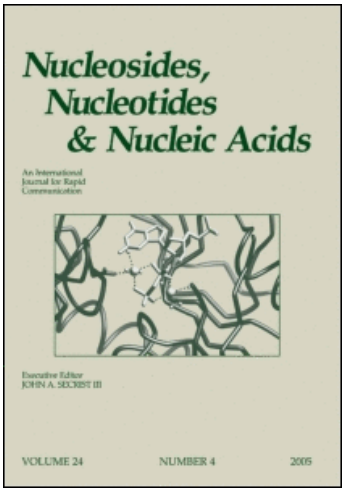

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MOLECULAR ANALYSIS OF HYPOXANTHINE GUANINE PHOSPHORIBOSYLTRANSFERASE (HPRT) DEFICIENCIES: NOVEL MUTATIONS AND THE SPECTRUM OF JAPANESE MUTATIONS

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□ *Inherited mutation of hypoxanthine guanine phosphoribosyltransferase, (HPRT) gives rise to Lesch-Nyhan syndrome or HPRT-related gout. We have identified a number of HPRT mutations in patients manifesting different clinical phenotypes, by analyzing all nine exons of the HPRT gene (HPRT1) from genomic DNA and reverse transcribed mRNA using the PCR technique coupled with direct sequencing. Recently, we detected two novel mutations: a single nucleotide substitution (430C> T) resulting in a nonsense mutation Q144X, and a deletion of HPRT1 exon 1 expressing no mRNA of HPRT. Furthermore, we summarized the spectrum of 56 Japanese HPRT mutations.*

Keywords HPRT; deficiency; mutations; Lesch-Nyhan syndrome; Kelley-Seegmiller syndrome

INTRODUCTION

Inherited mutations of a purine salvage enzyme, hypoxanthine guanine phosphoribosyltransferase (HPRT, EC 2.4.2.8; MIM308000), give rise to Lesch-Nyhan syndrome (MIM300322) or HPRT-related gout, the latter referred to as Kelley-Seegmiller syndrome (MIM300323). In contrast with the most severe phenotype of classical Lesch-Nyhan disease (LND), the least severe phenotype is characterized by hyperuricemia without any neurological or behavioral abnormality, and designated HPRT-related hyperuricemia (HRH). In between these two extremes are phenotypes

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involving hyperuricemia and varying degrees of neurobehavioral abnormality but without self-injury, designated HPRT-related neurological dysfunction (HRND). We have identified a number of HPRT mutations in patients manifesting different clinical phenotypes by analyzing all nine exons of the HPRT gene (*HPRT1*; located in Xq26.1) from genomic DNA and reverse transcribed mRNA using the PCR technique coupled with direct sequencing.[1] In this study, we identified two novel mutations and summarized the spectrum of Japanese HPRT mutations.

MATERIALS AND METHODS

Only 0.5–1.0 ml of peripheral blood from the subjects is enough to investigate the HPRT deficiency, covering the measurement of enzyme activities in the erythrocyte, and the molecular analyses of genomic DNA and cDNA reverse-transcribed from the mRNA. All the methods for *HPRT1* analysis, identification of the genomic mutation and the altered mRNA, were described previously.[1,2] DNA sequences were determined according to the simplified direct sequencing method as described previously.[2]

RESULTS

Two novel mutations were detected in two LND families after our last report.[1] One of them, a new single nucleotide substitution (430C>T) resulting in a nonsense mutation Q144X was detected in exon 6 of *HPRT1*. The expression of HPRT mRNA in the patient was decreased, since no cDNA fragment was amplified by usual RT-PCR method. However, the secondary PCR from the products of the first PCR using inner primers resulted in a small amount of two DNA fragments. In one fragment having normal size, the nonsense mutation Q144X was found. The shorter fragment was missing exon 6, which generated a frame-shift (135fs152X). The nucleotide substitution (430C>T) resulted in the creation of a new *Acc* I restriction site and the loss of a *Mwo* I site. PCR-RFLP analyses using these sites are effective for detection of mutations in prenatal genetic diagnoses. In the other LND patient, a deletion of exon 1 of *HPRT1* was revealed from genome PCR amplifications and no expression of HPRT mRNA was observed by RT-PCR. Detailed analyses of the breakpoint of this deletion are now in progress. Furthermore, we detected a 2 bp deletion (333delAG) resulting in a frame-shift (111fs120X) in exon 4 from another Japanese LND patient. This mutation has been reported previously in a Spanish patient.[3]

DISCUSSION

Marked genetic heterogeneity of HPRT deficiency is well known. More than 300 different mutations have been identified at the HPRT gene locus,

TABLE 1 Point mutations in *HPRT1* from Japanese HPRT-deficient families

Mutation	Location	mRNA	Amino acid	Phenotype
179A>G	exon 3	179A>G	H60R	Normal
68G>T	exon 2	68G>T	C23F	HRH
73C>A	exon 2	73C>A	P25T	HRH
215A>G	exon 3	215A>G	Y72C	HRH
370A>C	exon 4	370A>C	T124P	HRH
472G>T (2)	exon 6	472G>T	V158F	HRH
554A>G	exon 8	554A>G	D185G	HRH
575C>T	exon 8	575C>T	A192V	HRH
584A>G	exon 8	584A>G	Y195C	HRH
440T>C	exon 6	440T>C	L147P	HRND
475A>G	exon 6	475A>G	K159E	HRND
563T>C	exon 8	563T>C	V188A	HRND
27+5G>A	intron 1	normal; ins 49-bp	normal; 10fs27X	HRND
532+2T>C	intron 7	normal; ins 4-bp; skip exon 7	normal; 178fsl83X; 163fsl66X	HRND
29T>G	exon 2	29T>G	I9S	LND
74C>G	exon 2	74C>G	P24R	LND
131A>T	exon 2	131A>T	D44V	LND
151C>T (8)	exon 3	151C>T	R51X	LND
160A>C	exon 3	160A>C	M54L	LND
190G>C (2)	exon 3	190G>C	A64P	LND
194T>C	exon 3	194T>C	L65P	LND
208G>A	exon 3	208G>A	G70R	LND
209G>A (2)	exon 3	209G>A	G70E	LND
233T>A	exon 3	233T>A	L78Q	LND
415A>C	exon 6	415A>C	T139P	LND
419G>A	exon 6	419G>A	G140D	LND
430C>T	exon 6	430C>T; skip exon 6	Q144X; 135fsl52X	LND
486C>G	exon 7	486C>G	S162R	LND
27+1 G>T	intron 1	ins 49-bp	10fs27X	LND
28-lG>C	intron 1	skip exon 2	10fsl2X	LND
319-1G>T	intron 3	del 9-bp; skip exon 4	107del3aa; 107del22aa	LND
533-9T>G	intron 7	skip exon 8	178fsl83X	LND
538G>A	exon 8	skip exon 8	178fsl83X	LND
610-1G>A	intron 8	del 17-bp	H204X	LND

Newly detected mutations are indicated with **bold**. Numbers of families unrelated are shown in parentheses.

*Mutations in female patients.

including deletions, insertions, duplications, abnormal splicing, and point mutations at different sites of the coding region from exons 1 to 9.[1,4–6] A more complete list of *HPRT1* mutations is posted in the research sections at www.LESCH-NYHAN.ORG. The clinical differences between patients with HPRT deficiency and those with partial phenotypes cannot be explained by differences in locations of mutations, since these overlap considerably among the groups. However, patients with partial phenotypes are more likely to have mutations predicted to allow some residual enzyme function, and usually do not have early stop mutations, deletions, insertions, or more complex rearrangements. In two special HRND patients having splice site

TABLE 2 Insertion, deletion and other mutations in Japanese HPRT-deficient families

Mutation	Location	mRNA	Amino acid	Phenotype
330insA	exon 4	330insA	lllfs121X	LND
435insTTTG	exon 6	insTTTG	127fsl35X	LND
475ins6-bp	exon 6	insAAGGCT	159insKV	LND
SOdelA (2)	exon 2	SOdelA	17fs41X	LND
404delA	exon 6	404delA	135fsl36X	LND
635delG	exon 9	635delG	212fs250X	LND
317delGT(2)	exon 3 - intron 3	317delGT	C106X	LND
		skip exons 2&3	10del97aa	
333delAG	exon 4	333delAG	lllfsl20X	LND
22delGTC	exon 1	del 3-bp	VSdel	LND
82delTAT	exon 2	del 3-bp	Y28del	LND
319delAATG	intron 3 - exon 4	del 4-bp	107fsll3X	LND
		del 9-bp	107del3aa	
		skip exon 4	107del22aa	
609+ldelGT	exon 8 - intron 8	skip exon 8	178fsl83X	LND
610-16del74-bp	intron 8 - exon 9	del 58 ins 26-bp	204fs233X	LND
648del58-bp	exon 9		K217X (del 2aa)	HRH
de12969-bp (2)	~ exon 1~ intron 1	no mRNA	---	LND
de14131-bp	intron 4 - intron 6	del exons 5,6	129fsl43X	LND
		del 101 ins 28-bp	129fsl99X	LND
del ~ 15 kb	~ intron 1	no mRNA		LND
del exon 1	~ exon 1~ ?	no mRNA		LND
del exons 2,3	exons 2,3 ?	skip exons 2&3	10del97aa	LND
total deletion	whole	no mRNA		LND
translocation	intron 3	(-) splicing	107fsll3X	LND
		(+) splicing	107fsl09X	
536TTG>GTA	intron 8	536T>G & 538G>A	V179G & G180R	HRND

Newly detected mutations are indicated with **bold**. Numbers of families unrelated are shown in parentheses.

*Mutations in female patients.

mutations, major mRNA species were either absent or aberrant, but a small proportion of correctly spliced transcript allowed a very low level of normal HPRT protein to be translated. In the splice site mutations, we detected two or three types of abnormal mRNA Tables 1 and 2). Four main groupings within the HPRT deficiency spectrum was shown by Jinnah et al.[4] The spectrum of Japanese mutations fits that which emerged from the worldwide analysis. There are no racial differences for HPRT mutations, whereas the frequency of the nonsense mutation R51X (8/69, 11.6%) was bit higher than elsewhere in the world.

In contrast to some genetic diseases in which one or a small number of mutations account for the majority of patients, HPRT deficiencies are caused by multiple different mutations affecting nearly all parts of the HPRT gene. The nonsense mutation R51X, which is considered a hot spot for mutation of HPRT and detected in eight families, comprises less than 10% of all HPRT mutations described. Therefore, the identification of the mutation in each family with HPRT deficiency must be carried out in advance of prenatal

diagnosis. The appearance of new restriction sites and the loss of restriction sites by mutations are effectively used for the diagnosis by PCR-RFLP.

Since LND is a severe X-linked recessive neurological disorder, patients are usually males. However, seven unusual cases of LND in females including our two cases were found.[7–9] The genotypes of all seven female patients were heterozygous for mutations similar to the carriers with no symptoms. Nonrandom X-inactivation of the normal HPRT allele seems to cause LND in females.

In summary, the molecular study of the deficiency of HPRT associated with X-linked recessive neurological disorders has made remarkable progress. More than 300 mutations responsible for LND and the related partial syndrome have been identified in the world. A method for the prenatal diagnosis of the mutant gene has also been established. Future gene therapy approaches, which aims to augment the missing or defective HPRT gene, may be helpful in ameliorating the severity of the disease.

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