Detection of Hemophilia A Carriers
Using Intragenic Factor VIII:C DNA Polymorphisms


A DNA polymorphism for an XbaI site in intron 22 of the human factor VIII:C gene extends the utility of DNA methods for carrier detection in families segregating for hemophilia A. While the DNA polymorphism detected by a BclI site in intron 18 of the factor VIII:C gene was informative for 41% of females studied, the BglII/intron 25 polymorphism provided no additional information because of apparent linkage disequilibrium. In contrast, the XbaI intron 22 polymorphism was useful in 53% of women who were uninformative (homozygous) for either the BclI or BglII polymorphisms. Using the BclI/intron 18 and XbaI/intron 22 intragenic polymorphisms, we could provide highly accurate information for 68% of women we studied who were at risk for carriership. The carrier status of the remaining 32% could be determined utilizing the closely linked TaqI/St14 DNA polymorphism.

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Hemophilia A, an X-linked disorder producing absence or dysfunction of the factor VIII:C molecule, is the most common severe congenital coagulopathy. Despite advances in home infusion therapy, hemophilia A remains a potentially crippling and life-threatening condition. Therapy-induced complications include chronic hepatitis, inhibitor formation, and infection with the human immunodeficiency virus (HIV). Accordingly, many women at risk for being hemophilia carriers seek genetic counseling. Carrier detection (phenotype assignment) by statistical analysis of factor levels and pedigree probabilities is limited in its applicability to certain cases and includes significant risk for error, since misclassification rates vary from 5% to 15%. The discovery of restriction fragment length polymorphisms (RFLPs) within and closely linked to the factor VIII:C gene have recently provided markers for linkage analysis to determine the inheritance of normal or mutant (hemophilia) factor VIII:C genes. Use of these RFLPs enables carrier detection and prenatal diagnosis to be accomplished more accurately by actual genotype rather than phenotype assessment. Once the linkage phase for intragenic RFLPs is established, carrier assignment is basically nonprobabilistic if the consultand is heterozygous for the RFLP. Unfortunately, a single intragenic DNA polymorphism with two (+ and −) alleles can be informative for no more than 50% of women as predicted by the Hardy-Weinberg law. Additional intragenic RFLPs, if informative, may serve to extend this highly accurate approach to more women at risk for transmitting the hemophilia gene.

We have compared the utility of three intragenic RFLPs (BclI/intron 18, XbaI/intron 22, and BglII/intron 25) with a highly polymorphic, closely linked TaqI/St14 RFLP. Our results suggest a strategy for providing accurate genotype assignment for virtually all female relatives of hemophiliacs in an economical, efficient way.

Materials and Methods

Study group. Informed consent was obtained from normal volunteers and obligate carriers in accordance with policies of the Vanderbilt Committee for the Protection of Human Subjects. Leukocyte DNA samples from women at risk for transmitting the hemophilia A gene were obtained and the status of the closely-linked TaqI/St14 and intragenic BclI/intron 18 RFLPs were determined for 106 and 93 women, respectively. Forty-three women from 29 families had additional analyses performed for the BglII/intron 25 and XbaI/intron 22 RFLPs. Of these 43, 16 were relatives of nine sporadic cases. Samples needed to establish the linkage phase were obtained from affected males and other family members.

Isolation of DNA and Southern transfer. Whole venous blood (12 to 15 mL) was drawn using EDTA as an anticoagulant. Leukocyte DNA was isolated as previously described. DNA was digested with various restriction endonucleases according to the manufacturer’s instructions and subjected to electrophoresis for 16 hours at 25 volts in 1% agarose slab gels. DNA fragments were transferred to filters (Gene Screen Plus membranes, New England Nuclear, Boston MA) by Southern blotting.

DNA probes. Probes were radiolabeled with [32P] dATP and [35P] DCTP. Hybridization and autoradiography were carried out as previously described. The DNA sequences used in these studies were genomic fragments in exon 17-18, intron 22, exon 26, and St14 (anonymous, closely linked). Exon 26 analyses were kindly done by Dr Stylianos Antonarakis (Johns Hopkins University School of Medicine, Baltimore, MD) and the St14 clone was a gift from Prof J.L. Mandel, Strasbourg, France. The DNA probes for exon 17-18 and intron 22 are available through the NIH Human Repository, ATCC, Rockville, MD.

Results and Discussion

Frequency of informative RFLPs. A woman was considered to be informative if she was heterozygous for an RFLP and the linkage phase of the DNA polymorphism and the normal or abnormal factor VIII:C allele could be established. The extragenic TaqI/St14 RFLP, which is closely linked, was informative in 103/106 (97%) of women studied.

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In contrast, the BcI1/intron 18 RFLP was informative for only 38/93 (41%) of women. This latter result is in close agreement with the prediction of Gitschier et al1 of 42% heterozygosity from estimated allelic frequencies of 71% and 29% (2pq = 2 x 0.71 x 0.29 = 0.41).

The BgiI/intron 25 RFLP was informative for 14/44 women studied (32%). This agrees with prior estimates of heterozygosity that ranged from 11% to 38%.12

The XbaI polymorphic site in intron 22 was informative for 25/43 women (58%). The proportion of observed heterozygosity was slightly higher than the 48% expected from calculations based on previous reported allelic frequencies of 59% and 41% (2pq = 2 x 0.59 x 0.41 = 0.48).13

\[X^2 = \frac{(O - E)^2}{E}\]

In our experience, the intron 25 RFLP seems to have limited utility when compared to the intron 18 RFLP.

\[X^2 = \frac{(O - E)^2}{E}\]

In contrast to the BciI/intron 18 RFLP, the XbaI/intron 22 RFLP was often informative in those women who were homozygous for the BciI/intron 18 RFLP (Fig 1). Among 38 women uninformative for the BciI/intron 18 RFLP, 20 (53%) were informative with XbaI/intron 22. Similarly, among 35 women uninformative with BgiI/intron 25, 19 (54%) were informative with the XbaI/intron 22. These data suggest that the XbaI/intron 22 RFLP may be informative in about 1/2 of those females who are homozygous for the other two intragenic RFLPs. Assuming complete linkage equilibrium, analysis of the BciI/intron 18 and XbaI/intron 22 RFLPs should be informative for about 72% of the females (41% + 0.53 x 59% - 72%); however, results obtained in earlier studies demonstrated that these polymorphisms are not in correct equilibrium.15 In our study, this combination was informative for 25/39 (64%) women for whom we had complete data, suggesting that analysis of these two RFLPs could enable highly accurate carrier detection for about 2/3 of female relatives of males with hemophilia A. Moreover, our results suggest the optimal strategy for applying DNA techniques to both carrier detection and prenatal diagnosis of hemophilia A is to utilize analysis of the intron 18 and intron 22 RFLPs.

Uninformative with intragenic RFLPs. Of the 14 of 39 women who were uninformative with any of the three intragenic RFLPs studied, all were informative with the TaqI/Stl4 RFLP. Although there is a significant (3.85%) risk of recombination between this RFLP and the factor VIII:C gene (I. Peake, personal communication, January 1987), results obtained can be used to establish carriership with diagnostic accuracy that likely exceeds that obtained through conventional factor analysis.36
Detection of the carrier state of classic hemophilia using an enzyme-linked immunosorbent assay (ELISA). Blood 59:1163, 1982


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