Factor XII Gene Alteration in Hageman Trait Detected by TaqI Restriction Enzyme


A cDNA for coagulation factor XII has been used to investigate the presence of gene lesions and restriction fragment length polymorphisms in two brothers with Hageman trait and their family. A TaqI polymorphic fragment has been found in the two propositi and in 11 members of the paternal lineage. This polymorphism, absent in the normal population, is correlated with the reduction of factor XII activity and enables the identification of heterozygous factor XII deficiency. Factor XII gene deletion as the cause of Hageman trait in this family has been excluded. A restriction map has been constructed, and the TaqI polymorphic site has been localized within the 5' portion of the gene. The mutation in the polymorphic site is probably the cause of the factor XII deficiency. Data suggest the presence of one factor XII gene per haploid genome.

© 1987 by Grune & Stratton, Inc.

RESULTS

Coagulation and immunologic assays. The hemostatic study of the two brothers with Hageman trait showed a markedly prolonged activated partial thromboplastin time and normal bleeding and prothrombin and thrombin times. Specific assays for coagulation factors showed a severe deficiency of FXII clotting activity (XII: C < 1 U/dL) and a lack of factor XII antigen (XII: Ag < 10 U/dL).

RFLP detection. In order to investigate gene alterations of RFLPs of the FXII gene, DNA from the two brothers with Hageman trait (IV-1 and IV-2 of the pedigree in Fig 1) and from their parents was digested with several restriction enzymes and hybridized to cDNA probes for FXII.

The probes have been isolated from a human liver cDNA library (pEXI expression vector) by immunoblotting to an antibody specific for FXII. The DNA sequence of the inserts from their parents was digested with several restriction enzymes and hybridized to cDNA probes for FXII.

In the TaqI digests hybridized to probe 2 (Fig 2A) two bands of 3.3 and 1.1 kb were found in the four subjects as well as in the 35 unrelated normal individuals examined. An additional 2.1-kb band was found in the two brothers and...
their father. The polymorphic band (Fig 2B) was also detected in hybridizations with the 5' cDNA probe 1.

**Family studies.** The inheritance of the TaqI RFLP and the FXII activity have been studied in the family of the two Hageman trait subjects.

Figure 1 shows the pedigree of the family including the results of the RFLP analysis and of the FXII activity. The TaqI RFLP is present in addition to the Hageman trait subjects in seven males and four females, all of the paternal lineage; intermediate FXII activity levels are present in members of both the paternal and maternal lineages.

The levels of FXII activity in polymorphic and nonpolymorphic subjects of the paternal lineage together with IS normal control values are reported in Fig 3; activities are lower in the polymorphic subjects.

**RFLP characterization and localization.** Additional studies have been performed to determine the origin and to localize in the FXII gene the TaqI RFLP.

The intensity of the 3.3-kb band, using both cDNA probes, was reduced to half when the 2.1-kb band was present (data not shown), which suggests that the polymorphic band originates from the 3.3-kb band; thus two FXII genes should be present in the human diploid genome. Hybridizations of BamHI and BglII DNA digests with both probes (Fig 4) produced large FXII bands common to normal and polymorphic subjects, thus excluding that the polymorphism originates from a gene deletion detectable by Southern blot analysis. The polymorphic pattern is therefore explained by an additional TaqI site cleaving the 3.3-kb DNA fragment in the 2.1-kb band and in a 1.2-kb band not hybridizing to the cDNA probes used. This 1.2-kb DNA fragment is probably an intronic sequence.

In order to localize the TaqI polymorphic site in the FXII gene, double digestions with several restriction enzymes were performed. In Fig 5A NcoI/TaqI and BclI/TaqI double-digestion patterns are reported. Two polymorphic bands of 0.47-kb (BclI/TaqI) and of 1.2-kb (NcoI/TaqI) are present in a TaqI polymorphic subject. Analysis of the length of the DNA fragments obtained with additional double digestions (TaqI/BglII, HindIII, PstI, PvuII, RsaI) has located the TaqI polymorphic site in the 5' portion of the FXII gene (Fig 5B). Exonic sequences detected by the 5' FXII cDNA are...
FACTOR XII GENE ALTERATION IN HAGEMAN TRAIT

DISCUSSION

A TaqI RFLP within the gene for FXII has been identified in two brothers carrying the Hageman trait and is also found in 11 members of their paternal lineage, thus enabling the identification of the heterozygous condition and demonstrating that the autosomal pattern of inheritance of FXII deficiency is monogenic recessive.

The presence of the polymorphism only in the paternal lineage excludes the consanguinity of parents and indicates that the two Hageman trait subjects are double heterozygous for two different FXII gene lesions. Both paternal and maternal mutations should cause the complete impairment of gene expression, as indicated by the individuals with a potential and material absence of FXII. The presence of two different lesions within the same family may suggest that this asymptomatic gene alteration could be less infrequent than suspected.

The absence of this TaqI intragenic polymorphism in normal unrelated individuals and its association with the reduction of FXII activity suggests an intronic or a splice junction mutation, as is frequently found in genetic diseases.

ACKNOWLEDGMENT

We thank Dr Barbara Anderson for revising the manuscript.

REFERENCES

Factor XII gene alteration in Hageman trait detected by TaqI restriction enzyme

F Bernardi, G Marchetti, P Patracchini, L del Senno, M Tripodi, A Fantoni, S Bartolai, F Vannini, L Felloni and L Rossi