THE *Msp*I RESTRICTION FRAGMENT LENGTH POLYMORPHISM OF HUMAN ALDOLASE B GENE ON CHROMOSOME 9q21.3-q22.2

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Summary The probe containing the exon 9 of human aldolase B gene revealed *Msp*I polymorphism involving two fragments 6.5 and 3.0 kb long with the high frequency of heterozygosity (21%). The two alleles can be distinguished efficiently by the DNA PCR-restriction fragment length polymorphism procedure.

Key Words genetic linkage map, RFLP, human aldolase B, PCR

INTRODUCTION

Relatively few DNA markers have been assigned to chromosome 9q21-32 (Williamson *et al.*, 1990). Therefore, we have examined RFLP marker within this region for the construction of a detailed genetic linkage map.

MATERIALS AND METHODS

High molecular weight genomic DNA of 23 unrelated individuals from in and around Saga Prefecture was extracted from leukocyte nuclei of venous blood. Digests of the DNA samples with eight different restriction enzymes (Nippon Gene) were size-fractionated by electrophoresis, transferred to nylon membrane (Hybond-N, Amersham) and hybridized with the labeled probe (Southern, 1975). The polymerase chain reaction (PCR) (Saiki *et al.*, 1988) was performed by using a thermocycler (Program Temp Control System PC-700, Astech) as follows: 35 cycles of denaturation at 94°C for 1 min, annealing at 65°C for 2 min and extension at 72°C for 2 min.

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Fig. 1. Restriction map of the human aldolase B gene. At the top is the restriction map for the 5.75 kb genomic region containing the exon 7, 8, 9 of the aldolase B gene. Exons are numbered below the line where the restriction sites for enzymes, *Bam*HI (B), *Bgl*II (Bg), *Eco*RI (E), *Hind*III (H), *Kpn*I (K), *Pst*I (Ps), *Pvu*II (P), and *Xba*I (X) are shown. The polymorphic *Msp*I site, seen in 21% of the individuals, is indicated by an asterisk. Below the restriction map, the 1.6 kb 3' end fragment of the human aldolase B gene, position of primers (Fw1, Fw2, Rv1, and Rv2) for PCR, and sizes of *Msp*I digests of amplified DNA fragments are shown.

Fig. 2. Genotype analysis of six unrelated individuals by Southern blot. Peripheral blood DNAs from six individuals after digestion with *Msp*I, hybridized with the probe AB9 that overlaps the 925-bp *Xba*I-*Pvu*II fragment of the aldolase B genome DNA. Individuals 1 and 5 carry an extra 3.0 kb that is absent from individuals 2, 3, 4, and 6. Sizes of the human DNA fragments detected by the probe AB9 are indicated in kb on the right.

RESULTS AND DISCUSSION

A 925-bp *Xba*I-*Pvu*II fragment (AB9) of the aldolase B genome DNA containing the 3' end of intron 8, and exon 9 (Fig. 1) was tested for polymorphism against a panel of DNAs from several unrelated individuals digested with nine restriction enzymes. In 23 unrelated Japanese, the probe AB9 revealed *Msp*I polymorphisms involving two fragments 6.5 and 3.0 kb long (Fig. 2). Other eight enzymes (*Pvu*II, *Taq*I, *Pst*I, *Bgl*II, *Eco*RI, *Hind*III, *Bam*HI, *Rsa*I) failed to detect polymorphisms. Twenty one % of unrelated persons were heterozygous. The fragment lengths are consistent with their inheritances as Mendelian alleles (Fig. 3). Paolella *et al.*

Fig. 3. Panel A: A pedigree showing familial relationships of four individuals. Circles indicate females and boxes males. Panel B: Southern blot of the family of panel A. The DNAs from four members were examined, using *Msp*I and the probe AB9. The segregation of restriction fragment length shows Mendelian inheritance. Mother and her son are heterozygous. Father and his daughter show the homozygote. Sizes of the human DNA fragments detected by the probe AB9 are indicated in kb on the right.

Fig. 4. PCR analysis of the *MspI* polymorphism. Panel A: The aldolase B 3' flanking region was amplified by PCR from genome DNA with three pairs of the following primers: FW1 and RV1 (lanes 6 and 7), FW2 and RV2 (lanes 4 and 5), FW1 and RV2 (lanes 2 and 3). The amplification products were then digested with *MspI* (lanes 2, 4, and 6). Panel B: *MspI* digestions of PCR products from five unrelated persons (lanes 1, 2, 3, 4, and 5) who were heterozygous for the *MspI* polymorphism on Southern analysis. Lane 6, control (homozygous for the *MspI* polymorphism on Southern blot). FW2 and RV2 were used as the primers of the PCR. Molecular-weight standards of pUC 119/*HinfI* digest (in base pair) are shown on the right.

(1987) detected a RFLP in the intron 8 using *PvuII* digested genomic DNAs from 75 unrelated Caucasians, but we could not find out. The aldolase B 3' flanking region containing the presumptive site of *MspI* polymorphism was amplified by PCR from genome DNA with three pairs of primers (Fig. 1) and the PCR products were digested with *MspI* (Fig. 4A). Two pairs of primers (Fw1 and Rv2, Fw2 and Rv2) could define the *MspI* polymorphism. Five unrelated persons who were heterozygous for *MspI* polymorphism on Southern analysis showed 540 and 260 bp fragments in addition to the common band of 800 bp (Fig. 4B, lanes 1, 2, 3, 4, and 5). The DNA PCR-restriction fragment length polymorphism procedure would represent a rapid and efficient method for linkage studies and indirect carrier detection in families with a family history of hereditary fructose intolerance.

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