Factor XIII Deficiency: A Genetic Study of Two Affected Kindreds in Finland

By Jan McDonagh, R. P. McDonagh, G. Myllylä, and E. Ikkala

Factor XIII deficiency is a congenital defect of the blood coagulation system. Activated factor XIII is the fibrin cross-linking enzyme that catalyzes the formation of N\(^6\)-γ-glutamyllysyl bonds in fibrin. Congenital deficiency of factor XIII has been described in approximately 65 separate families. It is apparent that this is a hereditary trait, but the mode of inheritance is unclear. In this study two unrelated Finnish families with factor XIII deficiency were investigated in order to determine the pattern of inheritance. One family had only affected male members, and in the other both males and females were affected. Plasma factor XIII levels were measured by the fluorescent amine incorporation assay, which has been found to be sensitive to the detection of heterozygotes. In both families it was found that the parents of the affected children were indeed heterozygous for factor XIII, and as far as could be determined in the pedigrees each heterozygote had at least one heterozygous parent. This study indicates that in these two families the mechanism of inheritance of factor XIII deficiency is autosomal recessive. Comparison of these families with others reported in the literature suggests that an autosomal recessive pattern is the general mode of inheritance in factor XIII deficiency.

Factor XIII (fibrin stabilizing factor) deficiency is a rare, congenital defect of the blood coagulation system; it is characterized by a moderate to severe hemorrhagic diathesis. Factor XIII is a proenzyme that is present in plasma and blood platelets in trace amounts.\(^1\) The active form of the enzyme is a transamidase, which catalyzes the formation of N\(^6\)-γ-glutamyllysyl bonds in fibrin by a well-defined mechanism.\(^2,4\) The formation of these covalent bonds increases the resistance of fibrin to fibrinolytic degradation.\(^5\) Patients with factor XIII deficiency have greatly diminished, often undetectable, levels of factor XIII in their plasma and platelets.\(^6\)

Factor XIII deficiency appears to be a genetically transmitted trait, but the mode of transmission remains unclear. Various authors have suggested an autosomal recessive mechanism.\(^7,8\) However, the high frequency of consanguinity in families with affected females, coupled with a low frequency in families with male patients only, has led to the postulation of two modes of transmission, autosomal recessive and X-linked recessive.\(^9\) In this paper, data are presented on two nonrelated families from Finland with factor XIII deficiency; in one
family both sexes are affected and in the other only males are affected. Plasma factor XIII levels were measured in these kindreds by a quantitative fluorescence assay that is sufficiently sensitive for the detection of heterozygotes, a matter of special importance in studying the fathers of affected sons.

MATERIALS AND METHODS

All blood samples were collected in 0.109 M sodium citrate, one part anticoagulant and nine parts blood. Plasma samples were stored at -20°C or below and were not thawed until they were ready for assay. Control samples were obtained from 20 randomly selected adult donors who came to the Red Cross blood donor center in Helsinki. Blood samples were obtained from as many living members of the two kindreds as possible, 13 in family 1 and ten in family 2. Family relationships were carefully ascertained, and no evidence of any common ancestry for the two kindreds could be found.

Factor XIII activity was measured by the quantitative fluorescent amine incorporation assay in which a synthetic, fluorescent amine donor, monodansylcadaverine, is covalently bound to casein by activated factor XIII. The increase in fluorescent intensity of the casein, hence the incorporation of dansylcadaverine into casein, is a direct measurement of factor XIII activity. Since this is a rate assay, results are expressed in μmole dansylcadaverine incorporation per 30 min per 0.2 ml plasma. The dansylcadaverine (N-(5-aminopentyl)-5-dimethylamino-1-naphthalenesulfonamide) used in these studies was generously supplied by F. Hoffmann-La Roche, Basel, Switzerland. Bovine thrombin was used to activate factor XIII.

Analysis of plasma clots for fibrin cross-linking was performed by polyacrylamide gel electrophoresis in sodium dodecyl sulfate under reducing conditions. Aliquots of plasma were clotted by addition of thrombin and CaCl₂ and were incubated for 24 hr at 37°C. Clots were dissolved in a solution of phosphate buffer which contained urea and sodium dodecyl sulfate. After incubation the samples were subjected to electrophoresis as previously described. Non-cross-linked fibrin that has not been acted upon by factor XIII produces a pattern of monomeric α-, β-, and γ-chains. Fibrin that has been cross-linked by factor XIII yields α-polymer, monomeric β-chain and γ-dimer.

RESULTS

Control plasma samples were assayed individually for factor XIII activity, and the mean rate of dansylcadaverine incorporation per 30 min was found to be 6.13 ± 0.74 (SD). All control samples fell within 2 SD of the mean. There was no effect of sex on the level of factor XIII activity.

A partial pedigree of family 1, consisting of three generations, is shown in Fig. 1. This kindred has two affected male members, and no evidence of consanguinity in the last five generations has been found. Assayed values for plasma factor XIII, expressed as μmole dansylcadaverine incorporation per
30 min per 0.2 ml plasma are given for each member of the pedigree who could be tested.

Pedigree data for family 2, which has both male and female affected members, are shown in Fig. 2. In addition to those shown in the figure, another female, distantly related to the mother of the affected siblings, is also deficient in factor XIII. With the dansylcadaverine incorporation assay her plasma factor XIII level was found to be zero. Although there is a high degree of consanguinity in the mother’s family, there is no evidence that the mother and father of the two deficient children have any common ancestry in the preceding five generations. However, since both parents are from the same rural area, it is possible that the gene was derived from the same distant ancestor. A more complete pedigree of this family has been published.

A scatter diagram for factor XIII in the control population and in the two families is shown in Fig. 3. All family members whose plasma factor XIII levels were within 2 SD of the mean for the control group were classified as normal. Family members with intermediate assay values, less than 2 SD below the mean (4.65), but without clinical symptoms of factor XIII deficiency were regarded as heterozygous for factor XIII. The five patients have a classical history for factor XIII deficiency and have previously been diagnosed on the basis of the solubility of their plasma clots in urea. With the quantitative fluorescent amine incorporation assay, three of those patients had no detectable plasma
factor XIII activity. It appears therefore that these individuals do not produce any normal factor XIII protein. The two brothers in family I, who receive prophylactic factor XIII concentrate, equal to 600–800 ml plasma, at monthly intervals, had detectable but very low levels of plasma factor XIII. It is not possible to determine whether these low levels are the result of transfusion therapy or whether these persons are capable of producing trace amounts of normal factor XIII. However, it has been observed that before prophylactic treatment was started, the plasma clots of these patients dissolved more rapidly in urea. This indicates that the low levels of factor XIII, detectable with the fluorescent assay, are probably the result of transfusion therapy and not de novo synthesis.

Plasma clots of various family members were analyzed for fibrin cross-linking by polyacrylamide gel electrophoresis under reducing conditions. The patients’ clots had the typical pattern of non-cross-linked fibrin with monomeric α-, β-, and γ-chains. Only III-2 in family I had a trace amount of γ-dimer formation. Plasma clots from heterozygous family members yielded fibrin cross-linking patterns which could not be differentiated from normal, indicating that these individuals had sufficient plasma factor XIII for complete fibrin cross-linking.

DISCUSSION

In this report a quantitative analysis of plasma factor XIII levels has been made for two nonrelated kindreds with factor XIII deficiency. The results of the analyses, together with the pedigree data, suggest that in both families the mode of transmission is autosomal recessive. In the two families both parents of the affected members have approximately one-half the normal plasma level of factor XIII and therefore appear to be heterozygous for the gene locus controlling the synthesis of this proenzyme. Further, as far as could be determined, each heterozygote in the two kindreds also has one heterozygous parent.

In view of the hypothesis that the transmission of factor XIII may be either autosomal recessive or X-linked recessive, the study of family I is of particular interest. This kindred, with only affected male members and no consanguinity, would fit the hypothesis for X-linked transmission. However, quantitative analysis for plasma factor XIII in this kindred does not support this hypothesis, as both parents of the deficient patients were shown to have factor XIII levels approximately one-half that of normal.

Although the transmission of factor XIII deficiency in these two families has been shown to be autosomal recessive, the question of whether this is the mechanism of inheritance in all cases still remains. At the present time congenital factor XIII deficiency has been documented in approximately 65 separate families. The distribution of these kindreds with regard to consanguinity is shown in Table 1. Thirty-seven families have both male and female affected members; and in 21 of these, consanguineous unions are known to have occurred. Of the 28 families with affected male members only, there are six with consanguineous unions. An explanation for this rather bizarre distribution, as proposed by Ratnoff and Steinberg, is that there are two different groups of patients with factor XIII deficiency. In one group there is an autosomal re-
cessive mode of transmission, and in the other group the transmission is X-linked recessive. However, in the 28 kindreds with only male patients there are no patients with affected maternal uncles, as would be expected for classical X-linked transmission. Furthermore, with this report, six of these families in which there is no consanguinity have now been studied by quantitative means (dansylcadaverine or radioactive putrescine incorporation assays).\textsuperscript{16,17} Whenever both of the parents have been tested, they have been found to have intermediate factor XIII levels indicating an autosomal recessive mode of inheritance. In an additional case, only the mother of the male proband was available for testing; she had an intermediate level of factor XIII. Similar results have likewise been obtained in six additional families with both male and female affected members.\textsuperscript{8,16-18} Thus the only mode of transmission for this trait which is supported by pedigree data and by quantitative determination of plasma factor XIII levels in affected kindreds is an autosomal recessive mechanism. As yet there is no fully satisfactory explanation for the distribution of consanguinity in these families. It is probable that an explanation will be forthcoming only as an increasing number of affected families are analyzed for factor XIII by suitable, quantitative methods.

**REFERENCES**

13. Ikkala E, Myllylä G, Nevanlinna HR: Rare congenital coagulation defects in Finland. Scand J Haematol 8:210, 1971
14. Ikkala E, Nevanlinna HR: Congenital

**Table 1. Distribution of Consanguinity in Families with Factor XIII Deficiency**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Consanguinity</th>
<th>Yes</th>
<th>No</th>
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<td>Male only</td>
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<td>6</td>
<td>19</td>
<td>3</td>
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<tr>
<td>Female and male</td>
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<td>21</td>
<td>15</td>
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