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Brief Communication

A Structural Locus for Coagulation Factor XIIIA (*F13A*) Is Located Distal to the HLA Region on Chromosome 6p in Man

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SUMMARY

Linkage between the locus for coagulation factor XIIIA (*F13A*) and *HLA*-region genes has been revealed during a linkage study between *F13A* and approximately 40 other polymorphic marker genes. In males, the maximum lod score between *F13A* and *HLA*-region genes (*HLA-A, -C, -B, -DR; C4A,-B; Bf;* and/or *C2*) is 7.60 at $\theta_1 = .18$. To *GLO*, the maximum lod score is 2.37 at $\theta_1 = .19$; to *PGM3*, .22 at $\theta_1 = .35$. Female data indicate a clear sex difference in recombination frequency between *F13A* and *HLA*.

The present findings, in combination with earlier knowledge of *PGM3/GLO/HLA* localization and gene distances, show that *F13A* is distal to *HLA* on the short arm of chromosome 6 in man. It is thus likely that by including *FXIIIA* typing in linkage studies, the whole male 6p is within mapping distance of highly polymorphic, classical marker genes.

Earlier findings that the Hageman factor gene (*F12*) is located in the same chromosomal region may indicate the presence of a coagulation factor gene cluster in this region.

INTRODUCTION

Human coagulation factor FXIII is necessary for normal hemostasis. Plasma FXIII has a concentration of 10–20 μg/ml [1, 2] and consists of two pairs of

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noncovalently bound subunits a_2b_2 [3]. Activated a_2 dimer (a_2') acts as an enzyme catalyzing the formation of cross-linked fibrin.

Subunit a_2 (FXIIIA)-deficiency states causing hemorrhagic diathesis have been described in about 100 cases, and family studies have revealed an autosomal recessive mode of inheritance.

In 1979, Board [4] described a genetic polymorphism of plasma and platelet FXIIIA in several racial groups. The study of phenotypes in families showed an autosomal codominant inheritance pattern. Gene frequencies of about .8 and .2 for the *F13A*1* and *F13A*2* alleles, respectively, make this an informative marker for linkage studies in Caucasians.

We report linkage between the *F13A* locus and the *HLA* region and present data showing a *PGM3-GLO-HLA-F13A* gene sequence on human chromosome 6. Preliminary results of this study were presented at Human Gene Mapping Workshop 7, Los Angeles (quoted in [5]).

MATERIALS AND METHODS

Family Material

The family material used in our study is the OSLO-NHIK material. Families have been ascertained and blood samples collected from 1970 to the present time. The material comprises a total of 375 matings with 1,295 offspring usable in linkage studies.

Blood Samples

Ordinarily, venous blood was drawn into vacutainer tubes (one tube without additives, two heparin tubes, and two ACD tubes). Within 24 hrs of transport, the samples arrived at the laboratories, where aliquots of red cells, serum, and heparin plasma were frozen and stored at −70°C.

Marker Analyses

Most marker analyses were performed in fresh samples by conventional typing techniques. As new marker systems have become available, frozen samples have been used.

A total of some 40 different polymorphic markers have been typed in all or parts of this family material—*blood group markers:* ABO, Rh D, C, c, E, e, MNSs; Kell; Fy(a,b); Co(a); Jk(a,b); Do(a); Ch; Rg; Le(a,b), and P; *serum-type markers:* Hp (subt.), Gc (subt.), Tf (subt.), C3, C2, C4 (in desialized serum/plasma samples), Bf, C6, C8, FGG, ApoAI, ApoAIV, ApoE, Km, Gm, FXIIIB, and A2HS; *red cell enzymes:* PGM1 (subt.), GLO, ESD, ACP, GPT, AK, and PGP; and *white cell markers:* HLA-A,-B,-C,-DR; and PGM3.

Factor XIIIA Typing

Electrode and gel buffers were the Tris/glycine/barbital buffers described by O'Neill et al. [6]. One-mm-thick gels 0.5% in agarose (Seakem ME) were poured onto glass plates 20 × 27 cm. Samples of 3 μl of plasma stored at −70°C for 1–12 years were subjected to high-voltage agarose electrophoresis (3 hrs at 600 V). Proteins were then transferred to nitrocellulose sheets by passive blot for about 10 min.

Visualization of factor XIIIA bands was achieved as described by Whitehouse and Putt [7]. The nitrocellulose blot was soaked in rabbit antifactor 13A (Hoechst), diluted 1:500 with 0.15% Tween in PBS overnight, followed by a peroxidase conjugated goat antirabbit antiserum (Hoechst) diluted 1:1,000 with 0.15% Tween in PBS. Finally, peroxidase activity was developed using 3,3-diaminobenzidine.

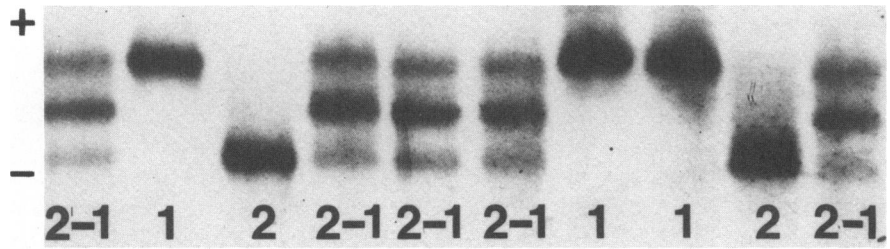

FIG. 1.—Coagulation factor XIIIA (FXIIIA) patterns in plasma as revealed by high-voltage agarose electrophoresis and immunoblotting procedures.

FXIIIA has been typed in most of the matings and in all offspring where one or both of the parents were heterozygous. Linkage studies were performed by the MOSM computer program based on the lod-score method, calculating lod scores for all recombination frequencies .01 . . . .49.

HLA-region genes included in this study were *HLA-A, -B, -C, -DR; C4A, -B; Bf,* and *C2*. In a very few intra-HLA recombination families, the recombinant child has been omitted.

RESULTS

Figure 1 shows a developed FXIIIA blot illustrating the common phenotypes FXIIIA 1, 2-1, and 2. We had no samples without typable FXIIIA pattern, and even in cases of very small aliquots (less than 1 μl), sufficient immunological FXIIIA activity to allow phenotyping was achieved in nonplatelet-enriched heparin plasma samples. *F13A* linkage information was obtained in 111 matings. Table 1 gives the data between *F13A* and chromosome 6p marker genes.

There is linkage between *F13A* and the *HLA* region, with a maximum lod score of 7.60 at recombination fraction $\theta_1 = .18$. Given linkage, the 95% confidence limits are $\theta = .12$ and $\theta = .26$.

Between *GLO* and *F13A*, the maximum lod score is 2.37 at $\theta_1 = .19$ in males. The maximum lod score for males is 0.22 at $\theta_1 = .35$ to *PGM3*. Lod scores rule out linkage between *F13A* and *PGM3* at all θ's below .12 (lod scores < -2.0).

In females, the maximum lod score is 0.19 at $\theta_1 = .42$ between the *F13A* and *HLA*-region loci, 0.01 at $\theta_1 = .44$ between *F13A* and *GLO*, and 0.14 at $\theta_1 = .34$ between *F13A* and *PGM3*.

Linkage studies between *F13A* and unassigned marker genes (*Jk, Co, Kell, DiaB, C6, GPT/EBS1,* and *F13B*) gave no indications of linkage. Linkage between *F13A* and *F13B* was excluded (lod score < -2) for all recombination fractions less than .19.

Linkage data between *F13A* and the marker genes known to be on chromosomes other than chromosome 6 are not presented. There were, however, no hints of linkage between *F13A* and any of these marker genes.

DISCUSSION

The data presented here map another important marker gene to chromosome 6 in man. Our study shows that *F13A* is linked to the *HLA*-region genes at a re-

TABLE 1

LINKAGE RELATIONS BETWEEN *F13A* AND CHROMOSOME 6 MARKER GENES

RELATION	SEX	PHASE-KNOWN R*	PHASE-KNOWN NR*	TWO-GENERATION Family	TWO-GENERATION Children	LOD SCORES AT RECOMBINATION FRACTIONS 0.05	0.10	0.15	0.20	0.30	0.40	PEAK AT Lods	PEAK AT θ	95% CONFIDENCE LIMITS GIVEN LINKAGE
F13A-HLA region	M	8	24	32	108	1.87	6.16	7.47	7.50	5.60	2.49	7.60	0.18	0.12–0.26
	F	17	22	33	120	−27.01	−14.35	−8.01	−4.31	−0.77	0.17	0.19	0.42	0.32–0.50
	M + F	25	46	65	228	−25.15	−8.19	−0.54	3.19	4.83	2.66	4.92	0.28	0.23–0.36
F13A-GLO	M	4	4	18	54	−0.67	1.49	2.23	2.36	1.72	0.61	2.37	0.19	0.11–0.34
	F	10	10	15	48	−12.12	−6.49	−3.67	−2.00	−0.42	−0.01	0.01	0.44	0.27–0.50
	M + F	14	14	33	102	−12.79	−5.00	−1.44	0.36	1.30	0.60	1.31	0.29	0.22–0.45
F13A-PGM3	M	3	3	14	44	−5.24	−2.41	−1.09	−0.38	0.17	0.20	0.22	0.35	0.20–0.49
	F	3	2	11	45	−5.05	−2.79	−1.30	−0.50	0.11	0.08	0.14	0.34	0.20–0.49
	M + F	6	5	25	89	−11.17	−5.20	−2.39	−0.87	0.29	0.28	0.37	0.34	0.24–0.49

NOTE: For sexes combined (M + F), $\theta_m = \theta_f$. R*: recombinant. NR*: nonrecombinant.

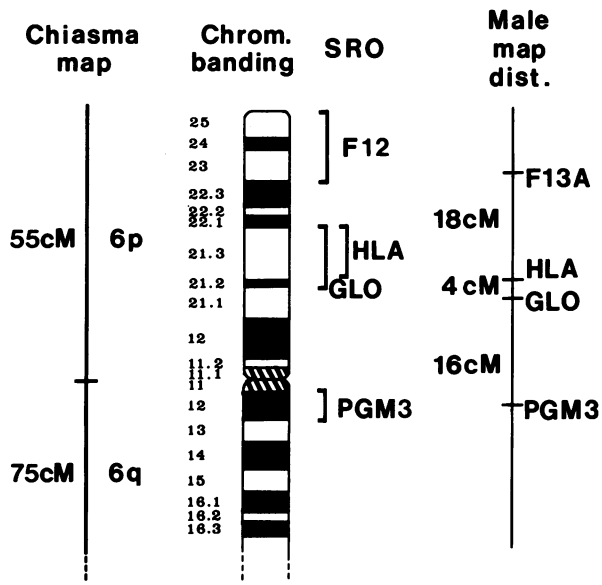

FIG. 2.—Map position of marker genes on chromosome 6. *On the left* is shown chiasma map [10], followed by chromosomal-banding pattern and smallest region of overlap (SRO) from compiled data on mapping by chromosomal breaks [5]. SRO for the Hageman factor gene (*F12*) is included. *On the right* is shown male map distances based on family studies.

combinational distance of about 0.18%. The male *HLA-PGM3* distance is very similar. It is estimated at 20% in pooled materials showing *HLA*-4%-*GLO*-16%-*PGM3* [8]. It is also 20% (11R, 43NR) in our phase known and 19% in our total Oslo-NHIK material [9]. Our present data exclude linkage below 0.12% between *F13A* and *PGM3*. This means that *F13A* must be located distal to *HLA*.

Figure 2 shows a genetic map of parts of chromosome 6 extending from the *PGM3* locus near the centromere on the long arm to the distal end of the short arm. For all genes except *F13A*, the data have been taken from the reviews given in [5] and [8]. Map distances (in cM) are indicated.

F12 was tentatively assigned to 6p from the findings in a translocation family [10]. Support for this was recently obtained in another family [11], in which a more precise chromosome assignment was achieved (6p23-pter). If a coagulation factor linkage group exists, however, it does not include the *F13B* gene.

Very recently, restriction fragment length polymorphisms (RFLPs) have been mapped that appear to cover most of the short arm of chromosome 6 [12]. As a consequence of the mapping of *F13A* to the distal end of 6p, it is reasonable to believe also that the polymorphic classical markers available (PGM3, GLO, *HLA*-region markers, and FXIIIA) cover the whole 6p in the context of linkage studies: *PGM3* is close to the centromere of chromosome 6 (on 6q) and about 38 cM from *F13A*, which would indicate a male map distance of about 17 cM between *F13A* and 6pter if 6p is 55 cM of length [13].

The female *HLA-F13A* distance of 42% is very approximate but reveals a clear sex difference and suggests a female/male sex ratio around .42/.19 = 2.2—or

from phase-known data, 1.8. In the same families, we have previously found the sex ratio for *PGM3/HLA* of .58/.20 = 2.9 for phase-known meioses [9].

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