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Factor XII Gene Mutation in the Hageman Family

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Hageman trait refers to an inherited deficiency of coagulation factor XII (FXII). The term was named by Oscar Ratnoff in 1955 after the index patient John Hageman, who had ‘incoagulable’ blood *in vitro* but no abnormal bleeding even after surgeries [1, 2]. Subsequently, Earl Davie and Oscar Ratnoff isolated Hageman factor, the protein missing in Hageman’s blood, which later was called FXII [3, 4]. This pioneer work contributed to the ‘Waterfall/Cascade’ hypothesis of blood coagulation presented in 1964 [5, 6], which established the basic principle that blood coagulation is mediated by the sequential activation of a series of plasma proteases.

John Hageman died of pulmonary thromboembolism during bed rest from a broken hip in 1968 before gene cloning techniques were available [7]. As a result, the genetic defect underlying his FXII-deficiency was never determined. In this study, we obtained blood samples from descendants of the Hageman family to analyze their *F12* gene that encodes FXII. The study was approved by the Cleveland Clinic Institutional Review Board. All participants provided written consent.

John Hageman was never married and had no known descendants (Fig. 1A). We obtained blood samples from III-1, III-3-5 and IV-3-5, who were family members of his siblings’ descendants. None of these individuals had a history of abnormal bleeding. PCR and DNA sequencing of all 14 *F12* gene exons in these individuals found no deletion, insertion or non-synonymous point mutations. In III-3 and IV-5, however, a G→A intronic mutation was identified at nucleotide position 11396 of the *F12* gene [8] (Fig. 1B), which was part of the splice acceptor site of exon 14 (Fig. 1C). The mutation abolished the original splice acceptor site and created a new site one nucleotide downstream. As a result, the exon 14 reading frame was shifted by one position, thereby encoding a new peptide that replaced the catalytic serine and the rest of the protease domain (Fig. 1C).

Interestingly, this 11396(G→A) mutation was reported previously in FXII-deficient individuals in Germany [9, 10]. PCR analysis of transcripts from the mutant *F12* gene confirmed the predicted fusion mRNA. The study also showed that the fusion protein, if

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The authors state that they have no conflict of interest.

translated from the fusion mRNA, was unstable, because individuals homozygous for this mutation had no detectable plasma FXII antigen or activity [9, 10].

By ELISA (Innovative Research Inc.), we found that plasma FXII antigen levels in III-3 and IV-5, who were heterozygous for the mutation, were ~50% of that in other members of the family (Fig. 1A). The antigen level in the normal individuals of this family was ~1.8 fold higher than that of pooled normal plasma. The levels in III-3 and IV-5 were at ~90% of pooled normal plasma level (data not shown). As measured by a modified one-stage activated partial thromboplastin time, plasma FXII coagulant activity in four family members (III-1 and IV-3-5), whose blood was collected in sodium citrate, were 1.31, 1.44, 1.28 and 1.21 U/mL, respectively. On immunoblots, plasma FXII protein in all samples had a similar molecular mass, although the level in samples from III-3 and IV-5 was lower (data not shown). No additional FXII protein bands were detected on immunoblots. The data were consistent with the genotype in these individuals.

These studies identified an *F12* gene mutation in the descendants of the Hageman family. We showed that there was a mutant allele in III-3 and IV-5, which was likely from II-3 who inherited it from I-1 or I-2. It is likely that John Hageman (II-1) also inherited this mutant allele. Given the fact that there was no detectable FXII activity in his blood [2], John Hageman was expected to be either homozygous for this mutant allele or compounded heterozygous with another unknown mutant *F12* allele. An inquiry of family history indicated no evidence that his parents were consanguineous but revealed that they emigrated from the central part of Germany, where the same mutant *F12* allele was reported in at least five unrelated families [9, 10]. This mutant allele also existed in another unrelated family in Switzerland [10]. All of the individuals with this allele were asymptomatic and identified only by chance during hospital visits. Apparently, this mutation originated for sometime in history and is present in a number of individuals in Europe, especially in Germany. Since no other *F12* mutations were found in the descendants of his family, the possibility cannot be excluded that John Hageman may have inherited two copies of the same mutant *F12* allele from his parents.

The chance encounter of John Hageman by Oscar Ratnoff more than half a century ago had a major role in the history of understanding the blood coagulation system [1, 3, 11–14]. Now FXII is known to be important in the contact activation system to regulate blood pressure, vascular permeability, complement activation, inflammatory responses, angiogenesis, and thrombosis risk [15–17]. Our finding of the *F12* gene mutation in the Hageman family, providing an explanation to the long-standing question of John Hageman's genetic defects, adds a fresh footnote to this important history in hematology.

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Fig. 1. (A) Hageman family pedigree. The pedigree was modified for confidentiality. J.H., John Hageman. Diamonds with a slash line represent deceased individuals. Diamonds with a dot represent heterozygous carriers. IV-1–2 were not available for the study. Numbers in parentheses below diamonds indicate plasma FXII antigen levels (μg/mL) in these individuals. Levels in III-3 and IV-5 were significantly lower ($p < 0.01$) than those in III-1, III-4, III-5, IV-3 or IV-4. (B) DNA sequencing data from III-1 (normal), III-3 and IV-5 (heterozygous). (C) Partial exon sequences are shown in boxes. The exon 14 splice acceptor site is in bold-type. Catalytic serine is indicated by an asterisk. The G→A mutation is indicated by an arrow. Predicted amino acids after frameshift are in red.