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Genetics

Regional localization on the human X chromosome and polymorphism of the coagulation factor IX gene (hemophilia B locus)

(blot hybridization/genetic screening/prenatal diagnosis/restriction site polymorphism)

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ABSTRACT **Hemophilia B is an X-linked disease caused by a functional deficiency in coagulation factor IX. A cDNA clone corresponding to factor IX has been used to detect homologous sequences in the human genome. All DNA fragments hybridizing to the probe, under medium- or high-stringency conditions, are X-linked, and the patterns obtained suggest that a single large (≥20 kilobases) gene is detected. The gene has been mapped to the q26–q27 region of the long arm of the X chromosome by hybridization to DNA from a panel of human–mouse hybrid cell lines. A search for restriction fragment length polymorphisms using seven restriction enzymes has led to the detection of a *Taq* I polymorphism, with allelic frequencies of about 0.71 and 0.29. This genetic marker should be useful for the detection of carriers of the hemophilia B trait and for prenatal diagnosis in informative families and, more generally, for the establishment of a linkage map of the human X chromosome.**

Coagulation factor IX (Christmas factor) is a protein in which maturation is dependent on vitamin K and which participates in the middle phase of the intrinsic pathway of blood coagulation (1). A functional deficiency in this protein leads to hemophilia B, a bleeding disorder with symptoms essentially identical to those of hemophilia A (the latter being caused by a lack of active factor VIII:C) (2). Both hemophilias are X-linked recessive disorders, but while family studies (3, 4) have shown a tight genetic linkage between the hemophilia A locus and the glucose-6-phosphate dehydrogenase (*G6PD*) gene located at the tip of the long arm of the X chromosome in the X q28 band (5), no measurable linkage could be established between hemophilia B and any of the available X chromosome genetic markers (6, 7). The recent cloning of genomic sequences (8) and cDNA sequences (9, 10) corresponding to human factor IX allows investigation of the structure of the gene and its regional localization on the X chromosome, analysis at the DNA level of the defects leading to hemophilia B, and a search for genetic markers linked to the gene. Such markers should be useful for genetic screening in families at risk for hemophilia B or other diseases with loci close to the factor IX gene (11).

We have used a human factor IX cDNA probe (10) to detect homologous fragments on the human genome. All of these fragments are located in the same region of the long arm of the X chromosome and probably correspond to a single large mosaic gene. A frequent restriction fragment length polymorphism has been found, which should be of use, in informative families, for the detection of heterozygous carriers of hemophilia B and for prenatal diagnosis.

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MATERIALS AND METHODS

Preparation of DNA and Blot Hybridization. DNA preparation (from cultured cell lines or from total leukocytes), digestion with restriction enzymes, blotting onto diazobenzyloxymethyl (DBM)-paper, and hybridization (in the presence of 4% dextran sulfate at 42°C) were as described (12, 13).

Cell Lines. A fibroblast cell line with the 48 XXXX karyotype (GM1415) was obtained from the Human Genetic Mutant Repository (Camden, NJ). Hybrid clones derived from fusions between RAG mouse cells and the following human diploid fibroblasts: PI (X;15) (p22;q21), GO (X;14) (p11;q32), Anly (X;9) (q12;p24) (see refs. 14 and 15), GM97 (X;1) (q26;q12), and GM194 (X;3) (q28;q21) (Human Genetic Mutant Repository) (see also refs. 16 and 17) were cultivated in hypoxanthine/aminopterin/thymidine (HAT) medium, and the karyotype and presence of X-linked enzymatic markers [G6PD, hypoxanthine phosphoribosyltransferase (HPRT), and phosphoglycerate kinase] were checked before preparing DNA. The hybrid cell lines A9-HRBC2 (containing the whole human X chromosome) (18) and A9-GM89 (X;19) (q22;q13) (16) were provided by M. Siniscalco (Sloan–Kettering Institute, New York) and cultivated in HAT medium, but they were not further karyotyped. Hybridization of DNA from this panel of cell lines with various X-linked probes gave internally consistent results with nine probes of previously unknown location and results consistent with the known location of other probes.

RESULTS

X-Linkage of Fragments Hybridizing to a Factor IX Probe. We have used a cDNA probe corresponding to the human coagulation factor IX (10) to detect homologous fragments in genomic DNA. This cDNA contains the entire protein coding sequence (including the signal peptide sequence) and about 800 nucleotides of 3′ untranslated region (Fig. 1). To determine whether all fragments recognized by the factor IX probe are X-linked, Southern blotting experiments were carried out using genomic DNA obtained from normal males (46 XY), from normal females (46 XX), and from cultured fibroblasts of a female with a 48 XXXX karyotype. In addition, we have blotted restriction digests of DNA from a mouse cell line (A9) and from a human–mouse hybrid line (A9-HRBC2) (18) containing the X chromosome as the only identified human chromosome. As shown in Fig. 2, several fragments hybridize to the factor IX probe in human DNA digested with either *Pvu* II or *Hin*dIII, all of which show a dosage effect demonstrating X-linkage (compare lanes 3 and 4 to lane 7).

Abbreviations: kb, kilobase(s); bp, base pair(s); G6PD, glucose-6-phosphate dehydrogenase; HPRT, hypoxanthine phosphoribosyltransferase; DBM-paper, diazobenzyloxymethyl-paper; NaCl/Cit, 0.15 M sodium chloride/0.015 M sodium citrate.

All of these bands are detected even after washing under high-stringency conditions [0.2× standard saline citrate (NaCl/Cit)/0.1% $NaDodSO_4$, 65°C; 1× NaCl/Cit = 0.15 M sodium chloride/0.015 M sodium citrate] (results not shown). These fragments are also present in the A9-HRBC2 hybrid cell line (Fig. 2, lane 2; see Fig. 4*A*, lane 8). In the mouse DNA control, crossreacting fragments can be detected when the hybridization reaction is carried out under medium-stringency conditions: a 10.5-kilobase (kb) fragment with *Pvu* II (Fig. 2, lane 1) and a 5.6-kb fragment with *Hin*dIII (the latter migrates at the same position as one of the human fragments; see Fig. 4*A*, lane 9). Under lower stringency conditions, more mouse bands appear (results not shown). Because we used DBM-paper to blot the DNAs, we were able to rehybridize them to autosomal probes to check that similar quantities of DNA were present in each lane, thus confirming the validity of the dosage effect (Fig. 2 *C* and *D*).

Complexity of the Gene. The sum of the sizes of the X-linked restriction fragments detected by the factor IX probe in *Hin*dIII or *Pvu* II digests is very large (40 and 28 kb, respectively) compared to the size of the cDNA (2.1 kb). With other enzymes (*Msp* I, *Taq* I, and *Xba* I), six or seven bands are also seen (summarized in Table 1) with a total fragment size for each enzyme of 16–18 kb. With *Bam*HI a single large (25–30 kb) fragment is detected. However, because of the poor resolution of the gel in this size range, one cannot eliminate the possibility that there are two *Bam*HI fragments of similar size. It should be noted that all these enzymes except *Taq* I and *Msp* I do not cut within the cDNA used as probe (10).

These results suggest either that the factor IX gene is a mosaic gene containing large introns or that there is a small family of related X-linked genes. To approach this problem, we used as probes smaller fragments obtained by *Taq* I digestion of the cDNA insert (see Fig. 1). We hybridized them to *Hin*dIII digests, which gives the largest sum of fragment size, and to digests done with *Taq* I, the enzyme used to generate the regional probes (Fig. 3). Probe 1, which is 480 base pairs (bp) long and corresponds to the 5′ quarter of the cDNA insert, hybridizes to four *Hin*dIII fragments. Probe 2 (400 bp long), which covers the center of the coding region, reveals three *Hin*dIII fragments. Probe 3 (1,200 bp long), which covers the 3′ end of the protein coding region and contains an additional 800 bp from the 3′ untranslated region, detects only the largest 14-kb fragment (Fig. 3*A*). Each pair of contiguous probes recognizes only one fragment in common: the 5.0-kb fragment is detected with both probes 1 and 2; the 14-kb fragment gives a faint signal with probe 2 and a strong signal with probe 3. Because the 5′ end of the cDNA (probe 1) gives the most complex hybridization pattern (revealing fragments with a total size of 20 kb), we then used as probes smaller sequences from this region (see Fig. 1). One

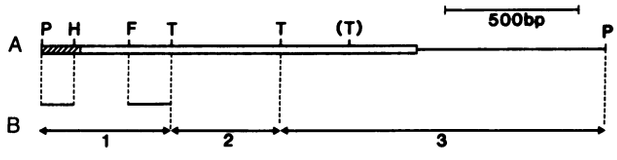

FIG. 1. Map of the factor IX cDNA clone. (*A*) Location of the protein coding sequence (thick line) and of 3′ untranslated sequence (thin line) in clone pTG397. The region coding for the signal peptide is represented by the hatched line, and the region corresponding to the mature protein is represented by the open line. Restriction sites used for the preparation of regional probes are indicated (P, H, F, and T represent *Pst* I, *Hae* III, *Fok* I, and *Taq* I, respectively). The *Taq* I site in parentheses exists in the sequence but cannot be cut in plasmid because it is contiguous with a *Sau*3A site and is thus methylated. (*B*) Location of regional probes used in Southern blotting experiments.

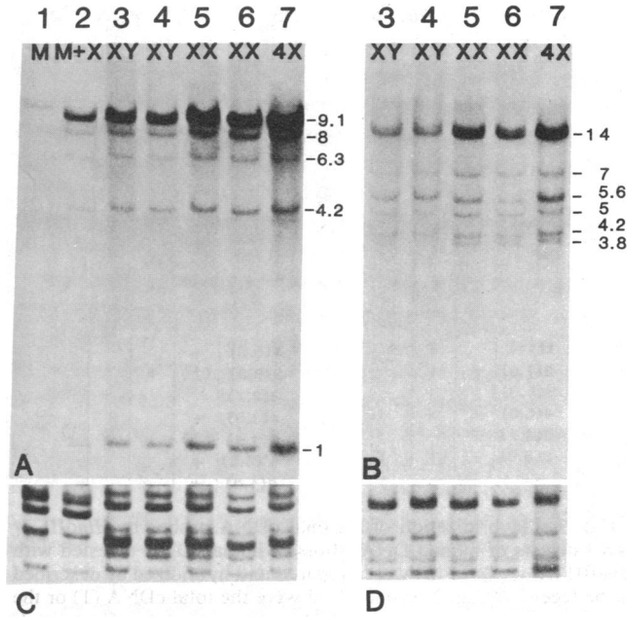

FIG. 2. Gene-dosage analysis of DNA fragments hybridizing to factor IX cDNA. DNA samples (15 μg) were digested with *Pvu* II (*A* and *C*) or *Hin*dIII (*B* and *D*), electrophoresed on a 0.9% agarose gel, and blotted onto DBM-paper (12, 13). The filters were hybridized to the nick-translated insert of the factor IX cDNA clone pTG397 (*A* and *B*). Control hybridizations of the same blots to a human skeletal α-actin cDNA probe (19) are shown in *C* and *D* (only part of the autoradiogram is shown). Blots were washed at 60°C in 0.5× NaCl/Cit (factor IX probe) or 2× NaCl/Cit (actin probe) in the presence of 0.1% $NaDodSO_4$. Sizes (in kb) of the human DNA fragments are indicated to the right of *A* and *B*. Source of DNA: Lane 1, mouse (M) A9 cells; lane 2, hybrid line A9-HRBC2 containing the human X chromosome in addition to the mouse genome (M+X); lanes 3 and 4, unrelated males (XY); lanes 5 and 6, unrelated females (XX); lane 7, fibroblast line from a 48 XXXX female.

such probe (*Pst* I/*Hae* III, nucleotides 1–129) corresponding to the signal peptide sequence detected both the 7- and the 4.2-kb *Hin*dIII fragments detected by probe 1. A *Fok* I/*Taq* I probe (nucleotides 327–483) detected the 5-kb fragment, which also hybridizes to the contiguous probe 2 (data not shown). With *Taq* I-digested DNA, probe 1 reveals either three or four fragments in DNA from different individuals (see below and Fig. 5). Probe 2 recognizes two fragments and probe 3 detects a single 2.7-kb fragment (Fig. 3*B*).

The sum of the observations that (*i*) probe 3 detects a single genomic fragment in both *Hin*dIII and *Taq* I digests; (*ii*) in the other parts of the gene, smaller probes recognize at most one or two fragments; and (*iii*) single *Hin*dIII fragments are found that correspond to the junction regions between probes 1 and 2, and 2 and 3 suggests that the complex hybridization patterns shown in Fig. 2 correspond to a single mosaic gene. The 5′ region appears to extend over 10–15 kb and probably contains small exons separated by very large introns. The apparent discrepancy between the sum of sizes of hybridizing *Hin*dIII fragments and that of fragments derived

Table 1. Sizes of restriction fragments in human DNA that hybridize to factor IX cDNA probe

Enzyme	Size of DNA fragments, kb	Total size, kb
*Bam*HI	25–30	25–30
*Hin*dIII	14, 7, 5.6, 5, 4.2, 3.8	39.6
Msp I	5.4, 4.6, 2.7, 2.5, 1.4, 1.2	17.8
Pvu II	9.1, 8, 6.3, 4.2, 1	28.6
Taq I	5, 3.6, 2.7, 1.8, 1.5, 1	15.6
Xba I	5.1, 4.5, 2.3, 1.8, 1.4, 1.2	16.3

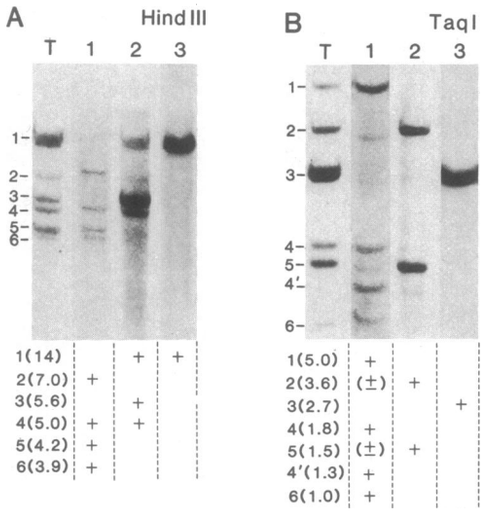

FIG. 3. Hybridization of regional cDNA probes to *Hin*dIII or *Taq* I digests of human DNA. Blots of human DNA digested with *Hin*dIII (*A*) or *Taq* I (*B*) were prepared and hybridized as described in the legend to Fig. 2. Probes used were the total cDNA (T) or the regional probes (see Fig. 1*B*), which correspond to the 5′ (probe 1) center of the protein coding region (probe 2) and to the rest of the cDNA insert including the 3′ untranslated region (probe 3). The interpretation (size of fragments in parenthesis and detection by the probes) is indicated below each lane. Fragment 4′ in *Taq* I digest (lane 1) corresponds to an alternative allele for fragment 4. A slight contamination of probe 1 by probe 2 sequences resulted in faint signals with the 3.6- and 1.5-kb *Taq* I fragments (lane 1, noted ±).

from digestion with other enzymes can be explained, at least in part, because the largest 14-kb *Hin*dIII fragment detected by probe 3 corresponds to a smaller hybridizing region of ≈2.7 kb revealed in the *Taq* I digest. Thus, most of this 14-kb fragment probably corresponds to a region downstream from the cloned cDNA sequences.

Regional Mapping of Factor IX Gene on the X Chromosome. To locate the factor IX gene on the X chromosome, we have used a panel of mouse–human hybrid cell lines derived from human parental cells carrying X-autosome translocations with various breakpoints on the X chromosome. The hybrids were selected in HAT medium to retain the translocation chromosome containing the human X-linked *HPRT* gene. The DNAs from the hybrid cells were digested with either *Eco*RI or *Hin*dIII, transferred onto DBM-paper, and hybridized to the complete cDNA probe. As shown in Fig. 4, the probe hybridized with all hybrid-cell DNAs giving the characteristic human fragment pattern with *Eco*RI. The same result was obtained with *Hin*dIII, where at least five of the six human factor IX fragments segregate together in the cell hybrids (the 5.6-kb human fragment comigrates with a mouse-specific fragment). When the same blots were hybridized with several other X-specific probes, different segregation patterns were observed without any discordance, thus establishing the validity of the panel (unpublished observations). In additional experiments (data not shown) we found that the human fragments were present in a hybrid containing the q13–qter region of the X chromosome but were absent from an azaguanine back-selected clone containing X pter–q13 (clones H and S; see ref. 21). From these results we conclude that the human factor IX gene is located between the breakpoint of the GM97 translocation at q26 and the breakpoint of the GM194 translocation within the q28 band (17).

It is interesting to note that, using this panel, we have found that mouse-specific fragments corresponding to both mouse HPRT and human factor IX probes are missing in the A9-HRBC2 cell line. As shown in Fig. 4*C* for *Eco*RI digestions, the HPRT probe detects at least four mouse-specific fragments in the control mouse cell line but only one in HRBC2 (the 3.5-kb fragment, noted M*, which might be autosomal). All four fragments are present in the other human–mouse hybrids. With the factor IX probe, the mouse-specific crossreacting band is detected in all mouse or mouse–human cell lines but not in A9-HRBC2. These results suggest that a part of the mouse X chromosome that contains both the genes for HPRT and factor IX is completely missing from the A9-HRBC2 cell line. Because the human X chromosome cannot be eliminated from A9-HRBC2 by an azaguanine back-selection (22), our observations support the interpretation that the human X chromosome is complementing vital functions normally carried out by the missing part of the homologous mouse chromosome in the A9-HRBC2 cell line.

Search for Restriction Fragment Length Polymorphism. From the preceding results, it appears that the factor IX cDNA probe detects a single gene. It is thus possible to use it to search for genetic markers linked to the gene. We have looked for polymorphisms in the factor IX gene using seven restriction enzymes (*Bam*HI, *Hin*dIII, *Msp* I, *Taq* I, *Pvu* II, *Bst*NI, and *Xba* I) to digest DNA from 10–15 individuals (mainly females). We have found one polymorphism with the

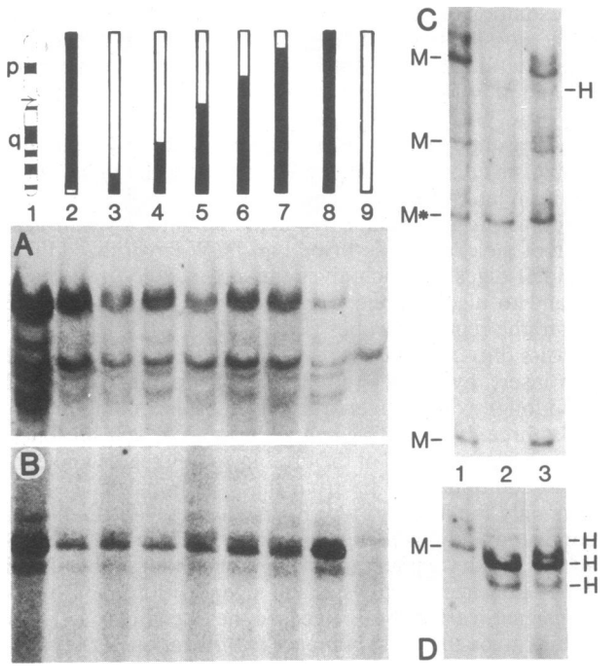

FIG. 4. (*A* and *B*) Regional localization of factor IX gene on the X chromosome. DNA was digested with *Hin*dIII (*A*) or *Eco*RI (*B*) and analyzed as described in the legend to Fig. 2. Source of DNA: lane 1, normal human 46 XX DNA; lanes 2–8, human–mouse hybrid cell lines [the portion of human X chromosome present in each cell line is also schematized above the corresponding lane (solid line)]: GM194-RAG7 (containing human X pter–q28 including the *G6PD* gene; ref. 17), GM97-RAG8-13 (X q26–qter), GM89-A9 (X q22–qter), Anly RAG1 (X q12–qter), GO RAG4 (p11–qter), PI RAG7-2 (X p22–qter), and HRBC2-A9 (X pter–qter), respectively; lane 9, mouse L cell (Cl1D). (*C* and *D*) Deletion of mouse HPRT and factor IX-related sequences in the HRBC2 human–mouse hybrid cell line. DNA was digested with *Eco*RI, treated as described in the legend to Fig. 2, and hybridized either to mouse HPRT cDNA clone pHPT2 (20) (*C*) or to human factor IX cDNA clone pTG397 (*D*). Source of DNA: lane 1, mouse L cells (Cl1D); lane 2, A9-HRBC2; lane 3, GM89-A9 hybrid cell line. M, mouse-specific fragment; M*, mouse-specific fragment that is not lost in the A9-HRBC2 hybrid cell line; H, human-specific fragment. The human HPRT fragment is barely seen with the heterologous probe under the hybridization conditions used.

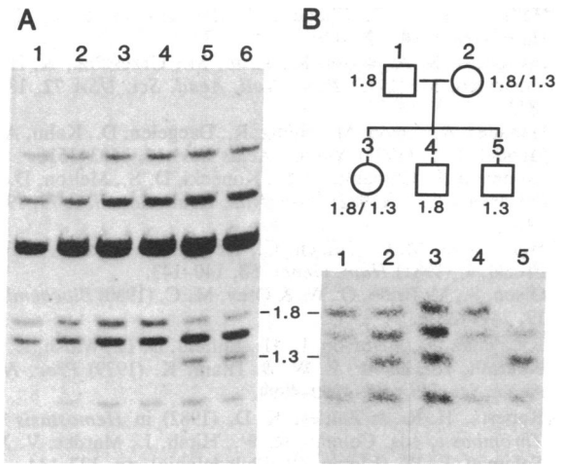

FIG. 5. *Taq* I polymorphism detected by the factor IX cDNA probe. DNA was digested with *Taq* I and analyzed as described in legend to Fig. 2. Source of DNA: (*A*) lanes 1 and 2, unrelated normal males; lanes 3–6, unrelated normal females. (*B*) Inheritance of *Taq* I polymorphism in an informative family (only part of the autoradiogram is shown).

enzyme *Taq* I. As shown in Fig. 5*A*, an additional 1.3-kb *Taq* I fragment is present in DNA from two unrelated women, and the intensity of a 1.8-kb fragment is decreased compared to that of neighboring samples. That this represents a *bona fide* genetic variation with mendelian sex-linked inheritance was proved by the fact that males carry only one or the other of the 1.8- and 1.3-kb *Taq* I fragments, but never both, and by analysis of the segregation pattern in an informative family (Fig. 5*B*). Analysis of DNA samples from 49 unrelated individuals (25 males and 24 females from various regions in France) gave a frequency of 0.29 for the 1.3-kb allele. Despite the rather large number of fragments detected with other restriction enzymes (except *Bam*HI) we have thus far not observed further evidence of restriction fragment length polymorphism.

DISCUSSION

Factor IX is a plasma protein that shows significant homology with other vitamin K-dependent plasma proteins [prothrombin, factor X, protein C (23)]. This homology is found throughout the entire protein sequence but is especially strong (60–70%) in a region 50 amino acids long at the NH_2 terminus of the mature protein (the so-called GLA region). In contrast to the sex-linked pattern of inheritance of hemophilia B, deficiencies in prothrombin and factor X are inherited as autosomal recessive diseases (24). The factor IX gene is thus a member of a dispersed multigene family. It was therefore important to determine whether factor IX cDNA probes hybridize to a single gene on the X chromosome or whether they also detect related sequences on autosomes or on the X chromosome. Gene-dosage experiments show that all the fragments detected under hybridization conditions of medium stringency are X-linked. This is in contrast to the results obtained with cDNA probes corresponding to two other X chromosome-encoded enzymes, HPRT (25) and phosphoglycerate kinase (26), where under similar conditions autosomal DNA fragments are also detected. Analysis of human DNA digested with several different restriction enzymes shows that the sum of fragment sizes hybridizing to the 2.1-kb factor IX cDNA probe is very large, ranging from 15.4 kb in *Msp* I digests to 38 kb in *Hin*dIII digests. We have used regional probes in an attempt to determine whether the large multiplicity of the fragments detected was due to the presence of more than one X-linked gene. Our data suggest that there is a single gene with large introns, especially in the 5′ region.

The regional localization on the X chromosome of factor IX gene was previously unknown, and family studies had failed to show genetic linkage to either the color blindness or the Xg blood group loci (6, 7). Using a panel of mouse–human hybrid cell lines containing various portions of the human X chromosome, we have shown that all of the fragments detected by the factor IX cDNA probe segregate together and can be mapped to the long arm of the X chromosome in the q26–q28 region. Localization of the factor IX gene within the q28 band can be excluded, however, taking into account both the results with the GM194 hybrid and previous family studies that showed a high proportion of recombination between the hemophilia B locus and the color blindness (6) and hemophilia A (27) loci, both of which are tightly linked to the *G6PD* gene at X q28. On the basis of our data, it should be interesting to search for linkage between the factor IX gene and the *HPRT* (Lesch–Nyhan) locus that maps in the same region of the X chromosome (5, 16). The recent cloning of a HPRT cDNA and the demonstration that both HPRT (25) and factor IX probes can detect restriction fragment polymorphisms should render possible such a study. Our results showing combined deletion of mouse sequences homologous to human factor IX probe and to a mouse HPRT probe in the hybrid line HRBC2 might also indicate that the two genes are located close to each other on the mouse X chromosome. Because it has been shown previously that the factor IX gene is also on the X chromosome in the dog (28), it would appear that this locus follows the rule of conservation of X-linkage in mammals (29).

The availability of a probe for factor IX gene should also be of use for genetic screening in families at risk for hemophilia B. Present immunological and functional assays for factor IX allow detection of carriers and prenatal diagnosis (after fetal blood sampling), but the methods are complex and may not be applicable to all cases (30). Thus, simpler methods based on analysis at the DNA level are desirable. The knowledge of the normal restriction pattern corresponding to the factor IX gene should be of use to detect eventual deletions or rearrangements in hemophilia B patients lacking factor IX antigen. More generally, the presence of restriction fragment length polymorphism within or in close proximity to the gene should allow prenatal diagnosis and carrier detection by linkage analysis. This would be particularly favorable in this case because linkage analysis is simpler for X-linked traits than for autosomal recessive disorders. Our finding of a frequent *Taq* I polymorphism in the factor IX gene is a first step in this direction, and it makes genetic screening for hemophilia B already feasible in informative families. This polymorphism also provides a useful genetic marker for the establishment of a linkage map of the human X chromosome.

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1. Hedner, U. & Davie, E. W. (1982) in *Hemostasis and Thrombosis*, eds. Colman, R. W., Hirsh, J., Marder, V. J. & Salzman, E. W. (Lippincott, Philadelphia), pp. 29–38.

2. Levine, P. H. (1982) in *Hemostasis and Thrombosis*, eds. Colman, R. W., Hirsh, J., Marder, V. J. & Salzman, E. W. (Lippincott, Philadelphia), pp. 75–90.
3. Boyer, S. H. & Graham, J. B. (1965) *Am. J. Hum. Genet.* **17,** 320–334.
4. Tsevrenis, H., Mandalaki, T., Volkers, W. S. & Meera-Khan, P. (1979) *Cytogenet. Cell Genet.* **25,** 213 (abstr.).
5. Pai, G. S., Sprenkle, J. A., Do, T. T., Mareni, C. E. & Migeon, B. R. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2810–2813.
6. Whittaker, D. L., Copeland, D. L. & Graham, J. B. (1962) *Am. J. Hum. Genet.* **14,** 149–158.
7. Race, R. R. & Sanger, R. (1975) *Blood Groups in Man* (Blackwell, Oxford), pp. 594–609.
8. Choo, K. H., Gould, K. G., Rees, D. J. G. & Brownlee, G. G. (1982) *Nature (London)* **299,** 178–180.
9. Kurachi, K. & Davie, E. W. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6461–6464.
10. Jaye, M., De La Salle, H., Schamber, F., Balland, A., Kohli, V., Findeli, A., Tolstoshev, P. & Lecocq, J. P. (1983) *Nucleic Acids Res.* **8,** 2325–2335.
11. Botstein, D., White, R. L., Scolnick, M. & Davis, R. W. (1980) *Am. J. Hum. Genet.* **32,** 314–331.
12. Alwine, J. C., Kemp, D. J. & Stark, G. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5350–5354.
13. Bellard, M., Kuo, M. T., Dretzen, G. & Chambon, P. (1980) *Nucleic Acids Res.* **8,** 2737–2751.
14. Grzeschik, K. H. (1976) *Somatic Cell Genet.* **2,** 401–410.
15. Hellkuhl, B., Mayr, W. R. & Grzeschik, K. H. (1978) *Cytogenet. Cell Genet.* **22,** 503–505.
16. Seravalli, E., DeBona, P., Velivasakis, M., Pagan-Charry, I., Hershberg, A. & Siniscalco, M. (1976) in *Human Gene Mapping 3, Birth Defects*, Original Article Series The National Foundation, ed. Bergsma, D. (Karger, New York), Vol. 12, pp. 219–222.
17. Hellkuhl, B., De La Chapelle, A. & Grzeschik, K. H. (1982) *Hum. Genet.* **60,** 126–129.
18. DiCioccio, R. A., Voss, R., Krim, M., Grzeschik, K. H. & Siniscalco, M. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 1868–1872.
19. Hanauer, A., Levin, M., Heilig, R., Daegelen, D., Kahn, A. & Mandel, J. L. (1983) *Nucleic Acids Res.* **11,** 3503–3516.
20. Brennand, J., Chinault, A. C., Konecki, D. S., Melton, D. W. & Caskey, C. T. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 1950–1954.
21. Hors-Cayla, M. C., Junien, C., Heuertz, S., Mattei, J. F. & Frezal, J. (1981) *Hum. Genet.* **58,** 140–143.
22. Olsen, S., McBride, O. W. & Otey, M. C. (1980) *Biochemistry* **19,** 2419–2428.
23. Katayama, K., Ericsson, L. H., Enfield, D. L., Walsh, K. A., Neurath, H., Davie, E. W. & Titani, K. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4990–4994.
24. Roberts, H. R. & Zeitler, K. D. (1982) in *Hemostasis and Thrombosis*, eds. Colman, R. W., Hirsh, J., Marder, V. J. & Salzman, E. W. (Lippincott, Philadelphia), pp. 127–144.
25. Nussbaum, R. L., Crowder, W. E., Nyhan, W. L. & Caskey, C. T. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4035–4039.
26. Singer-Sam, J., Simmer, R. L., Keith, D. H., Shively, L., Teplitz, M., Itakura, K., Gartler, S. M. & Riggs, A. D. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 802–806.
27. Robertson, J. H. & Trueman, R. G. (1964) *Blood* **24,** 281–288.
28. Brinkhous, K. M., Davis, P. D., Graham, J. B. & Dodds, W. J. (1973) *Blood* **41,** 577–585.
29. Ohno, S. (1967) *Sex Chromosomes and Sex Linked Genes* (Springer, New York).
30. Mibashan, R. S., Rodeck, C. H. & Thumpson, J. K. (1982) in *The Hemophilias*, ed. Bloom, A. L. (Churchill Livingstone, New York), pp. 176–196.