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CARRIER DETECTION AND PRENATAL DIAGNOSIS IN HAEMOPHILIA A BY GENE ANALYSIS. P. Moodie, I.R. Peake, M.B. Liddell and A.L. Bloom. Department of Haematology, University of Wales College of Medicine, Cardiff, U.K.

Restriction fragment length polymorphism (RFLP) analysis has been used to perform family studies, including prenatal diagnosis, in 21 haemophilia A kindred. Two intragenomic RFLPs were studied in conjunction with one linked RFLP. The intragenomic BglI RFLP, situation 3' to exon 26 was detected with cDNA probe C (Genetics Institute) giving bands of 20kb (17% of X chromosomes) and 5kb (83%), and the intragenomic BclI RFLP, situated 3' to exon 18, was detected with the genomic DNA probe p114.12 from Genentech. The frequency of this RFLP in the local population was 23% (1.1kb allele) and 77% (0.88kb allele). The linked probe DXS15 (DX13) was used to detect a BglII RFLP with alleles of 5.8kb (45%) and 2.8kkb (55%). A recombination rate of approximately 5% has been estimated between the factor VIII and DXS15 loci
Carrier studies were performed in 15 kindreds. 25 obligate carriers were identified and of these, 20 were potentially informative (heterozygous and phase known) for at least 1 RFLP (9 for BglI, 9 for BclI and 7 for BglII). 34 possible carriers were studied, of which 13 were diagnosed as normal (6 by BglI, 6 by BclI and 5 by BglII). 17 were diagnosed as carriers (2 by BglI, 12 by BclI and 10 by BglII) 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 (3 by BglI, 8 by BclI and 8 with BglII). 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. 1 fetus was diagnosed as being affected (BclI) and was electively terminated. Three other fetuses were diagnosed as normal by the BglII/DXS15 RFLP, but the two intragenomic RFLPs were non-informative. Because of the possibility of a crossover all three patients opted for mid-trimester fetoscopy and measurement of fetal factor VIII at Kings College Hospital, London (Dr Reuben Mibashan), where the diagnoses were confirmed. In the 4th case a normal fetus was diagnosed by the BglI RFLP analysis, but a spontaneous abortion at 12 weeks prevented confirmation of this result. In the final case of twin male fetuses, none of the RFLPs was informative and both were diagnosed as normal by fetal blood sampling at fetoscopy.

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CARRIER DETECTION OF HEMOPHILIA A BY DNA ANALYSIS IN AFFECTED JAPANESE FAMILIES. N. Suzuki(1), A. Iizuka(1), T. Nagao(1), Y. Nakahori(2), M. Yamada(2), and Y. Nakagome(2). Dept. of Hematology, Kanagawa Children's Medical Center, Yokohama, Japan(1) and Dept. 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 St14 (DXS52) 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 Taq I digests of Japanese DNA samples were hybridized with St14, 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 Caucasian. We have so far detected 10 alleles, and about 60% of Japanese women were heterozygous. Using these informations about Japanese population, we could 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/Bgl II, and two intragenic probes ; exon 14-26/Bcl I and exon 26/Bgl I. We thank Mandel J.L., Strasbourg, Davies K., Oxford and Genetics Institute, Cambridge for probes.

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CARRIER DETECTION IN JAPANESE FAMILIES WITH HAEMOPHILIA A USING FACTOR VIII GENE PROBE(F8A) AND ST 14-1 PROBE.
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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 analysed 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(F8A) and an extragenic(linked) DNA probe(St14-1).

The probe F8A revealed two variant bands after digestion by Bcl I. Of normal 60 X chromosomes(females) examined, about 85% bore the 879-bp fragment and 15% the 1165-bp fragment. Five of sixteen mothers of hemophiliacs, definite carriers, were found to be heterozygous for Bcl I polymorphism. Since the relationship between Bcl I alleles and hemophilia gene has been identified in the 5 families in which the mothers were heterozygous, we could diagnose the carrier status of two women whose brothers are hemophiliacs. On the other hand, we could identify that one "haemophilic woman" with less than 10% of F.VIII:C was a carrier status when we analysed the Bcl I alleles in the other members of the family.

The probe DNA(ST 14-1) revealed seven variant bands ranging from 5.5 kb to 3.4 kb after digestion by Taq I. In 6 out of 16 families, the RFLPs of ST 14 locus were informative for carrier detection.

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

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THE RELATIVE EFFICACY OF GENETIC ANALYSIS AND COAGULATION TESTING IN THE DIAGNOSIS OF CARRIERS OF HEMOPHILIA A.
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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 St14. 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 non-carriers by the BclI (11) and St14 (12) RFLP data. 39 women remained undiagnosed after gene analysis studies. 23 of these women were female relatives of sporadic hemophiliacs and thus RFLP segregation analysis was inappropriate. A further 9 potential carriers were undiagnosed because of homozygosity in key individuals in their families. In 31 potential carriers we have quantitated Factor VIII:C (one stage assay) and vWf:Ag (Laurell and ELISA) and derived probabilities for carrier status. In 3 women there was conflicting genetic and coagulation data. Meanwhile, in 12 undiagnosed women from sporadic families, carrier diagnostic probabilities of > 0.9 were obtained. These studies indicate that optimal carrier detection for hemophilia A requires more intragenic and closely linked RFLPs and the continuance of coagulation testing to assist women from sporadic families.