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Mutation Report

A NOVEL *DE NOVO* MUTATION IN HPRT GENE RESPONSIBLE FOR LESCH-NYHAN SYNDROME ($HPRT_{OSAKA}$)

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Summary A virtually complete deficiency of hypoxanthine guanine phosphoribosyltransferase (HPRT) causes Lesch-Nyhan syndrome. A novel mutation of HPRT gene in a Japanese Lesch-Nyhan family has been identified using mRNA and genomic DNA from peripheral blood cells. A single nucleotide substitution of T to C in exon 3 resulted in a mis-sense mutation, CTC (Leu) to CCC (Pro), at codon 65. Utilizing an *Mnl*I restriction site which was lost in the mutation as an indicator, a family study showed that the mother was normal not having the mutant gene. The mutation was a *de novo* event that had occurred in the germ cells of the mother or in the proband during the early phase of fetal development.
Key Words Lesch-Nyhan syndrome, hypoxanthine guanine phosphoribosyltransferase (HPRT), sequence analysis, *de novo* mutant, mis-sense mutation

A virtually complete deficiency of hypoxanthine guanine phosphoribosyltransferase [HPRT, EC 2.4.2.8] causes Lesch-Nyhan syndrome (Seegmiller *et al.*, 1967), whereas a partial deficiency generally presents as gout and uric acid overproduction in early adulthood (Kelley *et al.*, 1967). The marked genetic heterogeneity of HPRT deficiency is well known. As reviewed previously by

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Sculley *et al.* (1992), 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 two rare cases in female subjects (Ogasawara *et al.*, 1989; Yamada *et al.*, 1995a). By polymerase chain reaction of reverse-transcribed mRNA (RT-PCR) and the multiplex amplification technique of all nine HPRT exons from the genomic DNA coupled with direct sequencing, we identified HPRT mutants in Japanese (Yamada *et al.*, 1992, 1993, 1995a, 1996) and Korean (Choi *et al.*, 1993; Yamada *et al.*, 1995b). In this study, by the same means, we identified a new HPRT mutation in a Japanese family with Lesch-Nyhan syndrome.

The patient (13 mos) was suspected as having Lesch-Nyhan syndrome from the neurological manifestations with choreoathetosis, hyperuricemia (11.6 mg/dl), and hyperuricaciduria (46 mg/dl). HPRT activity was undetectable (<0.01, control value = 1.76±0.06 nmol/min/mg hemoglobin) and adenine phosphoribosyl-

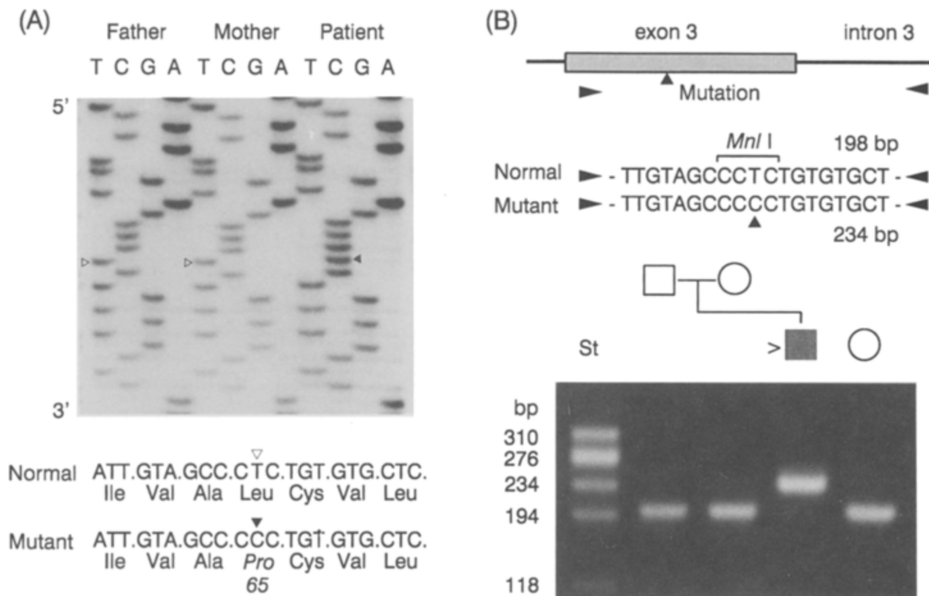

Fig. 1. Molecular analysis of a Japanese family with Lesch-Nyhan syndrome. (A) Direct sequencing of the HPRT genomic DNA from the father, the mother and the Lesch-Nyhan patient. The DNA fragments including exon 3 were amplified and were sequenced directly by the antisense primer HCB6, as described previously (Yamada *et al.*, 1992). (B) Family study by PCR-RFLP analysis. 234-bp DNA fragments were amplified using a sense primer HCA2 (Yamada *et al.*, 1992) and an antisense primer HG3E2 (5′-AGTATGGTTTGCAGAGATTCAAAG-3′), digested by *Mnl*I, and then agarose gel (1.5%) electrophoresis was carried out. The ϕX174/*Hae*III digest was used as DNA size markers. Closed triangles (▲) pointed the position of the mutation and an allow (>) showed the proband.

transferase (APRT) activity (0.62 nmol/min/mg hemoglobin) was increased about 1.5-fold compared with the control value (0.43±0.06) in the erythrocytes from the patient.

We carried out the molecular analysis of HPRT gene in this Japanese Lesch-Nyhan family. At first, the genomic DNA of the proband was analyzed. Multiplex amplification from genomic DNA (Yamada *et al.*, 1992) revealed no differences in product sizes between the patient and the normal control (data not shown). By direct sequencing of all nine amplified exons, a single nucleotide substitution of T to C was detected in exon 3 (Fig. 1A). No other change of the nucleotide sequence was detected on the entire coding region and on the region recognized for RNA splicing. RT-PCR amplification from mRNA isolated from the proband's peripheral blood resulted in a DNA fragment of the same size as that from the normal control. Direct sequencing analysis of the RT-PCR product showed a point mutation identical to that found in the genomic DNA. The substitution resulted in a mis-sense mutation, CTC (Leu) to CCC (Pro) at codon 65.

The *Mnl*I restriction site (CCTC) in exon 3 is lost in the mutant allele (CCCC). A family study was thus performed by PCR-RFLP analysis utilizing this *Mnl*I site (Fig. 1B). DNA fragments of 234 bp including exon 3 and intron 3 sequences were amplified using a sense primer HCA2 (on the exon 3, Yamada *et al.*, 1992) and an antisense primer HG3E2 (on the intron 3, 5′-AGTATGGTTTG-CAGAGATTCAAAG-3′), from the genomic DNAs of the proband, the mother, the father and a normal control female volunteer. The fragment from the normal allele should be digested by *Mnl*I to two bands of 198 and 36 bp. Analyses of the father and mother's samples showed the 198-bp band as the same as the normal control. Only the proband showed the 234-bp band from the mutant allele. Sequence analysis of the DNA fragments of the mother showed only normal T band at the mutation site (Fig. 1A). Therefore, the mutation was a *de novo* event that had occurred in the germ cells of the mother or in the proband during the early phase of fetal development.

The mutation at codon 65 in this study ($HPRT_{Osaka}$) can add a new information to over 50 different mis-sense mutations reported previously. This mutation (L65P) has not detected in any HPRT deficiencies previously, but we reported (Yamada *et al.*, 1992) that the single amino acid substitution of Ala (GCC) to Pro (CCC) at codon 64 caused Lesch-Nyhan syndrome (Case 2). In $HPRT_{Osaka}$, similar to in the Case 2, an increase in hydrophilicity and a change in secondary structure (α-helix to β-sheet) were predicted by the analysis systems of the protein structure (GENETYX, 9.0), and no other change of the nucleotide sequence was detected. Furthermore, the identified substitution has never been detected as a polymorphism in over 100 other alleles. Therefore, it is suggested that the amino acid substitution of L65P in $HPRT_{Osaka}$ must affect the enzyme activity, although it is necessary to confirm by expression studies.

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REFERENCES

Choi Y, Koo JW, Ha IS, Yamada Y, Goto H, Ogasawara N (1993): Partial hypoxanthine-guanine phosphoribosyltransferase deficiency in two Korean siblings -a new mutation. Pediatr Nephrol **7**: 739–740

Kelley WN, Rosenbloom FM, Henderson JF, Seegmiller JE (1967): A specific enzyme defect in gout associated with overproduction of uric acid. Proc Natl Acad Sci USA **57**: 1735–1739

Ogasawara N, Stout JT, Goto H, Sonta S, Matsumoto A, Caskey CT (1989): Molecular analysis of a female Lesch-Nyhan patient. J Clin Invest **84**: 1024–1027

Sculley DG, Dawson PA, Emmerson BT, Gordon RB (1992): A review of the molecular basis of hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency. Hum Genet **90**: 195–207

Seegmiller JE, Rosenbloom FM, Kelley WN (1967): Enzyme defect associated with a sex-linked human neurological disorder and excessive purine synthesis. Science **155**: 1682–1684

Yamada Y, Goto H, Suzumori K, Adachi R, Ogasawara N (1992): Molecular analysis of five independent Japanese mutant genes responsible for hypoxanthine guanine phosphoribosyltransferase (HPRT) deficiency. Hum Genet **90**: 379–384

Yamada Y, Goto H, Tamura S, Ogasawara N (1993): Molecular genetic study of a Japanese family with Lesch-Nyhan syndrome: a point mutation at the consensus region of RNA splicing ($HPRT_{Keio}$). Jpn J Human Genet **38**: 413–419

Yamada Y, Goto H, Yukawa T, Akazawa H, Ogasawara N (1995a): Molecular mechanisms of the second female Lesch-Nyhan patient. Adv Exp Med Biol **370**: 337–340

Yamada Y, Choi Y, Kim KJ, Koo JW, Ha IS, Goto H, Ogasawara N (1995b): Hypoxanthine guanine phosphoribosyltransferase (HPRT) gene mutations in Korean families with Lesch-Nyhan syndrome. Purine Pyrimidine Metab **19**: 13–21 (in Japanese)

Yamada Y, Suzumori K, Tanemura M, Goto H, Ogasawara N (1996): Molecular analysis of a Japanese family with Lesch-Nyhan syndrome: Identification of mutation and prenatal diagnosis. Clin Genet **50**: 164–167