A Unique 7p/12q Chromosomal Abnormality Associated With Recurrent Abortion and Hypofibrinogenemia

By Craig S. Kitchens, Amelia C. Cruz, and Jeffrey A. Kant

Recurrent first trimester abortions led to evaluation of a 25-year-old woman. Studies revealed she had hypofibrinogenemia (68 mg/dL) without evidence of dysfibrinogenemia or increased fibrinogen turnover. She was also found to have a unique 46,XX,t(7;12)(p15.2;q24.31) karyotype. Hypofibrinogenemia and identical chromosomal abnormalities were found in other members of her kindred. Southern blots of genomic DNA from the patient, her mother, and her daughter hybridized to human fibrinogen probes showed α, β, and γ fibrinogen genes to be present and without structural alterations when compared to normal controls. We conclude that the chromosomal abnormality and the hypofibrinogenemia are related but in an unclear manner. Because fibrinogen infusion in the proposita was associated with successful gestation, we also concluded that the chromosomal abnormality itself was not responsible for the repeated abortions but that fibrinogen concentration may be critical in securing implantation.

THE MECHANISMS by which the fertilized ovum invades the uterine wall during implantation and the subsequent repair of invaded tissue are subjects of active investigation.1-3 Presumably key in these processes are several proteases and other proteins4-7 regulating the activity of proteases. No doubt other processes are operative, including some yet to be identified. Coagulation factors appear to play a role in implantation. Clinical derangements of fibrin homeostasis that result in unsuccessful pregnancy8,9 suggest that fibrinogen and fibrin aid in securing the ovum and the morula in place following uterine invasion.9

In this report, we describe a woman with congenital hypofibrinogenemia, recurrent spontaneous abortion, and an unusual chromosomal abnormality. During one pregnancy, plasma fibrinogen levels were maintained by cryoprecipitate administration. That pregnancy was successful, whereas previous and subsequent pregnancies without cryoprecipitate resulted in spontaneous abortion. Hypofibrinogenemia and the unique chromosomal abnormality were shared by other members of her kindred, findings which suggest an association among the hypofibrinogenemia, fetal wastage, and the chromosomal abnormalities.

MATERIALS AND METHODS

All coagulation studies were performed using platelet-poor plasma prepared from whole blood, which was anticoagulated with 1/10th volume 3.8% sodium citrate. Coagulation studies and determination of antithrombin III, plasminogen, and fibrinogen degradation products were done as previously described.12 Plasma fibrinogen concentration was determined by three methods: chromometric,14 gravimetric,15 and immunoreactive (Cal Biochem, San Diego).

Fibrinogen was isolated from plasma, reduced with 2-mercapto-ethanol, and electrophoresed on SDS-PAGE (7.5% acrylamide for the separating gel and 4.0% for the stacking gel) by the Laemmli method.16 Cytogenetic analyses were performed using peripheral blood lymphocytes grown by a microculture technique.19 Geimsa banding was done by a modification of the technique of Seabright.20 Fibrinogen gene structure was determined in several subjects. EDTA-anticoagulated blood (10 to 50 mL) was used for preparation of leukocyte genomic DNA by lysis and washing of cells in JG buffer (0.25 mol/L sucrose, 0.005 mol/L MgCl2, 0.025 mol/L KCl, 0.05 mol/L Tris-HCl, pH 7.4) containing 0.25% Triton X-100. Nuclei isolated in this fashion were washed in JG buffer and disrupted in 20 mmol/L EDTA, 1% SDS, and then digested overnight with 100 to 500 µg/mL protease K. After phenol/CHCl3 extraction, 10 µg of genomic DNA was digested for 2 to 3 hours using two additions of restriction endonuclease (3 U enzyme/µg DNA). The digests were fractionated by size on a 0.7% agarose gel at 2 volts/cm overnight, denatured, neutralized, and the contents transferred to BA85 nitrocellulose paper essentially as described by Southern.21 This blot was hybridized to 5 to 20 x106 cpm of 32P-nick translated human α, β, or γ fibrinogen cDNAs at 65 C. in 6 x SSC (1 x SSC = 0.15 mol/L NaCl, 0.015 mol/L Na citrate) for 18 hours, then washed at 65 C. successively in 6 x SSC-0.1% SDS, 1 x SSC-0.1% SDS, and briefly in 0.1 x SSC-0.1% SDS. The cDNAs used contained coding information from the carboxy terminus extending 5' to residue 154, 241, and 255 of the α, β, and γ chains, respectively. The blots were exposed to Kodak XAR-5 x-ray film at −70°C.

CASE REPORT AND SPECIAL STUDIES

A 25-year-old woman consulted us following two spontaneous first-trimester abortions. She now wished to become pregnant again. No records were available concerning the first abortion, which took place in her 12th week of gestation. Her plasma fibrinogen level early in the second pregnancy was 59 mg/dL. She experienced vaginal spotting in the 4th week and spontaneously aborted in the 8th week. No transfusions were administered. Several weeks later her fibrinogen level was 51 mg/dL. Neither she nor other family members reported other hemorrhagic events. When she consulted us, she was in normal health and not on contraceptives. Multiple
Table 1. Coagulation Studies on Proposita

<table>
<thead>
<tr>
<th>Test</th>
<th>Normal Range</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin time (s)</td>
<td>9.5-14.5</td>
<td>13.9</td>
</tr>
<tr>
<td>Partial thromboplastin time (s)</td>
<td>25-40</td>
<td>30</td>
</tr>
<tr>
<td>Thrombin time (s)</td>
<td>17-33</td>
<td>60</td>
</tr>
<tr>
<td>Fibrin degradation products (titre)</td>
<td>≤1:4</td>
<td>1:2</td>
</tr>
<tr>
<td>Fibrinogen, chromometric (mg/dL)</td>
<td>150-400</td>
<td>68</td>
</tr>
<tr>
<td>Fibrinogen, gravimetric (mg/dL)</td>
<td>150-400</td>
<td>76</td>
</tr>
<tr>
<td>Fibrinogen, immunoreactive (mg/dL)</td>
<td>150-400</td>
<td>80</td>
</tr>
<tr>
<td>Factor II activity (% normal)</td>
<td>70-150</td>
<td>100</td>
</tr>
<tr>
<td>Factor V activity (% normal)</td>
<td>70-150</td>
<td>88</td>
</tr>
<tr>
<td>Factor VII activity (% normal)</td>
<td>70-150</td>
<td>94</td>
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<tr>
<td>Factor VIII activity (% normal)</td>
<td>60-200</td>
<td>120</td>
</tr>
<tr>
<td>Factor VIII antigen (% normal)</td>
<td>60-200</td>
<td>115</td>
</tr>
<tr>
<td>Factor IX activity (% normal)</td>
<td>70-150</td>
<td>100</td>
</tr>
<tr>
<td>Factor X activity (% normal)</td>
<td>70-150</td>
<td>82</td>
</tr>
<tr>
<td>Factor XI activity (% normal)</td>
<td>70-150</td>
<td>95</td>
</tr>
<tr>
<td>Factor XII activity (% normal)</td>
<td>70-150</td>
<td>120</td>
</tr>
<tr>
<td>Factor XIII screen, 5 mol/L urea solubility</td>
<td>negative negative</td>
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</tr>
<tr>
<td>Plasminogen CTA/mL</td>
<td>2.3-3.8</td>
<td>2.8</td>
</tr>
<tr>
<td>Antithrombin III (% normal)</td>
<td>80-120</td>
<td>94</td>
</tr>
</tbody>
</table>

coagulation studies were performed (Table 1). Hypofibrinogenemia was repeatedly confirmed at times when levels of serum fibrinogen degradation products were normal.

Because of the possibility that her previous abortions had been the result of chromosomal abnormalities, chromosomal analysis was done, which revealed a karyotype of 46, XX, t(7;12) (p15.2;q24.31) (Figs 1 and 2).

Pedigree analysis was carried out on all available members (Fig 3). Note the correlation of the 7p/12q translocation and hypofibrinogenemia. The proposita’s mother had not experienced spontaneous abortion, although her plasma fibrinogen level (140 mg/dL) was consistently higher than that of her daughter (68 mg/dL).

We wished to see if the 7p/12q or other chromosomal aberrations were generalized in hypofibrinogenemia and related disorders. We collected blood and successfully karyotyped three patients with hypofibrinogenemia and one patient each with afibrinogenemia and dysfibrinogenemia. Karyotypic analyses of these five patients were normal. Whereas these findings do not exclude the possibility that other fibrinogen abnormalities are associated with other translocations, it appears that 7p/12q abnormalities are not generalized in disorders of fibrinogen.

Fibrinogen isolated from the proposita’s plasma was reduced with 2-mercaptoethanol into α, β, and γ chains. SDS-PAGE electrophoresis was performed using reduced normal fibrinogen as a control. No differences in mobility of this patient’s fibrinogen chains were observed (Fig 4). Fibrinogen isolated from the proposita’s plasma and normal plasma showed identical reptilase and thrombin clotting times at several different concentrations. The normal half-life of infused fibrinogen (see below) and absence of increased serum fibrinogen degradation products in this patient served as evidence against increased fibrinogen turnover, thus supporting the probability of decreased fibrinogen production. The nearly identical results of plasma fibrinogen concentrations using three quantitative methods and the normal electrophoresis and normal thrombin clotting times support but do not prove that the fibrinogen abnormality in this kindred represents hypofibrinogenemia rather than dysfibrinogenemia.

The hypofibrinogenemia associated with this syndrome prompted us to examine the structure of these subjects’ fibrinogen genes. Genomic DNA was isolated from periph-

![Fig 1. Karyotype analysis of patient.](image-url)
eral blood leukocytes, digested with restriction endonucleases, and size fractionated on agarose gels. The size of genomic DNA fragments hybridizing to human α, β, and γ chain cDNAs was compared with similarly processed samples from normal individuals. When digested with restriction endonucleases EcoRI or HindIII, DNA from the proband’s mother gave hybridizing bands of exactly the same size as DNA from a normal subject. Figure 5 illustrates a representative experiment comparing the proband’s mother with a normal individual. Fibrinogen cDNA probes for the α, β, and γ chain genes bind to identically sized fragments in each, suggesting no major alterations of gene structure in the regions detected by the probes. Normally a faintly hybridizing 600 bp EcoRI fragment from the extreme 3′ end of the αα fibrinogen gene is present. The absence of this band in both the patient and normal individual suggests the common problem of poor retention of small restriction fragments on nitrocellulose membranes. Identical results were seen with the proband and her daughter.

Following consent of both the patient and the University of Florida Investigational Review Board, she was admitted to the Clinical Research Center for further studies. Thirty units of cryoprecipitate containing 6 g fibrinogen were administered, which increased her plasma fibrinogen level from 80 mg/dL to 340 mg/dL; analysis of subsequent fibrinogen levels determined the plasma fibrinogen half-life to be normal at 96 hours. She became pregnant. Vaginal spotting began in the fifth week at a time when her plasma fibrinogen level was 71 mg/dL. She was immediately transfused with cryoprecipitate, 10 U, after which time spotting ceased. She was infused weekly with cryoprecipitate from the fifth to the twelfth week of pregnancy at which time her plasma fibrinogen level was maintained in the range of 105 to 275 mg/dL (averaging 150 mg/dL). From the 12th week to parturition, her plasma fibrinogen level increased to 200 mg/dL without further cryoprecipitate infusion consistent with the increase in fibrinogen concentration experienced in pregnancy. She had no further spotting. She delivered a normal female infant.

One year later, she became pregnant a fourth time. The patient elected not to receive cryoprecipitate infusion. Spotting occurred in the fourth week and she spontaneously aborted in the ninth week at a time her fibrinogen concentration was less than 100 mg/dL. Analysis of the abortus and its blood was unsuccessfully attempted.

Plasma fibrinogen concentration (mg/dL)
The coagulation proteins, particularly fibrinogen and fibrin, seem to play a role in implantation and maintenance of the fertilized ovum in the uterine wall. Evidence to support the hypothesis that fibrinogen and fibrin homeostasis are important in pregnancy are presently supported by clinical observations. At least six publications report hypofibrinogenemia or dysfibrinogenemia associated with spontaneous abortion, including placental abruption. The abortions seen with fibrinogen Metz are restricted to the homozygous state. The patient reported by Gralnick and colleagues also experienced successful gestation when functional plasma fibrinogen levels were supported during early gestation by cryoprecipitate infusion. Women congenitally lacking coagulation factor XIII, the enzyme that cross-links fibrin and links fibrin to fibronectin, habitually abort. One pregnancy in a woman lacking factor XIII was successfully carried to term when factor XIII levels were maintained by chronic infusion of cryoprecipitate. Reid and colleagues described abortion in pregnant women who developed acquired afibrinogenemia following envenomation by the Malayan pit viper. There is no evidence to suggest that this kindred also has a dysfibrinogenemia. To exclude exhaustively that possibility, other studies to include immunoelectrophoresis and turbidity studies of fibrin assembly would be necessary. Accordingly, at this time we assume the fibrinogen abnormality is purely quantitative.

Experimental models also emphasize the role of hemostatic proteins in pregnancy. Mouse trophoblasts produce a plasminogen activator. Peak production coincides with the timing of the uterine invasion. If one blocks fibrinolysis with epsilon aminocaproic acid in pregnant rats, fetal wastage increases sixfold. This effect seemed to be dose-related but could be neutralized with plasmin administration.

The observation that fibrinogen genes from our patients appear to be structurally normal is not surprising, since the human α, β, and γ fibrinogen genes have been found linked in a small region of chromosome 4 at bands 4q23-4q32. Small structural changes that might affect the levels of expression of one or more genes would not be detected by our studies. Moreover, since cDNA probes used to probe the Southern blots do not span the entire gene, it is possible that structural changes at the 5’ ends of each gene might not be appreciated. The basis for the hypofibrinogenemia in this kindred is thus unclear. The 7p/12q translocation seems to be associated with low or low-normal fibrinogen concentration in each of the observed generations, and it is possible there is a gene on chromosome 7 or 12 whose disruption by the translocation might adversely affect fibrinogen production. Conversely, the translocation could affect other genes important in maintaining pregnancy or have no causal role. Since the proposita successfully sustained a pregnancy when her fibrinogen concentration was supported by cryoprecipitate infusion, it seems less likely that the chromosomal abnormality in and of itself is the cause of her recurrent fetal wastage.

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REFERENCES


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