Molecular Analysis of Hypoxanthine Guanine Phosphoribosyltransferase (HPRT) Gene in Five Korean Families with Lesch-Nyhan Syndrome

Lesch-Nyhan syndrome is caused by the complete deficiency of hypoxanthine guanine phosphoribosyltransferase (HPRT). By the analysis of genomic DNA and mRNA using the polymerase chain reaction (PCR) technique coupled with direct sequencing, five independent mutations in HPRT genes have been identified in Korean Lesch-Nyhan families. Two novel mutations and three previously reported mutations have been found in five independent families. Heterozygous carriers were detected in all the families, and prenatal diagnosis was carried out in two families.
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Key Words : *Lesch-Nyhan syndrome, Hypoxanthine guanine phosphoribosyltransferase (HPRT) gene*

Ki Joong Kim, M.D.[1], Yasukazu Yamada, Ph.D.[2], Kaoru Suzumori, M.D.[3], Yong Choi, M.D.[1], Sei Won Yang, M.D.[1], Hae Il Cheong, M.D.[1], Yong Seung Hwang, M.D.[1], Haruko Goto[2], and Nobuaki Ogasawara, M.D.[2]

Department of Pediatrics, Seoul National University, College of Medicine, Seoul 110-744, Korea[1]
Department of Genetics, Institute for Developmental Research, Aichi Human Service Center, Kasugai, Aichi 480-03, Japan[2]
Department of Obstetrics and Gynecology, Nagoya City University Medical School, Nagoya 467, Japan[3]

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Address for correspondence
Yong Choi M.D., Ph.D., Department of Pediatrics, Seoul National University, College of Medicine, Seoul 110-744, Korea
Tel : 82-2-760-3624, Fax : 82-2-743-3455

INTRODUCTION

Lesch-Nyhan syndrome is an X-linked recessive disorder characterized by hyperuricemia, choreoathetosis, psychomotor retardation and compulsive self-injurious behavior (1). This disease is caused by the complete absence of activity of an enzyme involved in purine metabolism, hypoxanthine guanine phosphoribosyltransferase (HPRT, EC 2.4.2.8) (2). The HPRT gene is located on the long arm of the X chromosome and consists of nine exons (3). The complete sequence totaling 57 kb nucleotides was determined completely by Edwards et al. (4). Transcription of this gene produces a mRNA of 1.6 kb, which contains a protein-encoding region of 654 nucleotides (5).

The marked genetic heterogeneity of HPRT deficiency is well known. As Sculley et al. reviewed (6), many different mutations at the HPRT gene locus (deletions, insertions, duplications, abnormal splicings, and point mutations at different sites in the coding region from exon 1 to 9) have been reported including rare female cases (7, 8). Using RT-PCR of the mRNA and multiplex PCR of all 9 exons from the genomic DNA coupled with direct sequencing, we have identified five HPRT mutations in 5 families with Lesch-Nyhan syndrome. Furthermore we performed prenatal diagnosis of the gene mutation in 2 families.

MATERIALS AND METHODS

Subjects

Case 1 and 2 were male siblings (Family A) who manifested with choreoathetosis, spasticity, psychomotor retardation, hyperuricemia and hyperuricaciduria. Case 3 (Family B), Case 4 (Family C) and Case 6 (Family E) subjects were diagnosed as having Lesch-Nyhan syndrome from the neurologic manifestations of motor developmental delay, spasticity, choreoathetosis, characteristic self-injurious behavior, hyperuricemia, and hyperuricaciduria with orange sand in urine. Case 5 (Family D) had presented with red crystalluria at 1 month of age. Diagnostic work-up revealed hyperuricemia, hyperuricaciduria and renal stone. Later he showed motor developmental delay and nephrocalcinosis. The HPRT activities in the erythrocytes from Case 1, 2, 3, 4 and 6 were undetectable. The HPRT activity from Case 5 was 0.011 nmol/min/mg hemoglobin.

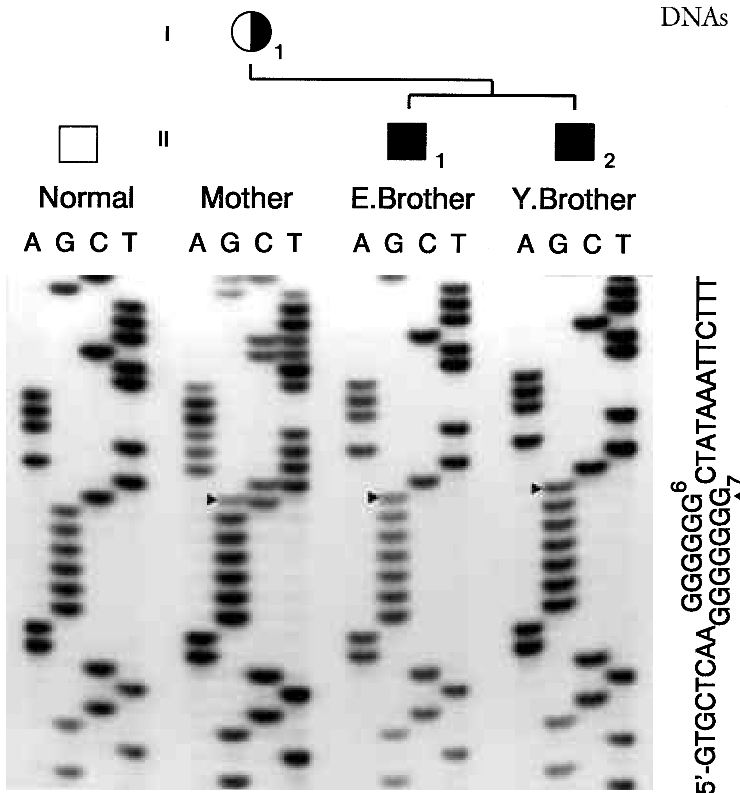
Methods

All the methods of HPRT gene analysis, identification of the genomic mutation and the altered mRNA have been described previously (9). B-lymphoblastoid cell lines were established and maintained in RPMI 1640 medium containing 15% fetal calf serum (Gibco Laboratories), penicillin (100 units/ml), and streptomycin (100 μg/ml) at 37℃ in 5% CO_2. Selection for HPRT-negative 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 previously (9), and were recorded into a personal computer and analyzed with GENETYX version 9.0 (SDC, Japan) gene analysis software. The names and sequences of used primers are adopted from Yamada et al. (9).

RESULTS AND DISCUSSION

In Family A (Fig. 1), insertion of 1 bp (G) around cDNA nucleotide position (nt.) 310 was found by analyses of genomic DNAs and cDNAs from Case 1 and 2. This mutation, 310insG, resulted in frameshift and a premature stop codon (TAA) appearing at codon 73 following a change of Tyr to Leu at codon 72. The mother was heterozygous having both the normal and the mutant genes. The same mutation has been reported already in two unrelated patients, RJK866 (10) and 1650 (11).

A mutation resulting in a splicing error was identified in Family B. A single nucleotide substitution of T to A at 9 bp upstream from 5'-end of exon 8 (533-9TA) was found. The *Hinf* I restriction site at the 3'-end of intron 7 is lost in the mutant allele. The family study was performed by digestion of PCR product of the genomic DNA using *Hinf* I (Fig. 2A). DNA fragments of 499 bp including exon 7 and 8 and its flanking intron sequences were amplified by PCR from the genomic DNAs of the family members. The fragment from the



Normal
GTG.CTC.AAG.GGG.GGC.TAT.AAA.TTC.TTT 225
Val Leu Lys Gly Gly Tyr Lys Phe Phe 75
GTG.CTC.AAG.GGG.GGG.CTA.TAA.ATT.CTT 225
Val Leu Lys Gly Gly Leu stop 72
Mutant insertion (G)

Fig. 1. Analysis of Family A. The genomic DNA fragments including exon 3 were PCR-amplified using a primer pair of HGE3A and HGE3B(14) from a normal control, the patients' mother and two sibling patients (Case 1 and 2), and then sequenced directly using the primer HCA2.

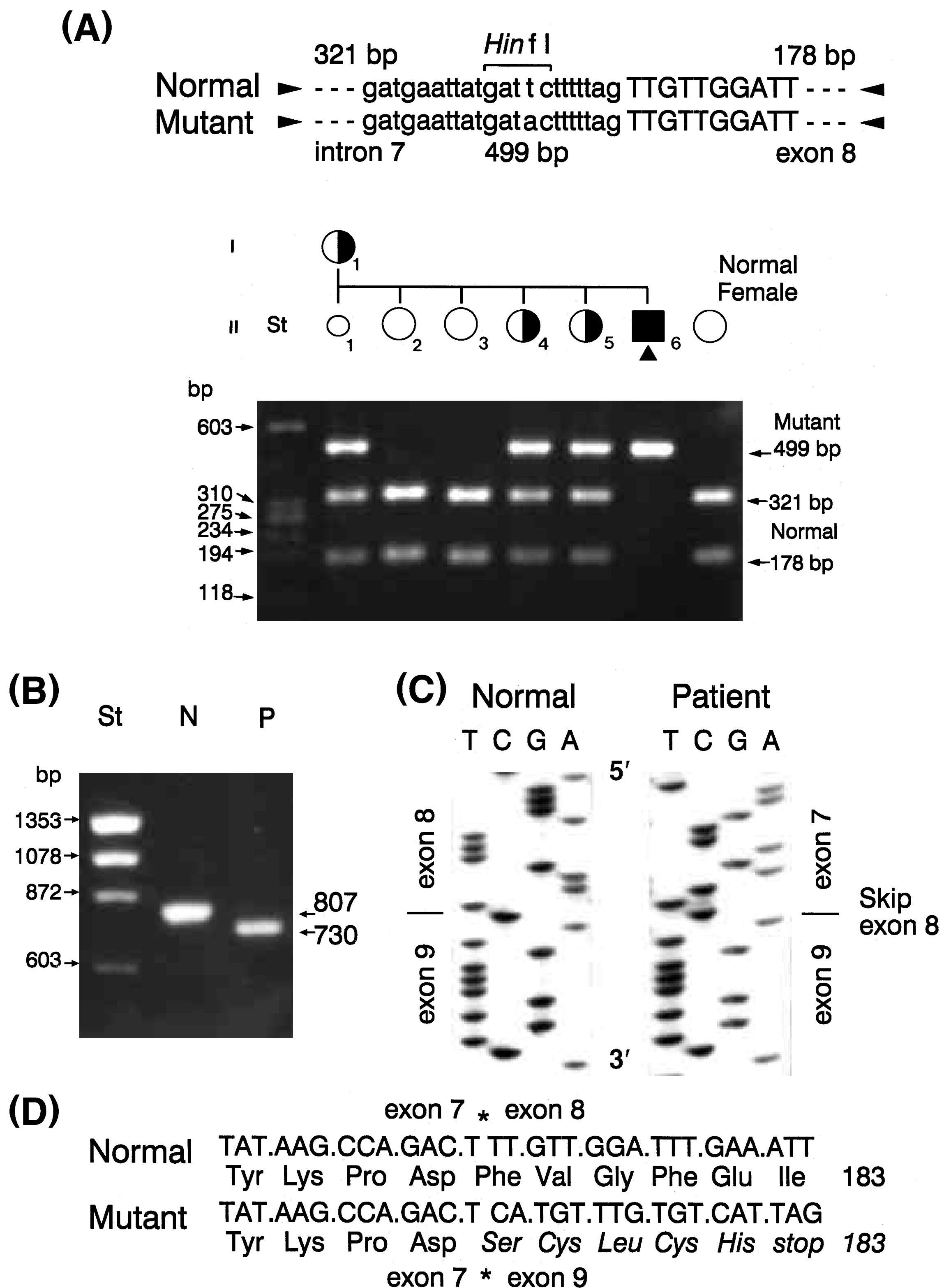


Fig. 2. Analysis of Family B. **A :** Electrophoresis(3.0% NuSieve 3 : 1 agarose gel) after *Hin*f I digestion of the HPRT genomic DNA from Family B. The genomic DNA fragments were amplified using primers HGE78A and HGE78B from a normal control and the family members, and then digested by *Hin*f I. **B :** RT-PCR amplification of the entire coding region of HPRT cDNA from a normal subject and the patient. Electrophoresis was carried out on a 1.5% agarose gel. **C :** Direct sequencing of cDNA fragments from normal control and the patient using primer HCB4. **D :** Nucleotide and deduced amino acid sequence of normal and mutant HPRT cDNA. The ϕX174/*Hae* III digest was used as standard DNA size markers (St).

normal allele was digested to 321 and 178 bp fragments by *Hinf* I, but that from the mutant allele was not digested. The mother and two of four sisters who showed three bands of both two digested normal fragments and one undigested mutant fragment were diagnosed as heterozygous carriers. The sequence analysis of the DNA fragments confirmed the diagnosis (data not shown). To clarify the alteration of mRNA generated by the identified mutation, we analyzed the HPRT cDNA amplified by RT-PCR. The amplified product from the patient was a much shorter cDNA fragment compared to normal (807 bp) (Fig. 2B), and deletion of the entire exon 8 in the cDNA was identified by direct sequencing of the fragment (Fig. 2C). The 533-9TA substitution generated the exon 8 skipped mRNA, and the predicted size of the translated protein is 182 amino acids consisting of 177 normal and 5 different amino acids (Fig. 2D). The mutants skipping exon 8 have been reported in four subjects (10, 12, 13, 14). In one subject, RJK888 (13), a single nucleotide substitution (G to A) at splicing donor site in intron 8 caused the exon 8 skipping, but another 3 subjects were not analyzed on genomic DNA. This mutation can add new information to the mutant HPRT genes.

A nonsense point mutation (Q109X) of C to T at nucleotide 424 replacing Gln-109 (CAG) with a stop codon (TAG), the same as RJK1930 (14), was found in Family C. The family study and prenatal gene diagnosis were performed utilizing *Spe* I (ACTAGT) restriction site created in the mutant gene. The DNA fragments including exon 4 were amplified from genomic DNAs of family members and also from the chorionic villus samples at the second conception, then the fragments were digested by *Spe* I (Fig. 3A). The analysis of the mother's sample showed three DNA bands, an undigested band and two bands digested by *Spe* I. The sequence analysis of the mother's DNA fragment showed two bands (C and T) at the mutation site. The grandmother and two aunts did not have the mutant gene confirmed by both *Spe* I digestion of the fragments and direct sequencing analysis (data not shown). Therefore, the mutation must be a *de novo* one occurring in the grandmother's or grandfather's germ cell. Furthermore, a male fetus inherited the mutant HPRT gene from the mother, the same as the patient. By RT-PCR analysis of the B-lymphoblasts from the patient, an additional minor DNA fragment shorter than the main normal size fragment was also observed. Direct sequencing of the fragments showed that the main normal size fragment (Type A) had the same substitution observed in the genomic DNA analysis and the minor short fragment (Type B) was skipping the exon 4 (Fig. 3B). Type A generates a premature enzyme protein consisting of 108 amino acids and Type B results in the protein deleted 22 amino acid residues of the exon 4. The existence of Type B mRNA with this mutation (Q109X) was detected first in this study. The amount of Type B is less than 1/10 amount of Type A, however, it is not the artifacts of PCR, since the skipping of exon 4 can be found only in mRNA from mutants having mutations at the 3-splice acceptor site. The extra mRNA skipped exon 4 had been also observed in two previously reported Japanese mutants, a 4 bp deletion at 5'-end of exon 4 (9) and a single point mutation at 3'-end of exon 3 (15). Thus, in these cases, RNA splicing might be missing because of the mutation at the 3-splice acceptor site.

In Family D, a 2 bp deletion of GT at nucleotide position 289 and 290 on the exon 3, 289 delGT, has been identified by the analysis of genomic DNA from the patient. By utilizing *Bfa* I restriction site which was created in the mutation as an indicator, the family study and prenatal diagnosis were carried out (Fig. 4A). The mother was predicted to be a heterozygous carrier. The amniotic cells of the mother's second conception were resistant for 6-thioguanine. Further, the genetic analysis of the cells revealed that the male fetus inherited the mutant HPRT gene from the mother. The cDNA analysis demonstrated that this mutation generated two types of abnormal mRNA, similar to that in Family C. One altered mRNA (Type A) showed reading frameshift from the mutation site (codon 97) resulting in a premature stop codon (TAA) at codon 106, and the other (Type B) mRNA skipped the exons 2 and 3 (Fig. 4B). The detected mutation has been reported already in two different patients, RJK1332 (10) and $HPRT_{Cheltenham}$ (16), but the existence of two types of abnormal mRNA was discovered first in this study.

A one bp deletion of adenine at nucleotide 631 (631delA) was detected in Family E. Since this mutation neither creates a new restriction site nor destroys any restriction sites, we designed a mismatch primer, HXCM1 (5'-ATAGCATGTTTGTGcCATTAGTGA-3'), constructing a new *Xcm* I restriction site (CCANNNNNNNNNNTGG) in the mutant fragment for the family study (Fig. 5A). DNA fragments (normal 155 bp ; the mutant 154 bp) including the mutation site were amplified using a primer pair HXCM1 and HCB1 (9) from the genomic DNAs of the patient, his mother, a normal brother, and a normal control. The fragment from the mutant allele was digested by *Xcm* I to 22 and 132 bp, but that from the normal allele (155 bp) was not digested. The mother was diagnosed as a heterozygous carrier and the brother inherited the normal allele from the mother. The RT-PCR amplification resulted in almost the same size cDNA fragment as normal, and the direct sequencing analysis of the cDNA

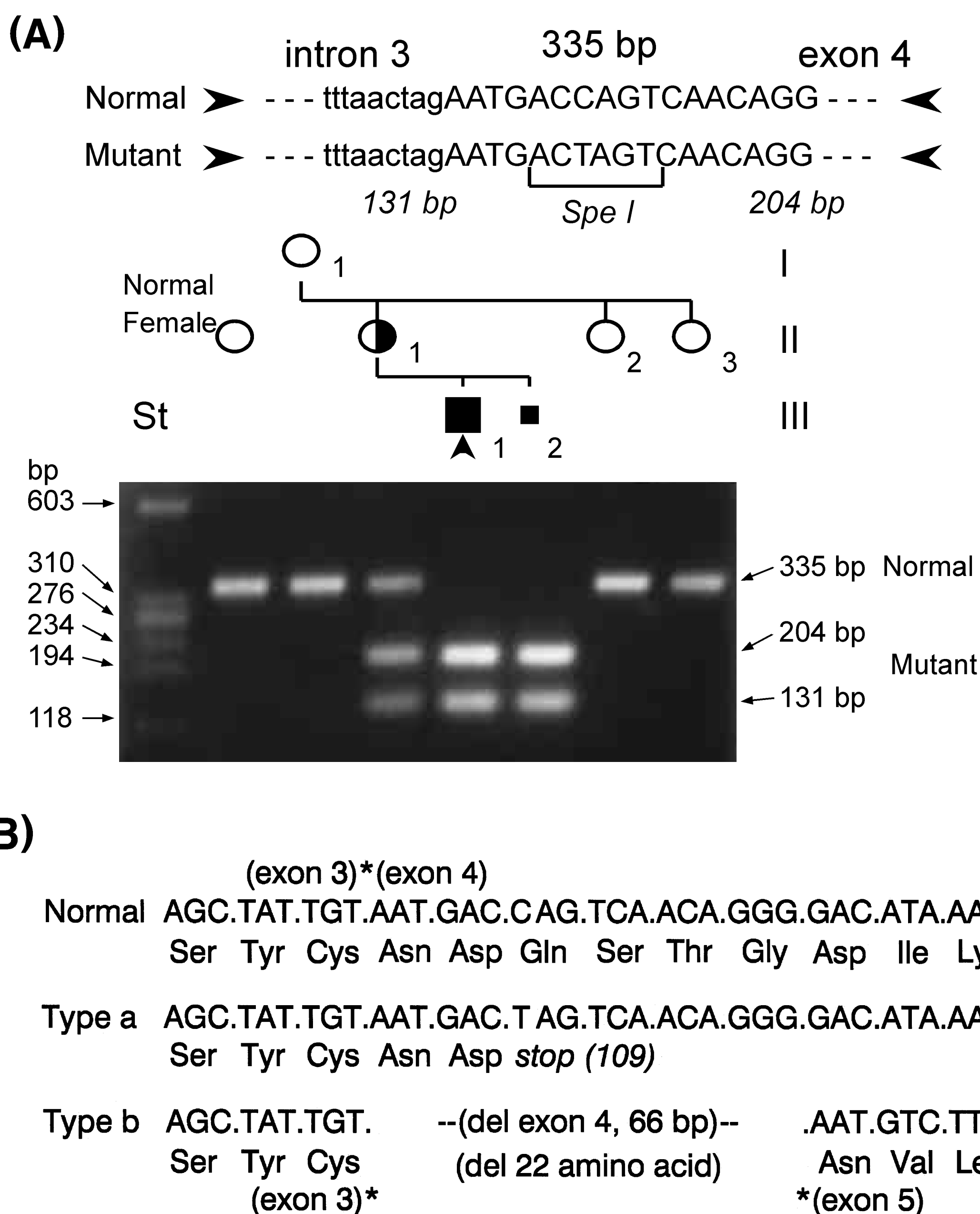


Fig. 3. Analysis of Family C. **A :** Electrophoresis(3.0% NuSieve 3 : 1 agarose gel) after *Spe* I digestion of the HPRT genomic DNA. The genomic DNA fragments were amplified using primers HGE4A and HGE4B from a normal control, the family members and the chorionic villus samples of the mother's second conception, and then digested by *Spe* I. The *ϕ*X174/*Hae* III digest was used as standard DNA size markers (St). **B :** Alterations in the cDNA.

fragment showed the 1 bp deletion of A, the same as in the genomic DNA. The altered mRNA generate a larger mutant protein having a 39 amino acid chain at the C terminal instead of the normal 8 amino acid chain due to the frame shift of the codon at codon 211 (Fig. 5B). This mutation has not been reported previously. The precise mechanism of formation of exon skipped Type B mRNA is unclear. In this case, Type B mRNA seems to be expressed as the same amount as Type A from the results of PCR. The cDNA skipped exons 2 and 3 has never been observed in the analysis of normal subjects or the other mutants. Thus, it was not considerable that the exons skipped fragment was the artifacts of PCR.

The mutations on the HPRT gene identified in this and the previous study (17) are summarized in Table 1. Of the five mutations detected in this study, three had

(A)

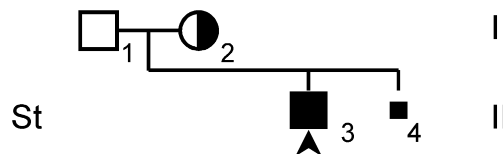
664 bp Normal 1059 bp

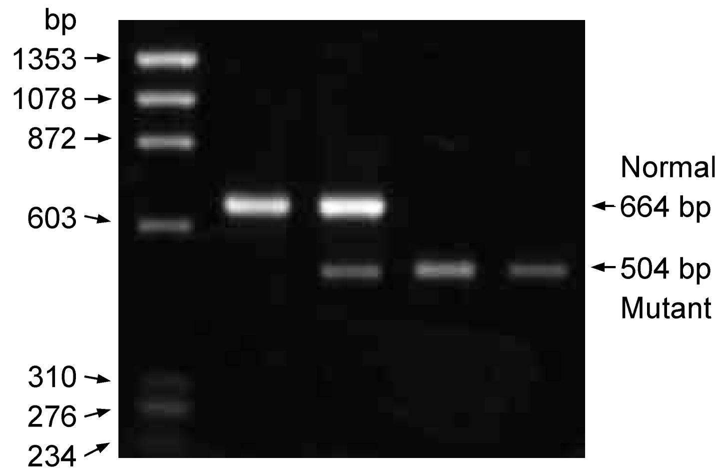
➤ - - ATTCCTATGACTGTAGATTTTATCAGA - - ➤

➤ - - ATTCCTATGACT - - AGATTTTATCAGA - - ➤

504 bp *Bfa* I Exon 3

Mutant





(B)

	(exon 1)*(exon 2)	(exon 3)	(exon 3)*(exon 4)
Normal	GTC.GTG.ATT.AGT. -	ACT.GTA.GAT -	.TAT.TGT.AAT.GAC
	Val Val Ile Ser	Ser Val Asp	Tyr Cys Asn Asp
Type a	GTC.GTG.ATT.AGT. -	ACT. AGA.T -	TA.TTG.TAA.TGA.C
	Val Val Ile Ser	Ser *Arg*	*Leu stop*
Type b	GTC.GTG.	- - skip exon 2 & 3, 291 bp - -	.AAT.GAC
	Val Val	- - - del. 97 amino acids - - -	Asn Asp

Fig. 4. Analysis of Family D. **A:** Electrophoresis(1.5% agarose gel) after *Bfa* I digestion of the HPRT genomic DNA. The genomic DNA fragments were amplified using primers HGE3A and HGE3B from a normal control, the family members and the amniotic cells of the mother's second conception, and then digested by *Bfa* I. The ϕ X174/*Hae* III digest was used as standard DNA size markers (St). **B:** Alterations in the cDNA.

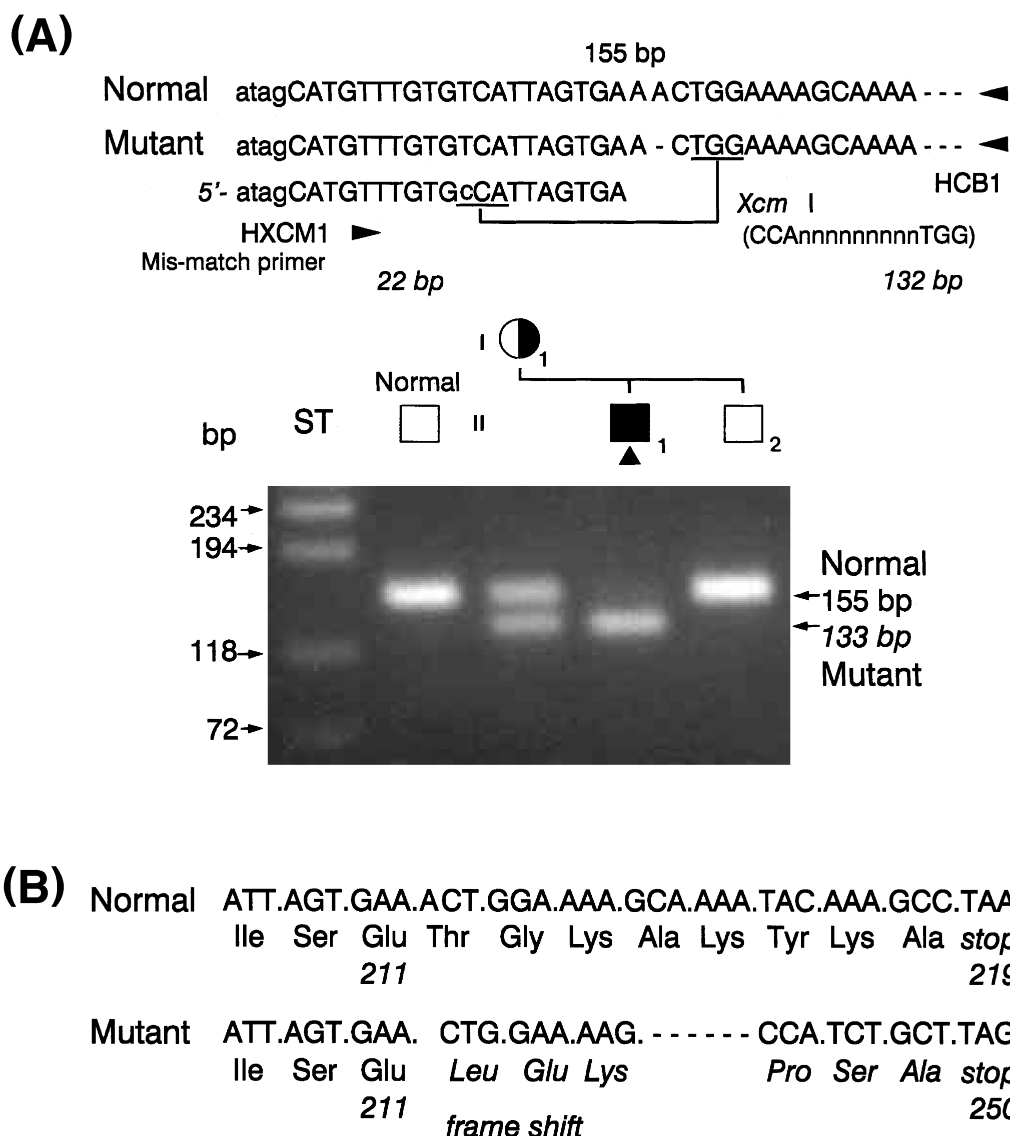


Fig. 5. Analysis of Family E. **A :** Electrophoresis(3.0% NuSieve 3 : 1 agarose gel) after *Xcm* I digestion of the HPRT genomic DNA. The genomic DNA fragments were amplified using a mis-match primer HXCM1 and HGE9B from a normal control and the family members, and then digested by *Xcm* I. The *ϕ*X174/*Hae* III digest was used as standard DNA size markers(St). **B :** Alteration in the cDNA.

been reported previously (10, 11, 14, 16), but we first discovered the existence of secondary abnormal mRNAs with splicing error in the two reported mutations (Q109X and 289delGT). Two mutants (533- 9TA and 631delA) are represented as new mutations for HPRT deficiency.

Acknowledgments

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Table 1. Mutations responsible for HPRT deficiencies

Case	Mutant	Phenotype	Genomic DNA change (No. gnt.[a])	Restriction site change	cDNA change (No. cnt.[b])	Amino acid change (codon)	References
	Y72C	acute renal failure	A to G (16683)	–	A to T (214)	Tyr to Cys (72)	Choi et al. 1993 (17)
1 & 2	310insG	L-N	G insertion (16675)	–	G insertion (310)	frame-shift (72) stop (73)	Family A (present study)
3	533-9T→A	L-N	T to A (40024)	lost *Hinf* I	Skip exon 8	frame-shift (178) stop (183)	Family B (present study)
4	Q109X	L-N	C to T (27897)	created *Spe* I	A) C to T (325) B) Skip exon 4	Gln to stop (109) 22 amino acid deletion	Family C (present study)
5	289delGT	L-N	GT deletion (16757-8)	created *Bfa* I	A) GT deletion (289 & 290) B) Skip exon 2 & 3	frame-shift (97) stop (106) 97 amino acid deletion	Family D (present study)
6	631delA	L-N	A deletion (41473)	–	A deletion (631)	frame-shift (211) stop (250)	Family E (present study)

[a] Base 1 of the genomic nucleotide sequence(gnt.) is G in the first *Eco* RI site 5' upstream from exon 1 in the HPRT gene(4).
[b] Base 1 of the cDNA nucleotide sequence(cnt.) is A in the initiation codon(ATG) in HPRT cDNA(5).

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