DETECTION OF CARRIER STATUS OF HEMOPHILIA B USING DNA MARKERS

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Abstract. Hemophilia B is an X-linked recessive disorder of the hemostasis involving a defective clotting factor IX. Amplification of the regions containing restriction fragment length polymorphisms (RFLP) can be achieved by the use of polymerase chain reaction (PCR). This paper describes the analysis of 2 RFLPs involving the *Ddel* and *Taq1* restriction sites within the factor IX gene in a family with hemophilia B. Digestion of the PCR products with *Taq1* revealed a 163bp fragment in all the family members. This finding suggests the absence of restriction site for *Taq1* enzyme. However, the *Dde1* digest results in bands 369bp and 319bp seggregated amongst the family members. The pattern of inheritance of the 369bp fragment in this family suggested that both the patient's mother and aunt are not carriers and that the patient's factor IX gene could have undergone a *de novo* mutation producing a defective factor IX gene responsible for the hemophilia B. This is supported by the fact that no family history of hemophilia B is indicated in the other male members within the family.

INTRODUCTION

Hemophilia B is an X-linked, recessive disorder of hemostasis, due to a defect in coagulation factor IX (FIX). Detection of restriction length polymorphisms (RFLPs) by Southern blotting procedures has been employed for carrier status assessment and prenatal diagnosis Misbashan *et al.* We examined two RFLPs, namely *Taq1* poly-morphism resulting from a point mutation and a polymorphism that is detected by the enzyme *Dde1* which gives rise to two allelic forms that differ by a 50bp element of inserted DNA. The ability to amplify the regions containing the RFLP loci *Dde1* (Winship *et al*, 1984) and *Taq1* (Camerino *et al*, 1984; Giannelli *et al*, 1984) by PCR followed by digestion with appropriate restriction enzymes has enabled us to evaluate presence of DNA polymor-phisms in a hemophilia B family studied for the purpose of carrier detection.

MATERIALS AND METHODS

DNA from five members of hemophilia B family were phenol-chloroform extracted (Kunkel *et al*, 1977). All PCRs were performed in a 100 µl reaction volume containing 1 µg of genomic DNA, 1.25 mM each of dNTPs, 50 pmol each of primers specific for *Dde1* and *Taq1*, 10 µl of 10 × PCR reaction buffer (100 mM Tris HCl, p.H 8.3, 0.5 M KCl, 15 mM $MgCl_2$ and 0.01% gelatin). The above mixture was covered with 60 µl mineral oil to prevent evaporation. PCR was performed using 30 cycles of incubation at 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds. A total of 10 µl of PCR products were digested with the appropriate enzymes for 4 hours at 65°C. The digested material was then electrophorized on a 2.5% agarose gel and visualized under ultraviolet light following ethidium bromide staining.

RESULTS

The amplified Factor IX fragment after digestion with restriction enzyme *Taq1* resulted in only the 163bp fragment for all the family members investigated. This fragment, obtained when there is absence of an RFLP restriction site, is not informative as a DNA marker. However, unlike most RFLPs which result from the presence or absence of a specific restriction endonuclease recognition sequence, the *Dde1* RFLP results from the presence or absence of a 50 nucleotide fragment of DNA in intron 1 of the FIX gene. Analysis of the *Dde1* digest showed the presence of 369 and/or 319bp fragments segregated amongst the family members investigated. The inheritance pattern for the two RFLPs is given in Table 1.

Table 1

The PCR products and their associated digestion products for the two RFLP loci of the FIX gene.

Sample type	PCR product size (bp)	
	RFLP locus *Dde1*	RFLP locus *Taq1*
Grandfather	369	163
Grandmother	319/319	163/163
Mother	369/319	163/163
Auntie	369/319	163/163
Hemophilia B patient	369	163

DISCUSSION

The presence of differences between DNA of normal healthy people inherited as RFLPs is a useful marker for detection of carrier status amongst family members of sex-linked disorders such as hemophilia B.

Findings from the restriction analysis of PCR products after digestion with *Dde1* from five members of a hemophilia B family revealed that the 369bp fragment found in the patient is inherited from his mother. Since this fragment is passed down from his grandfather who does not have the disease, both his mother and aunt are therefore not carriers of hemophilia B. This would then suggest that the factor IX in the patient could have undergone a *de novo* mutation which resulted in the hemophilia B. Accurate diagnosis of carrier status for his mother and aunt could not have been made if the family/clinical history of his grandfather was not available. Digestion with *Taq1* enzyme however, did not provide added information as only a fragment of 163bp was found in all the family members studied.

In conclusion, in any molecular diagnosis it is essential that an appropriate pedigree analysis is available in order to arrive at a confirm and accurate diagnosis and a marker such as DNA restriction fragment length polymorphisms is only useful in carrier status detection if it is highly polymorphic and transmitted through a pedigree.

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