HAEMOPHILIA

CARRIER DETECTION AND PRENATAL DIAGNOSIS IN HAEMOPHILIA

A.L. Bloom. Department of Haematology, University of Wales

Carrier detection has been used to perform family studies, including prenatal diagnosis, in 21 haemophilia A kindreds. Two intragenic RFLPs were studied in conjunction with one linked RFLP. The intragenic Bell RFLP, situation 3' to exon 26 was detected with cDNA probe C (Genetics Institute) giving bands of 29kb (7% of X chromosome) and 3kb (83%), and the intragenic Bell RFLP, situated 3' to exon 18, was detected with the genomic DNA probe pl1.12 from Genentech. The frequency of this RFLP in the local population was 73% (1-kb allele) and 27% (0.8-kb allele). The linked probe DXN15 (DX13) was used to detect a BglII RFLP with alleles of 3.8kb (6%) and 2.8kb (93%). A recombination rate of approximately 3% has been estimated between the factor VIII and DXN15 loci.

Carriers were performed in 13 kindreds. 25 obligate carriers were identified and of these, 20 were potentially informative (heterozygous and phase known) for at least 1 RFLP (for BglI, 9 for BclI and 7 for BgIII). 34 possible carriers were studied, of which 13 were diagnosed as normal (6 by BglI, 6 by BclI and 1 by BgIII). 17 were diagnosed as carriers (2 by BglI, 15 by BclI and 10 by BgIII). BglI and diagnosis was not possible in a further 4 cases. Of these diagnosed as carriers 3 were non-informative for all RFLPs and 14 informative for at least one RFLP. BglI. 8 by BclI and 3 with BgIII.

Prenatal diagnosis was attempted by analysis of DNA extracted by chorionic villus sampling in 6 cases of male fetuses at risk of having haemophilia A. Three patients opted for mid-trimester fetoscopy and measurement of the normal fetus was diagnosed by the BglII RFLP, but the two intragenic RFLPs were non-informative for analysis of the material. In 4 out of 5 fetuses, all three patients opted for mid-trimester fetoscopy and measurement of the normal fetus was diagnosed by the BglII RFLP analysis, but a spontaneous abortion at 12 weeks prevented confirmation of this result. In the final case of two male fetuses, none of the RFLPs was informative and both were diagnosed as normal by fetal blood sampling at fetoscopy.

CARRIER DETECTION IN JAPANESE FAMILIES WITH HAEMOPHILIA A USING FACTOR VIII GENE PROBE(S) AND ST 14-1 PROBE.


Recently, the gene structure for human F.VIII protein was clarified, and F.VIII DNA probes have been used for carrier detection and prenatal diagnosis of haemophilia A. In order to make sure that the phenomena are universal, we have analyzed the RFLPs of F.VIII gene in 16 Japanese families with haemophilia A, including a female haemophiliac case, using an intragenic F.VIII DNA probe(FSA) and an extragenic linked DNA probe(D5S13). Of 85% bore the 879-bp fragment and 15% the 1165-bp fragment. Of these diagnosed as carriers, 2 were by BglI, 10 by BclI and 7 for BgIII. 17 by BglII.

From these data, it was concluded that the Bcl I polymorphism of F.VIII gene and the Tag I polymorphism of 14 loci were informative for carrier detection in 8 out of 16 families with haemophilia A.

CARRIER DETECTION OF HEMOPHILIA A BY DNA ANALYSIS IN AFFECTED JAPANESE FAMILIES.

N. Suzuki(1), A. Iizuka(1), T. Nagao(1), T. Nakatani(2), Y. Nakahori(2), M. Yasuda(2), and Y. Nakagome(2). Dept. of Hematology, Kangawa Children’s Medical Center, Yokohama, Japan(1) and Div. of Congenital Abnormality Research, National Children’s Medical Research Center, Tokyo, Japan(2).

Several DNA probes have been isolated to detect Factor VIII gene and a DNA segment which locates very close to the gene. They have been successfully used to detect carriers and patients of hemophilia A. We analyzed DNA samples of Japanese population to see whether these probes are also useful for carrier detection of hemophilia A in affected Japanese families, since the size and frequency of allelic fragments detected by a DNA probe are sometimes different in various ethnic groups. A probe of BclI (DSS52) is thought to be one of the best probes for such analysis in Caucasian population because it detects very polymorphic DNA fragments containing a minisatellite. When Tag I digests of Japanese DNA samples were hybridized with StI, several DNA fragments with a range from 1.7 kb to 5.5 kb were detected, where at least 6 fragments were polymorphic. A notable difference between Japanese and Caucasian was that a band of 5.5 kb was variable in Japanese while it was constant in Caucasians. We have so far detected 18 alleles, and about 60% of Japanese women were heterozygous. Using these informations about Japanese population, we can detect carriers in several families. Other RFLPs data are necessary to increase information content. Similar studies are in progress using different probes i.e. an extragenic probe; DX13/BglII, and two intragenic probes; exon 14-26/BclI and exon 14-26/BglII. We thank Mandel J.L., Strasbourg, Davies K., Oxford and Genetics Institute, Cambridge for probes.

THE RELATIVE EFFICACY OF GENETIC ANALYSIS AND COAGULATION TESTING IN THE DIAGNOSIS OF CARRIERS OF HEMOPHILIA A.

D. Lilllicrap(1), A.R. Giles(1), J.J.A. Bolden(2), B.N. White(2), and the Ontario Hemophilia Study Group. Department of Pathology, Queen’s University, Kingston, Ontario, Canada(1). Department of Biology, Queen’s University, Kingston, Ontario, Canada(2).

This study has assessed the relative benefits of restriction fragment length polymorphism (RFLP) linkage and coagulation testing in the diagnosis of carriers of hemophilia A. 221 samples from 55 families have been studied for intragenic and flanking RFLPs. All samples were tested for the Factor VIII intragenic BclI RFLP and for the flanking marker StI14. 83% of obligate carrier females were heterozygous at one or both of these two polymorphic sites. However, only 38% of these women were heterozygous at the intragenic site and might safely be offered prenatal diagnosis using this marker for the hemophilia mutation. Carrier diagnosis was obtained in 52% of 81 potential carriers tested. Diagnosis was based on intragenic RFLP information in only 48% of these cases. Genetic diagnosis was possible in 27% at risk women from families with no prior history of hemophilia. Four of these women were diagnosed as carriers on the basis of a gross Factor VIII gene deletion and the remaining 23 women were identified as carriers using genetic analysis. In 31 potential carriers we have sequenced Factor VIII/C (one stage assay) and WfA4 (Laurell and ELISA) and derived probabilities for carrier status. In 3 women these were conflicting, but in 28 the data were consistent. Meanwhile, in 12 undiagnosed women from sporadic families, carrier diagnostic probabilities of > 0.9 were obtained. These studies indicate that genetic analysis for hemophilia A requires more intragenic and closely linked RFLPs and the continuance of coagulation testing to assist women from sporadic families.