to provide a precise molecular diagnosis for patients as well as prenatal diagnosis/counselling for the families concerned.

Marguerite Neerman-Arbez, Associate Editor