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Brief Communication

Structural Gene Encoding Human Factor XII Is Located at 5q33-qter

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Abstract—*The gene encoding human factor XII (*F12*) or Hageman factor has been mapped to 5q33-qter. This has been achieved by analyzing the results obtained from hybridizing a cloned fragment from the factor XII gene to a panel of human–hamster somatic cell hybrid DNAs and also by in situ hybridization to normal human metaphase cells. The previously reported results localizing* F12 *to 6p23 are discussed.*

INTRODUCTION

Factor XII (Hageman factor) is a glycoprotein (mol wt 76,000) that circulates in plasma as an inactive serine protease (1). Although the role of FXII is not completely understood, it has been implicated in the surface activation of a number of cascade reactions resulting in either blood coagulation, fibrinolysis, bradykinin generation, or the formation of angiotensin (2–6). No pathological state has been clearly attributed to factor XII deficiency, but a deficiency of factor XII results in a marked delay in plasma coagulation which is usually only detected by chance laboratory testing (7). The deficiency is inherited in an autosomal recessive manner (8). Factor XII is activated by limited proteolysis by kallikrein in the presence of high-molecular-weight kininogen and a negatively charged surface (9). Surface-bound and activated FXII (alpha-FXIIa) cleaves its substrates, prekallikrein and FXI, resulting in the initiation of the plasma cascade reactions (9–11). The complete amino acid sequence (596 residues) of the zymogen form of human plasma FXII has been determined (12, 13). The amino acid sequence has also been predicted from the nucleotide sequence of a cloned *F12* cDNA isolated from a human liver cDNA library (14).

Since 1974 there have been several publications (15–22) concerning the location of the *F12* gene, and these have been based on dosage of different chromosome regions and correlation with levels of factor XII activity. This study was undertaken to localize the gene for FXII by hybridizing a cloned genomic sequence from the *F12* gene to a somatic cell hybrid panel of DNAs and to further regionally localize the gene by in situ hybridization to normal human metaphase cells.

MATERIALS AND METHODS

Recombinant Probe. A human genomic phage library was kindly provided by Dr. P.

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Table 1. Human Chromosomes in Human X Hamster Hybrids and Human Sequences Homologous to pu.λ.HXII2.5B/H

Cell line	Response to pu.λ.HXII2.5B/H[a]	Human chromosome number[b]																						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
41.06	+	−	−	+	−	+	−	−	−	−	+	−	−	+	+	+	+	+	+	−	+	−	−	+	−
45.01	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
45.43	−	+	−	−	−	−	−	−	−	+	+	+	−	−	−	−	+	+	+	+	+	+	−	+	−
76.14	+	−	−	+	−	+	+	−	−	−	−	+	−	+	+	−	+	+	+	−	+	+	−	+	+
76.31	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
76.33	−	+	−	+	+	+	−	−	+	−	+	−	−	+	−	+	+	−	+	+	−	+	−	+	−
79.05b	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	+	−	+	−
80.05d	−	−	−	−	−	−	+	+	−	−	−	−	+	−	−	−	+	−	−	−	+	−	−	+	+
80.14c	+	−	−	−	+	+	+	+	−	−	+	−	−	−	−	−	−	−	−	−	+	+	−	+	−
80.17a	+	−	−	−	+	+	−	−	−	+	−	+	−	−	−	+	+	−	+	+	+	+	+	+	−
82.82a	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
85.16a	+	−	+	+	+	+	−	−	+	−	+	+	−	+	−	+	−	+	−	−	−	+	−	+	−
89.27	+	−	−	−	−	+	−	−	−	+	−	−	+	−	−	+	+	+	+	−	+	−	+	−	−
100.02b	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	+	−	+	−	−	+	−	−	−
102.05b	+	+	−	−	−	+	+	−	−	−	−	−	−	+	−	−	+	+	−	−	+	−	+	−	−
103.04	+	−	−	−	+	+	−	−	−	−	−	−	+	−	−	+	+	−	+	−	+	+	−	−	−
111.02a	+	+	−	−	−	+	+	−	−	−	−	−	+	+	+	+	−	+	−	+	−	+	+	−	−
112.10a	+	+	−	−	+	+	−	−	+	−	−	+	+	+	−	+	+	−	−	+	+	−	−	+	−
120.33	+	−	−	−	−	+	+	−	−	−	−	−	+	+	−	−	+	+	−	+	−	+	+	+	−
120.35	+	−	−	+	−	+	+	−	+	−	−	−	+	+	+	−	−	+	+	+	−	−	+	+	−
133.05	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+	−
134.02a	+	+	−	−	+	+	−	+	+	−	+	+	−	+	−	+	+	+	+	+	+	+	−	+	−
1103	−																								
P23	+																								
Concordancy (%)		50.0	45.5	45.5	63.6	95.5	59.0	45.5	50.0	45.5	50.0	59.0	59.0	72.7	50.0	63.6	59.0	72.7	50.0	54.5	68.2	59.0	59.0	54.5	36.4

[a]Presence (+) or absence (−) of human EcoRI-digested sequences homologous to the genomic probe for factor XII.
[b]Presence (+) or absence (−) of human chromosomes as determined by cytogenetic and confirmed by isozyme analysis.

Leder (Harvard University) from which a clone containing the *F12* gene was isolated (23). A 2.5-kb BamHI/HindII subfragment was isolated from the cloned genomic DNA and ligated into the pUC-13 plasmid cloning vector (13). *E. coli* JM83 was transformed with the ligation mixture and grown in the presence of 0.05% X-gal (13) on Luria broth agar plates. White colonies were selected and the plasmid DNA isolated and tested for 2.5-kb inserts. A positive colony (pu.λ.HXII2.5B/H) was amplified and the plasmid DNA isolated and purified by cesium chloride gradient centrifugation (24).

Hybrid Cell Lines. The human chromosome content of the human–hamster somatic cell hybrid lines (Table 1) was identified by cytogenetic analysis and confirmed by isozyme analysis (25).

Southern Blot Analysis. Two hundred nanograms of the plasmid pu.λ.HXII2.5B/H were labeled by random priming with oligonucleotides (26) to a specific activity of 9.5×10^8 cpm/μg using [^{32}P]dATP and [^{32}P]dCTP. The labeled probe was then hybridized to a Southern blot of HindIII digests of somatic cell hybrid DNAs (27). The Southern blot was washed in 0.5× SSC and 0.1% SDS at 65°C.

In Situ Hybridization. The plasmid was labeled by nick translation to a specific activity of 2.55×10^7 cpm/μg using [^{3}H]dATP, [^{3}H]dCTP, and [^{3}H]TTP. In situ hybridization was carried out as previously reported (27, 28). The chromosomes were identified by the BrdU–Hoechst 33258–UV–Giemsa method for replication banding (29; E. Jenkins, personal communication).

RESULTS AND DISCUSSION

The autoradiogram shown in Fig. 1 shows the response of the panel of somatic cell hybrid DNAs to the probe for the factor XII gene. The control lane of human DNA (P23) shows a single band of approximately 5.6 kb, and this band is also present in a number of human–hamster hybrid DNAs. The control lane of hamster DNA (CHW1103) does not hybridize to the human genomic probe for the *F12* gene. The band of 4.15 kb seen only in the hybrid DNA 100.02b is thought to be a result of the plasmid sequences of the labeled probe, pu.λ.HXII2.5B/H, hybridizing to a low level of plasmid contamination in the hybrid DNA. Among the 22 somatic cell hybrid DNAs analyzed, 21 (95.5%) were concordant for the presence or absence of human chromosome 5 and a positive or negative response to the probe for *F12* (Table 1). The discordant hybrid cell line, 7633, had only seven copies of human chromosome 5 among the 20 cells analyzed, and it was also negative for the human isozyme hexosaminidase B which is

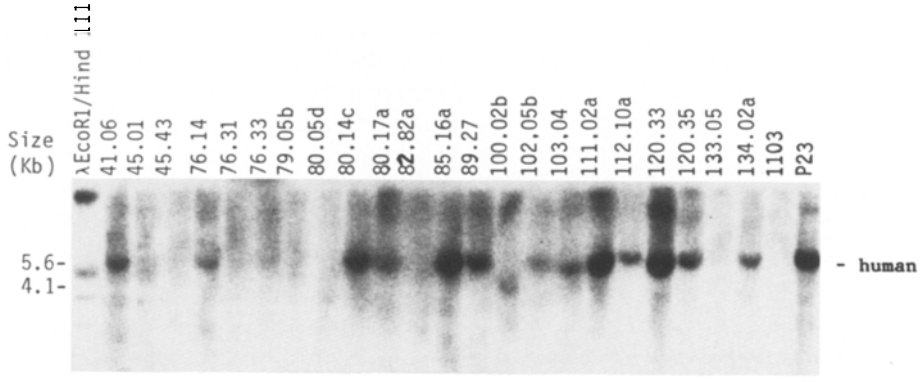

Fig. 1. Segregation of sequences homologous to the human genomic probe for the *F12* gene. The single band of 5.6 kb is present in DNAs from the normal human (P23) and some of the hybrid cells.

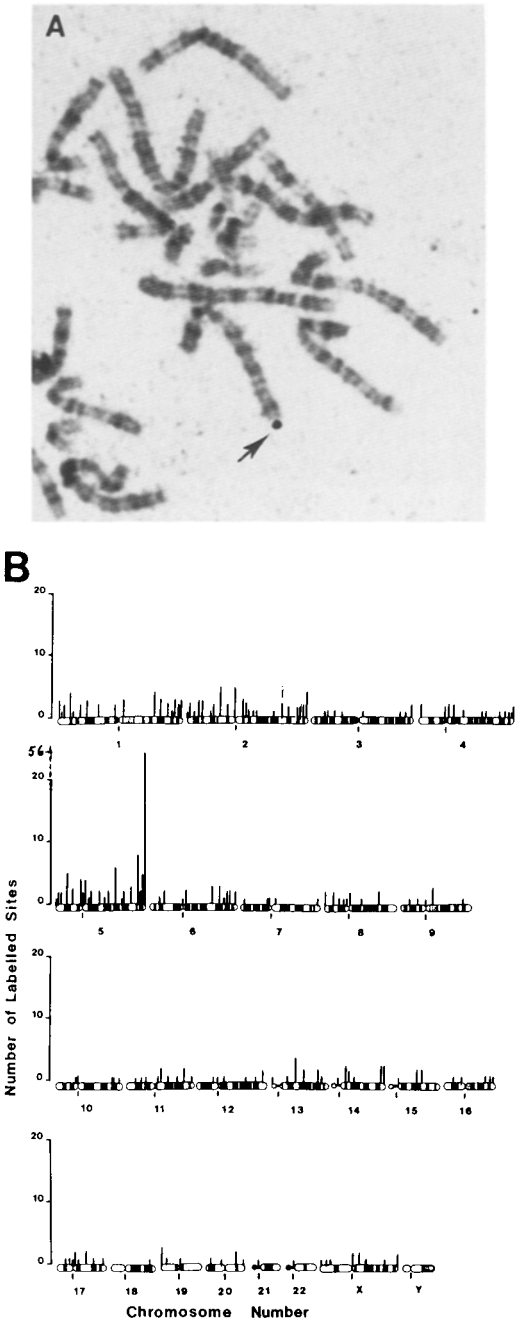

Fig. 2. (A) Partial metaphase spread G-banded by the Hoeschst 33258–UV–Giemsa method showing a silver grain at 5qter after hybridization to the genomic probe for *F12*. (B) Distribution of labelled sites over the normal human idiogram. The peak at 5q33-qter represents 19.2% of the total number of labeled sites scored.

located at 5q13. The negative response to the probe for *F12* is probably because there are few copies of human chromosome 5 in the hybrid cell line 7633 and these sequences were not detected under the conditions used.

The location of the structural gene for factor XII on chromosome 5 was confirmed and further localized by in situ hybridization to human metaphase cells. Figure 2 shows the results of the analysis of 277 cells, and among the 369 silver grains scored, 19.2% were clustered in the region 5q33-qter. It has also been shown that 62% of the 114 silver grains scored on chromosome 5 were located in the region 5q33-qter. These results map the structural gene for factor XII (*F12*) to 5q33-qter.

In 1974, dosage studies in two patients were used by de Grouchy and Turleau (15) to provisionally map *F12* to 7q35, but this was later dispoved by a number of research groups (11–20) who all showed normal levels of FXII activity in patients with deletions of 7q. The most recent location of *F12* reported by Pearson et al. in 1981 (21) was also based on the measurement of reduced factor XII activity in a patient known to have only one copy of the region 6p23-p25. Since that report, Niebuhr, et al. (22) showed that a patient with a deletion 6p23-pter had normal levels of factor XII activity, and these authors excluded this region, leaving the provisional location of *F12* at 6p23.

The present study has used a probe for the structural gene for factor XII to locate the gene on chromosome 5q33-qter by the analysis of the response of a panel of human–hamster somatic cell hybrid DNAs and by in situ hybridization to human metaphase cells. The location of *F12* at 5q33-qter does not exclude the possibility that there is a gene on the short arm of chromosome 6 that modifies the expression of *F12* on 5q33-qter.

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