Congenital afibrinogenemia is a rare autosomal recessive disorder characterized by the complete absence of detectable fibrinogen. We previously identified the first causative mutations for this disease, homozygous deletions of approximately 11 kb of the fibrinogen alpha chain gene (FGA). Subsequent analyses revealed that most afibrinogenemia alleles are truncating mutations of FGA, although mutations in all 3 fibrinogen genes, FGG, FGA and FGB have been identified. In this study, we performed the first prenatal diagnosis for afibrinogenemia. The causative mutation in a Palestinian family was a novel nonsense mutation in the FGB gene, Trp467Stop (W467X). Expression of the Trp467Stop mutant FGB cDNA in combination with wild-type FGA and FGG cDNAs showed that fibrinogen molecules containing the mutant beta chain are not secreted into the media. The fetus was found to be heterozygous for the Trp467Stop mutation by direct sequencing and by linkage analysis, a result that was confirmed in the newborn by intermediate fibrinogen levels. (Blood. 2003;101:3492-3494)

Study design

Description of family

Informed consent was obtained from the adults participating in the study. The parents were a healthy consanguineous Palestinian couple with no history of bleeding tendency. Their first conception was a miscarriage during the first trimester. The second and third conceptions produced 2 daughters who were born at term with birth weights of 2.9 and 3 kg, respectively. The bleeding tendency in the 2 daughters was noted during midinfancy (between 4 and 5 months of age) in the form of serious intracranial bleeding after nonsignificant trauma. Fibrinogen levels were undetectably low (<50 mg/dL), establishing the diagnosis of congenital afibrinogenemia. Following diagnosis, treatment consisted of infusions of fresh frozen plasma (FFP) following any significant bleeding, approximately once every 5-6 months. No infusion was required for the last 2 years because of increased parental awareness of accident and trauma prevention. Fibrinogen levels were determined for the parents and were found to be 170 and 150 mg/dL for the mother and the father, respectively.

Mutation screening in family members

Mutation screening of the FGA, FGG, and FGB gene was performed by polymerase chain reaction (PCR) amplification of all exons and intron-exon junctions, followed by sequencing as previously described.

Microsatellite analysis

The FGA intron 3 tetranucleotide repeat (FGAi3) was analyzed by PCR amplification with oligonucleotides FGA PCR2.1 (5’CATAGGGTTT-GAACTCACAG3’) and FGA PCR2.2 (5’TTTCTCAGATCCTCTGAC-CA3’) followed by denaturing polyacrylamide-urea gel electrophoresis according to standard procedures.

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A consanguineous Palestinian couple (first cousins) with 2 daughters with congenital afibrinogenemia requested a prenatal diagnosis for their ongoing pregnancy (Figure 1A). In the first step, DNA was extracted from fresh EDTA (ethylenediaminetetraacetic acid) blood samples from parents and affected children for identification of the causative mutation transmitted in this family. Screening of all the coding regions and exon-intron junctions for FGA (α isoform, exons 1-5) and FGG (α and β isoforms, exons 1-8) and FGB (β isoform, exons 1-8) by PCR and sequencing showed no mutation. Analysis of the FGB gene revealed a previously unreported nonsense mutation in the last exon, exon 8, Trp467Stop (TGG->TAG) (numbered from the first ATG codon according to guidelines for human mutation nomenclature11,12), which was found in heterozygosity in each of the parents and in homozygosity in each of the 2 affected daughters (Figure 1B).

Microsatellite analysis of the FGA intron 3 tetrancleotide repeat (FGA13) was also performed. Both parents were found to be heterozygous for the FGA13 microsatellite marker, whereas the 2 affected daughters were homozygous (data not shown).

Mutation analysis was performed on DNA isolated from cultured amniocytes, and the fetus was found to be heterozygous for the familial Trp467Stop mutation both by sequencing and FGA13 microsatellite analysis. This result was confirmed in the newborn when the mother gave birth to a healthy baby boy weighing 2.9 kg at birth, and fibrinogen determination revealed a level of 120 mg/dL, consistent with heterozygosity for an afibrinogenemia mutation.

The Trp467Stop mutation was predicted to lead to the production of a truncated fibrinogen β chain, with 25 amino acids missing from the C-terminus (Figure 1C). Alternatively, the truncated beta polypeptide might be unstable or the mutation might cause a defect in the FGB mRNA by aberrant splicing (nonsense-associated alternative splicing) or by affecting the stability of the fibrinogen beta chain, with 25 amino acids missing from the C-terminus (Figure 1C).
mRNA through nonsense-mediated mRNA decay. However, because the Trp467Stop mutation lies within the last exon of FGB, these mechanisms are not thought to be activated.

Previous studies in COS-1 cells expressing normal fibrinogen alpha and gamma chains in combination with beta-chain deletion mutants had led to the conclusion that the C-terminal portion of the beta chain, notably residues 238-491 (numbering from the initiator methionine), was not essential for fibrinogen assembly and secretion. In order to prove the causative nature of the mutation, Trp467Stop mutant and wild-type FGB cDNAs were transiently cotransfected with wild-type FGA and FGG cDNAs in COS-7 cells. Cells were lysed 18 hours after transfection and the conditioned media harvested. Individual fibrinogen chains and assembled hexamers were detected by Western blot analysis with a polyclonal antifibrinogen antibody (Figure 2).

When COS-7 cells are transfected with the 3 normal fibrinogen cDNAs, all 3 chains are correctly expressed and assembled inside the cell, and the fibrinogen hexamers are secreted into the media. When cells are transfected with normal FGA and FGG cDNAs and the Trp467Stop FGB mutant cDNA, again all 3 chains are correctly and stably expressed inside the cells. The mutant beta chain is incorporated into the fibrinogen hexamer inside the cell (Figure 2B, “Cells,” lane 3) but is not secreted: only incomplete forms containing α and/or γ chains and no fibrinogen hexamers are detectable in the supernatant (Figure 2A and B, “Media,” lane 3). These results closely resemble those previously described in COS cells transfected with different combinations of the 3 normal fibrinogen cDNAs.

When cells are transfected with equal amounts of wild-type and mutant FGB cDNAs (and normal FGA and FGG cDNAs), imitating heterozygosity for the Trp467Stop mutation, both beta chains are clearly distinguished in the Western blot of cell lysates, with the normal chain being more abundant (Figure 2A, “Cells,” lane 2). By contrast, in the cell medium only the wild-type beta chain is found (Figure 2A, “Media,” lane 2).

The data demonstrate that truncation of the last 25 amino acid residues from the fibrinogen beta chain C-terminus does not inhibit hexamer assembly but eliminates its secretion, as previously reported for 2 missense mutations in FGB exons 7 and 8 identified in afibrinogenemia patients. These results are apparently in contradistinction with the experimental observation that fibrinogen beta chains truncated at amino acid position 238 were able to assemble with alpha and gamma fibrinogen chains and were secreted into the media. However, the assembly and secretion of such a severely truncated polypeptide may not be physiologically relevant.

Interestingly, Homer et al reported a very similar mutation (Trp440Stop, amino acids numbered without the signal peptide, or Trp470Stop according to our nomenclature), which occurs only 3 codons downstream of the Trp467Stop mutation. The Trp470Stop mutation “fibrinogen Mount Eden” was identified in heterozygosity in a patient following laboratory investigations prior to a liver biopsy for hepatitis C. The patient had reduced fibrinogen levels (0.7 mg/mL) and a prolonged activated partial thromboplastin time. Other than mild epistaxis and gum bleeding, the patient was asymptomatic. The authors showed that the truncated fibrinogen beta chain was not found in the patient’s plasma and suggested that removal of the C-terminal 22 residues does not allow incorporation of the mutant chain into mature fibrinogen hexamers. No fibrinogen inclusion bodies were detected in the liver biopsy, indicating that molecules containing the mutant chains do not accumulate in the patient’s hepatocytes. We suggest that the molecular mechanism may be at the level of secretion, as with the Trp467Stop mutation we describe. The authors state that the mutation causes hypofibrinogenemia in heterozygosity; however, one can consider it an afibrinogenemia mutation because a homozygous individual for this mutation will most certainly have no circulating fibrinogen.

In conclusion, the Trp467Stop mutation identified in this study, along with the Trp470Stop mutation and 3 missense mutations identified in the same region in afibrinogenemia patients, confirms the necessity of intact C-terminal portions of the fibrinogen beta chain for the secretion of functional fibrinogen hexamers.

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References