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CASE REPORT

Carrier analysis of a moderately affected haemophilia B family

N. HOLOSHITZ, K. KURACHI and S. KURACHI
Department of Human Genetics, the University of Michigan Medical School, Ann Arbor, Michigan, USA

Summary. Here we report the successful genetic diagnosis of a pregnant caucasian female patient whose family has a history of moderate haemophilia B. While restriction fragment length polymorphism (RFLP) analysis was not informative, nucleotide sequencing of the factor IX genes of the patient's family members determined that her mother and one of her two sisters were carriers of the mutation C31008T, which causes a Thr296Met transition. In contrast, the pregnant female herself and her other sister were found to carry only normal alleles. Plasma factor IX activity and antigen levels supported these findings.

Keywords: carrier detection, CpG hotspots, direct sequencing, factor IX, mutation, RFLPs.

Introduction

In families of patients mildly to moderately affected by haemophilia B, plasma factor IX (FIX) activity and antigen level analyses alone may give ambiguous diagnoses of carrier status. A combination of protein and gene analysis may provide the most reliable method of carrier detection. Highly dependable mutation analysis and carrier detection are possible due to the wealth of previous studies of the human FIX gene [1,2]. In this paper we describe the identification of the molecular defect in a moderately affected haemophilia B family and successful diagnosis of a pregnant member for her carrier status.

Patients and methods

We studied a caucasian family, moderately affected by haemophilia B that has been traced as far back as an obligatory carrier maternal grandmother. After obtaining appropriate written consent, whole blood samples (~5 mL) from the pregnant patient whose carrier status we hoped to determine (II-3), her two sisters (II-1 and II-2) and their obligate carrier mother (I-1) were drawn by standard methods with acid citrate dextrose anticoagulant (10% in the final volume). Unfortunately, all male members of the family, including the patient's affected brother and uncle, were not available for blood collection in this study. Plasma samples were prepared from whole blood by brief centrifugation, and genomic DNA was extracted from the cell fraction using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN, USA). Polymerase chain reaction (PCR) amplified fragments of the coding and exon–intron junctions were subjected to direct sequencing using the Thermosequenase sequencing kit (Amersham Life Science, Arlington Heights, IL, USA). Haplotypes of alleles, as defined by RFLPs, were determined by Southern blot analysis of restriction fragments generated by digestion with the restriction enzymes *Hinf* I, *Xmn* I, *Taq* I, *Hha* I, and *Bam*H I and probing by FIX cDNA (data not shown) [3–6]. Plasma FIX activity and antigen levels were assayed by obtaining activated partial thromboplastin times (APTT) using the ST4Bio clot detection system (American Bioproducts, Parsippany, NJ, USA) and FIX-specific enzyme-linked immunosorbent assay (ELISA), respectively [7].

Correspondence: Sumiko Kurachi, Department of Human Genetics, the University of Michigan Medical School, C580 MSRB II, Ann Arbor, MI 48109-0618, USA.
Tel.: + 1 734 647 3152; fax: + 1 734 647 3158;
e-mail: kurachis@umich.edu

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Results and discussion

By direct sequencing of PCR-amplified fragments covering the entire transcribed region and splice junctions of the obligatory mother (I-1), heterozygous nucleotide sequences, cytidine (C) and thymine (T), were found at nucleotide (nt) 31008 in exon VIII (see reference 2 for nt numbering) (Fig. 1). The normal and abnormal alleles have C or T respectively at this position. nt 31008 was then analysed in the other female family members, II-1–3, using PCR-amplified fragments prepared with a specific PCR primer pair (30731-CCAATTAGGTCAGTGGTCC-CAAG-30753 and 31050-GTCGGTGAATGTAT-CGGTCT-31031). One sister (II-1) was also found to be heterozygous at this site, while the pregnant member (II-3) and another sister (II-2) had only the normal allele (C) at this position, thus diagnosing both II-2 and II-3 as noncarriers. In agreement, plasma FIX activity and antigen levels for both II-2 and II-3 were in the normal range (100 and 104%, and 133 and 122%, respectively), while heterozygous female members (I-1 and II-1) had significantly lowered activity and antigen levels (85 and 74%, and 42 and 41%, respectively). The reason why carriers have significantly lowered FIX activity and antigen levels, while normal female members have full levels of activity, remains to be determined. This could be due to skewed X-chromosome inactivation [9], or a hitherto unidentified mechanism.

The mutation identified in this family results in the amino acid change T296M [1,2] and is at a CpG dinucleotide, a site known to be a hotspot for mutations [8]. To date, 86 cases of C31008T mutations and two cases of C31008A mutations have been reported [1]. Our RFLP analysis showed that both normal and mutant alleles have the same haplotype, and thus, RFLP analysis with the set of restriction enzymes used was not informative for determining carrier status in this family. Whether the mutant allele identified in this family was generated by *de novo* mutation or by a founder effect remains to be determined.

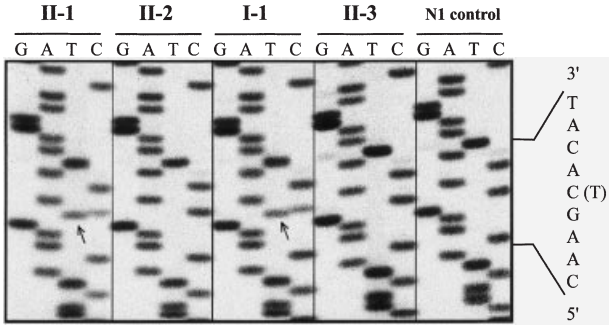

Fig. 1. DNA Sequence around the mutation site at nt 31008. Roman numerals above each lane indicate patient number. Nl control indicates an unrelated normal male whose DNA was used as a control. Letters on the right of the figure indicate the nucleotide sequence spanning from 31004 to 31012. C (T) labels the normal C and the abnormal T in the carriers. Both C and T were observed at nt 31008. Arrows mark the site of the mutated allele.

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References

1 Giannelli F, Green PM, Sommer SS *et al.* Haemophilia B. database of point mutations and short additions and deletions – eighth edition. *Nucl Acids Res* 1998; **26**: 265–8.
2 Yoshitake S, Schach BG, Foster DC, Davie EW, Kurachi K. Nucleotide sequence of the gene for human factor IX (anti-hemophilic factor B). *Biochemistry* 1985; **24**: 3736–50.
3 Chen S-H, Zhang M, Lovrien EW, Scott CR, Thompson AR. CG dinucleotide transitions in the factor IX gene account for about half of the point mutations in hemophilia B patients: a Seattle series. *Hum Genet* 1991; **87**: 177–82.
4 Mariani G, Chistolini A, Hassan HJ *et al.* Carrier detection for hemophilia B. evaluation of multiple polymorphic sites. *Am J Hematol* 1990; **33**: 1–7.
5 Graham JB, Kunkel GR, Egilmez NK, Wallmark A, Fowlkes DM, Lord S. The varying frequencies of five DNA polymorphisms of X-linked coagulant Factor IX in eight ethnic groups. *Am J Hum Genet* 1991; **49**: 527–44.
6 Ketterling RP, Bottema CDK, Phillips JA III, Sommer SS. Evidence that descendants of three founders constitute about 25% of Hemophilia B in the United States. *Genomics* 1991; **10**: 1093–6.
7 Kurachi S, Deyashiki Y, Takeshita J, Kurachi K. Genetic mechanisms of age regulation of human blood coagulation factor IX. *Science* 1999; **285**: 739–43.
8 Green PM, Montandon AJ, Bentley DR, Ljung R, Nilsson IM, Giannelli F. The incidence and distribution of CpG→TpG transitions in the coagulation factor IX gene. A fresh look at CpG mutational hotspots. *Nucl Acids Res* 1990; **18**: 3227–31.
9 Plenge RM, Tranebjaerg L, Jensen PKA, Schwartz C, Willard HF. Evidence that mutations in the X-linked DDP gene cause incompletely penetrant and variable skewed X inactivation. *Am J Hum Genet* 1999; **64**: 759–67.