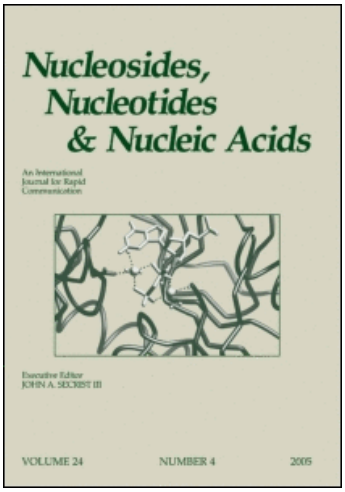

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Molecular Analysis of Two Enzyme Genes, *HPRT1* and *PRPS1*, Causing X-Linked Inborn Errors of Purine Metabolism

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MOLECULAR ANALYSIS OF TWO ENZYME GENES, *HPRT1* AND *PRPS1,* CAUSING X-LINKED INBORN ERRORS OF PURINE METABOLISM

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□ *Inherited mutation of hypoxanthine guanine phosphoribosyltransferase (HPRT) gives rise to Lesch-Nyhan syndrome or HPRT-related gout. On the other hand, PRPS1 mutations cause PRPP synthetase superactivity associated with hyperuricemia and gout, sometimes including neurodevelopmental abnormalities. We have identified two mutations in two Lesch-Nyhan families after our last report. One of them, a new single nucleotide substitution (130G> T) resulting in a missense mutation D44Y was detected in exon 2 of HPRT1. RT-PCR amplification showed not only a cDNA fragment with normal size, but also a small amount of shorter fragment skipping exons 2 and 3. The other missense mutation F74L (222C > A) was detected in a Japanese patient but has been reported previously in European families. In four hyperuricemic patients with mild neurological abnormality, no mutations responsible for partial HPRT deficiency were identified in HPRT1. In these four patients, we also performed molecular analysis of PRPS1, but no mutations in PRPP synthetase were found.*

Keywords HPRT; PRPP synthetase; mutations; hyperuricemia; Lesch-Nyhan syndrome

INTRODUCTION

Deficiencies in the activity of a purine salvage enzyme, hypoxanthine guanine phosphoribosyltransferase (HPRT, EC 2.4.2.8; MIM308000) are associated with Lesch-Nyhan syndrome (MIM300322) and HPRT-related gout (MIM300323). 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 at Xq26.1) from genomic DNA and reverse

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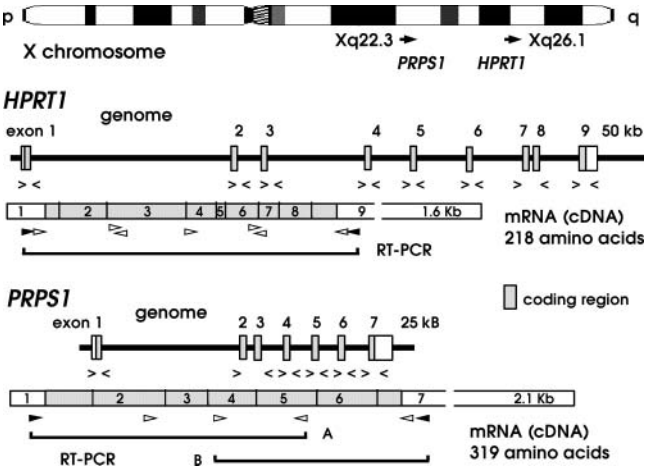

FIGURE 1 Molecular analysis of *HPRT1* and *PRPS1*. All nine exons of *HPRT1* are amplified as eight separate DNA fragments, and seven exons of *PRPS1* as 6 separate fragments, using specific primer pairs (> <), respectively. The entire coding region of HPRT cDNA is amplified as an 871-bp DNA fragment, and two fragments (A: 760 bp, B: 621 bp) covering the PRS1 cDNA are amplified.

transcribed mRNA using the PCR technique coupled with direct sequencing.[1,2] On the other hand, PRPP synthetase superactivity (MIM300661) is an X-linked inborn error of metabolism in which increased enzyme activity is associated with hyperuricemia and gout, sometimes including neurodevelopmental abnormalities. Human PRPP synthetase (PRPS; EC 2.7.6.1) exists as heterogeneous aggregates composed of two catalytic subunits (PRSI and PRSII) and two associated proteins. Mutations of *PRPS1* (PRSI subunit gene; MIM311850) were found in patients with PRPS superactivity.[3,4] Recently, in affected males with X-linked recessive Charcot-Marie-Tooth disease-5 (CMTX5; MIM311070), *PRPS1* mutations responsible for decreased enzyme activity were identified.[5] Furthermore, the mutations resulting in a loss of PRPS activity with hypouricemia were also identified in patients with Arts syndrome (MIM301835).[6] In this study, we have performed molecular analysis of two enzyme genes, *HPRT1* and *PRPS1,* causing X-linked inborn errors of purine metabolism (Figure 1).

MATERIALS AND METHODS

All the methods for molecular analysis of *HPRT1* and *PRPS1*, identification of the genomic mutation and the altered mRNA, have been described previously.[7] The primers for the analysis of *PRPS1* was designed according to the previous reports.[3–6] Only 0.5 ml of peripheral blood from the subjects is enough to investigate the two genes, covering analyses of genomic DNA

and cDNA reverse-transcribed from the mRNA. DNA sequences were determined according to the simplified direct sequencing method as described previously.[7]

RESULTS AND DISCUSSION

A list of more than 300 different *HPRT1* mutations is posted in the research section at www.lesch-nyhan.org. Marked genetic heterogeneity of HPRT deficiency is well known. In this study, performed after our last report,[2] we detected two mutations in families with children with Lesch-Nyhan syndrome. One mutation is a new single nucleotide substitution (130G > T) resulting in a missense amino acid shift (D44Y) in exon 2 of *HPRT1*. RT-PCR amplification showed not only a cDNA fragment with normal size, but also a small amount of shorter fragment skipping exons 2 and 3. In prior splice site mutations, we have sometimes detected two or three types of abnormal mRNA.[2] Expression of abnormal mRNA skipping exons 2 and 3 was also found in other reported mutations. The expression of mRNA with normal size may be decreased in the patient with the mutation D44Y. As suggested by Garcia et al.[8] the exons 2 and 3 deleted mRNA may be normal constitutions of human cells and a decrease in normal mRNA expression could explain their presence. Our second missense mutation F74L (222C > A) was detected in a Japanese patient, but has been reported previously in European families.[9,10] In four hyperuricemic male patients with mild neurological abnormalities, no mutations in *HPRT1* responsible for partial HPRT deficiency were identified. Consequently, we also performed molecular analysis of *PRPS1* in these individuals, using PCR and direct sequencing of genomic DNA and mRNA. No mutations or abnormalities likely to generate PRPS superactivity were found.

CONCLUSION

We have identified two *HPRT1* mutations (D44Y, F74L) in two Lesch-Nyhan families. One of them, D44Y is a newly detected mutation. No *HPRT1* mutations were detected in four hyperuricemic patients with mild neurological abnormalities (resembling partial HPRT deficiency). In those four patients, however, no abnormalities in *PRPS1* were detected.

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