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MOLECULAR GENETIC STUDY OF A JAPANESE FAMILY WITH LESCH-NYHAN SYNDROME: A POINT MUTATION AT THE CONSENSUS REGION OF RNA SPLICING ($HPRT_{KEIO}$)

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Summary Complete deficiency of hypoxanthine guanine phosphoribosyltransferase (HPRT) causes Lesch-Nyhan syndrome. A single nucleotide substitution of G to T at the 3′-end of intron 3 in the splicing consensus region has been identified in one allele of the HPRT gene from a mother predicted to be a heterozygous Lesch-Nyhan carrier. Utilizing a *Bfa*I restriction site which was lost in the mutation as an indicator, family study showed that the mother and her only daughter were heterozygotes but the mother's sister did not have the mutant allele. The mutation generated splicing error and resulted in two types of abnormal mRNA. The major altered mRNA, named Type I, skipped the exon 4 and is predicted to produce a protein deleted of 22 amino acid residues. The other, Type II, having a 9-bp deletion at the 5′-end of exon 4, can result in a protein lacking 3 amino acids, from codon 107 to 109.
Key Words Lesch-Nyhan syndrome, hypoxanthine guanine phosphoribosyltransferase, sequence analysis, point mutation, altered RNA splicing

INTRODUCTION

Deficiency of a purine salvage enzyme, hypoxanthine guanine phosphoribosyltransferase [HPRT, EC 2.4.2.8], is associated with two distinct clinical disorders which are inherited as an X linked recessive trait. Complete deficiency of HPRT leads to Lesch-Nyhan syndrome (Seegmiller *et al.*, 1967), whereas partial deficiency causes a severe form of gout (Kelley *et al.*, 1967). The HPRT gene is located on

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the long arm of the X chromosome and consists of nine exons and eight introns (Patel *et al.*, 1986). The complete sequence totalling 57 kb nucleotide was determined completely by Edwards *et al.* (1990). Transcription of this gene produces a mRNA of 1.6 kb, which contains a protein-encoding region of 654 nucleotides (Jolly *et al.*, 1983). The marked genetic heterogeneity of HPRT deficiency is well known. As Sculley *et al.* (1992) reviewed, many different mutations at the HPRT gene locus; deletions, insertions, duplications, abnormal splicing, and point mutations at different sites of the coding region from exon 1 to 9, have been reported, including a rare case of female (Ogasawara *et al.*, 1989). The technique of polymerase chain reaction (PCR) amplification of reverse-transcribed mRNA has recently been used to identify the molecular basis of HPRT deficiency in a number of subjects (Davidson *et al.*, 1988, 1989, 1991; Gibbs *et al.*, 1989, 1990; Igarashi *et al.*, 1989; Fujimori *et al.*, 1990, 1992; Lightfoot *et al.*, 1992; Sculley *et al.*, 1991; Tarle *et al.*, 1991). Further, the multiplex amplification technique of all nine HPRT exons from the genomic DNA (Gibbs *et al.*, 1990; Gordon *et al.*, 1991; Yamada *et al.*, 1992) has enabled the genomic analysis of HPRT mutations. We report here the identification of a point mutation at the consensus region for splicing of the HPRT gene in a Japanese family with Lesch-Nyhan syndrome. The mutation generated two types of abnormal mRNA because of the altered RNA splicing.

MATERIALS AND METHODS

All the methods of HPRT gene analysis, identification of the genomic mutation and the altered mRNA, were described previously (Yamada *et al.*, 1992). B-lymphoblastoid cell lines were established and maintained in RPMI 1640 medium containing 15% FCS (Gibco Laboratories), penicillin (100 units/ml), and streptomycin (100 μg/ml) at 37°C in 5% CO_2. Selection for $HPRT^-$ cells was carried out in a medium containing 20 μM 6-thioguanine (6-TG). DNA sequences were determined according to the simplified direct sequencing method described by Yamada *et al.* (1992), and were recorded into a personal computer and analyzed with a software of gene analysis, GENETYX version 8.0 (SDC, Japan).

RESULTS AND DISCUSSION

We investigated the molecular genetic basis of the Lesch-Nyhan syndrome in a Japanese family with the disease. The mother was predicted to be a heterozygous carrier since her two sons who were dead were both diagnosed as Lesch-Nyhan syndrome. The genomic DNA of the mother was first analyzed. The multiplex amplification from genomic DNA revealed no differences in product sizes between the mother and normal control (data not shown). By direct sequencing of all nine amplified exons, a single nucleotide substitution of G to T at the

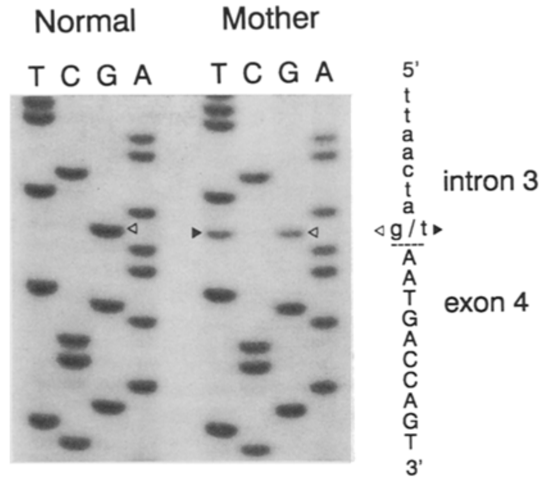

Fig. 1. Direct sequencing of the HPRT genomic DNA from a normal subject and the mother predicted to be a heterozygous Lesch-Nyhan carrier. The genomic DNA fragments were sequenced using an antisense primer HCB5 (5′CTTTCCAGTTAAAGTTGAGA-3′).

3′-end of intron 3 has been identified in one allele of the HPRT gene (Fig. 1). The mutation should result in splicing error due to the alteration of the splice acceptor site, since the 5′-splice consensus sequence AG was changed to AT.

The *Bfa*I restriction site (CATG) at the 3′-end of intron 3 is lost in the mutant allele (CTAT). Family study was performed by the PCR amplification of the exon 4 fragment and digestion by *Bfa*I (Fig. 2). DNA fragments of 334 bp including exon 4 and its franking intron sequences were amplified from the genomic DNAs of the mother, her daughter, the mother's sister, and a normal control female. The fragment from the normal allele was digested to 34, 98, and 202 bp by two *Bfa*I sites, but that from the mutant allele to 34 and 300 bp by only one *Bfa*I site. In Fig. 2, the 34-bp band was not detected in electrophoresis because of its small size. The analysis of the mother's and her daughter's samples showed three DNA bands, the 300 bp band from the mutant allele and the two bands, 98 and 202 bp, from the normal allele. The sequence analysis of the DNA fragment of the daughter showed both G and T bands at the mutation site (data not shown), as seen in the DNA sequence analysis of the mother (Fig. 1). The mother's sister had two normal a lleles. Prenatal diagnosis in this family can be performed easily if genomic DNA is isolated from chorionic villus samples (CVS), as carried out previously in another family (Yamada *et al.*, 1992).

To clarify the alteration of mRNA generated by the identified mutation, we analyzed the HPRT cDNA amplified by RT-PCR. Amplification of the entire coding region or portions of HPRT cDNA from random B-lymphoblasts derived from the mother's blood cell resulted in same-sized DNA fragments as a normal

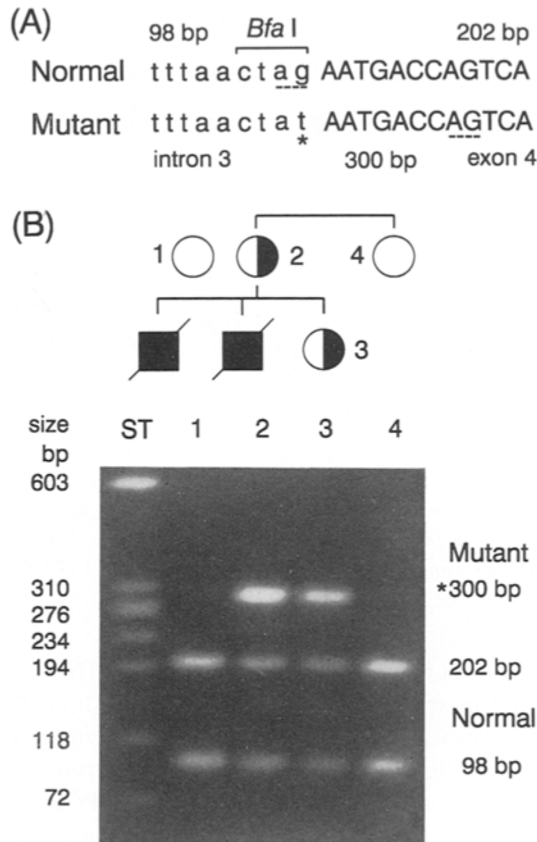

Fig. 2. Family study to detect the mutant allele. (A) The alteration of the HPRT genomic DNA. (B) Agarose gel electrophoresis (3.0% NuSieve 3:1 agarose). The 334-bp DNA fragments were amplified using a sense primer HGE4A (5′-TAGCTAGCTAACTTCTCAAATCTTCTAG-3′, on the intron 3) and an antisense primer HGE4B (5′-ATTACCTAGACTGCTTCCAAGGG-3′, intron 4), and digested by *Bfa*I. ST, DNA size marker (ϕX174/*Hae*III digest); 1, normal female; 2, the mother; 3, her daughter; 4, mother's sister.

subject (data not shown). Direct sequencing of the DNA fragments or sequencing of the cloned recombinants could not detect any alterations. Most transcripts seemed to be normal. Therefore, $HPRT^-$ cells were selected from the mother's B-lymphoblasts in the presence of 6-TG. The cDNA amplification from the $HPRT^-$ cells are shown in Fig. 3A. The amplification from the $HPRT^-$ cells using the primers, HCA2 on the exon 3 and HCB3 on the exon 6/7 junction resulted in a main fragment (M23) which was much shorter than the normal fragment (N23, 348 bp), and small amounts of fragment close to normal size. Further, the fragment (M25) amplified from the $HPRT^-$ cells by HCA2 and HCB5 on the exon 4 was slightly shorter than that from normal cells (N25, 238 bp). Direct sequencing

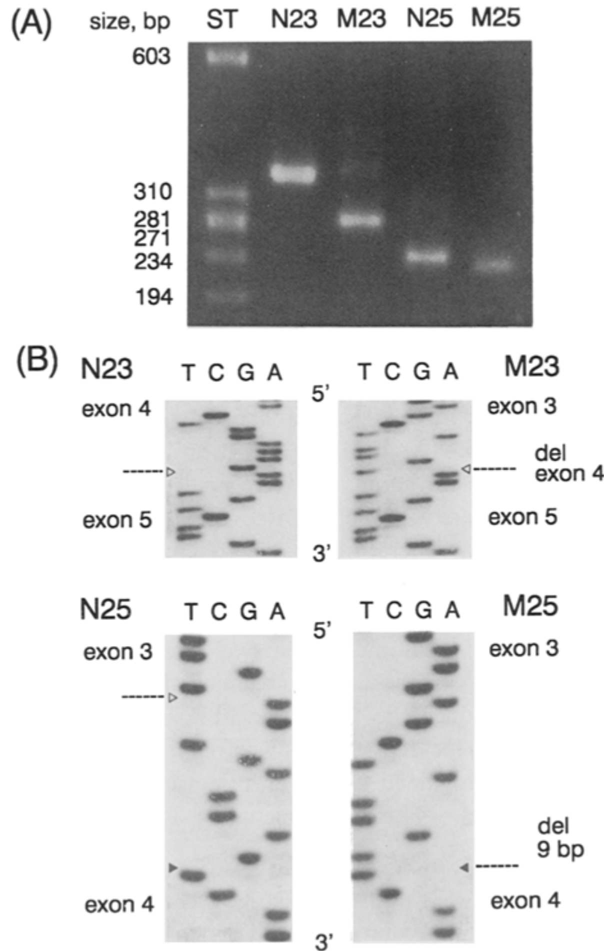

Fig. 3. Analysis of HPRT cDNA from the mother's HPRT⁻ cells. (A) Agarose gel electrophoresis (3.0% NuSieve 3:1 agarose) of PCR product from normal and the mutant cell. The cDNA fragments N23 (normal) and M23 (mutant) were amplified using a sense primer HCA2 (5′-TGCTCGAGATGTGATGAAGG-3′, on the exon 3) and an antisense primer HCB3 (5′-ACCAGCAAGCTTGCGACC-TT-3′, exon 6/7). N25 (normal) and M25 (mutant) were amplified using the primers HCA2 and HCB5 (5′-CTTTCCAGTTAAAGTTGAGA-3′, exon 4). ϕX174/*Hae*III digest was used as standard DNA size markers (ST). (B) Direct sequencing of the PCR products from cDNA. The fragments N23 and M23 were sequenced directly using the antisense primer HCB3, and N25 and M25 using HCB5.

of these cDNA fragments demonstrated that M23 had a deletion of all 66 bp of the exon 4 and M25 lost 9 bp at the 5′-end of exon 4 (Fig. 3B). Thus, there are

two types of abnormal mRNA: Type I which is the major altered mRNA skipped the exon 4, and Type II revealed a 9-bp deletion at the 5′-end of exon 4. In Type I, exon 4 might be skipped because of splicing error of the primary transcript due to a mutated 5′-splice site of exon 4. And, Type II was probably spliced by recognizing AG:TC 8–11 bp downstream from the mutation site (Fig. 2A). Type I mRNA results in a protein deleted of the 22 amino acid residues of exon 4 and Type II produces a protein lacking 3 amino acids, Asn-Asp-Gln, from codon 107 to 109.

Five cases of point mutation at the splice sites in introns that may lead to altered HPRT splice patterns were reported (Gibbs *et al.*, 1990; Gordon *et al.*, 1991): at the splice accepter sites of exons 2 and 9, and the donor sites of exons 6, 7, and 8. However, a new finding of a single nucleotide substitution at the accepter site of exon 4 has been identified in this study. The deletion of exon 4 has been found in 3 different families by cDNA analyses (Davidson *et al.*, 1991; Tarle *et al.*, 1991), but the genomic mutations in these cases were unclear. Some of them might have been caused by a splicing error. We reported previously a mutant with 4-bp deletion at the 5′-end of exon 4 generating altered splicing (Yamada *et al.*, 1992). The mutation resulted in the formation of three different types of abnormal mRNA. One of them, type A, revealed a 4-bp deletion at the 5′-end of exon 4, and the other two, types B and C, were the same as the two abnormal mRNAs shown in this study, Type II and I, respectively. It is presumed that mutations at the 5′-splice site of exon 4 always produce the latter two abnormal mRNAs. The secondary junction AG:TC which is located 9-bp downstream from the normal junction of intron 3 and exon 4 is recognized as the cryptic splice site in only mutant genes, but never in normal genes. The mechanisms of RNA splicing deserves further study.

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