Identification of a New Point Mutation in Hypoxanthine Phosphoribosyl Transferase Responsible for Hyperuricemia in a Female Patient

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A 29-year-old woman was referred to our department because of gout. Routine laboratory data showed hyperuricemia, a high level of plasma oxypurines, increased urinary uric acid excretion, and increased urinary oxypurine excretion, with decreased hypoxanthine phosphoribosyl transferase (HPRT) activity in the erythrocytes. From these findings, the patient was diagnosed with a partial deficiency of HPRT. To determine its properties, a cDNA sequence encoding HPRT and the androgen receptor AR XIST minimal promoter gene, as well as methylation of the AR gene were investigated. The HPRT cDNA sequence revealed a point mutation of G to A in nucleotide 40, which changed codon 14 from GAA (Glu) to AAA (Lys) in the mutant gene. In addition, the HPRT genomic DNA sequence, including the mutation site, revealed the same point mutation, indicating that the patient was heterozygote. Further analysis of the AR gene on the X chromosome suggested nonrandom X-chromosome inactivation, whereas the AR XIST minimal promoter gene was normal. Such results have not been previously reported in a female with partial HPRT deficiency.
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HYPOXANTHINE phosphoribosyl transferase (HPRT) is an important enzyme that converts hypoxanthine to inosine monophosphate (IMP), as well as guanine to guanosine monophosphate (GMP), using phosphoribosyl pyrophosphate (PRPP). Since HPRT is an enzyme that salvages hypoxanthine and guanine, most of these purine bases are metabolized to uric acid, which leads to hyperuricemia in HPRT deficiency. A complete deficiency of HPRT is called Lesch-Nyhan disease, first reported by Lesch and Nyhan,[1] and is characterized by mental retardation or self-mutilation, choreoathetosis, gout, renal damage, and urolithiasis. Lesch-Nyhan disease is an inherited sex-linked recessive trait. Therefore, female patients with Lesch-Nyhan disease are very rare.[2-4] Each of those cases was heterozygous, in whom a mutant HGPRT gene was expressed and normal *HPRT* gene was not. On the other hand, no female patient with partial deficiency of HPRT has been reported, whereas males with the partial deficiency and characterized by gout have been occasionally seen.[5,6] We recently observed a female with partial HPRT deficiency, which provided an opportunity to investigate HPRT, as well as the androgen receptor (AR) genes and promoter genes of AR.

MATERIALS AND METHODS

Patient

A 29-year-old woman (body weight, 52 kg; height, 158 cm) was referred to our department because of gout, as she occasionally suffered from gouty attacks of the left ankle joint and had a history of urinary calculi, although there was no family history of gout. A physical examination showed an inflamed left foot joint, which disturbed her walking without support. Routine laboratory findings showed no abnormal data for serum AST, serum ALT, serum alkaline phosphatase, serum creatinine, serum cholesterol, serum triglyceride, or complete blood cell count, while serum uric acid (548 μmol/L), C-reactive protein (0.4 mg/dL), and erythrocyte sedimentation rate (28 mm/h) levels were elevated.

Erythrocyte HPRT, PPRP, and Adenine Phosphoribosyl Transferase Activities

Erythrocyte HPRT, adenine phosphoribosyl transferase (APRT), and PRPP activities were determined using high-performance liquid chromatography (HPLC) as described previously.[7,8]

Determination of Hypoxanthine, Xanthine, and Uric Acid Concentrations

The concentrations of hypoxanthine and xanthine were determined by HPLC as described previously,[9] while that of uric acid was determined by a uricase method, using a Wako Uric Acid Kit (Wako Pure Chemical Ltd, Osaka, Japan).

Direct Sequencing of cDNA

Direct sequencing of cDNA was performed as described as previously.[10] Total RNA was obtained from mononuclear cells using a chloroform-phenol extraction method. The forward and reverse primers for −46 to 351 of the HPRT cDNA were CCTCCTCTGCTCCGCCACCG and GTTGAGAGATCATCTCCACC, respectively, and the forward and reverse primers for 247 to 692 of the HPRT cDNA were CTGACCTGCTGGATTACATC and ACTGGCGATGTCAATAGGAC, respectively.

The polymerase chain reaction (PCR) primers and conditions for AR cDNA amplifications have been described previously.[11]

Direct Sequencing of Genomic DNA Including Point Mutation Site

Genomic DNA was extracted from blood using a DNA Quick II kit (Dainippon Pharmaceuticals, Osaka, Japan). PCR was performed as described previously with the forward primer (TGGCCTGATGTTACGTCTGC) designed from intron 1of HPRT gene, and the reverse primer (CCTGGCCTATTAATCCAGCG) designed from intron 2 of HPRT gene. The forward and reverse primers for the XIST minimal promoter were GTTGTGACTCCTGGTCTAGA and CATAAAGGGTGTGGGGGAC, respectively.

Methylation at the AR Locus

One hundred fifty nanograms of DNA was digested in a 30-μL reaction volume with 10 U of *Rsa*I and 15 U *Hpa*II in buffer 1 (Roche,

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Manheim, Germany) for 16 hours at 37°C. The enzyme was heat-inactivated for 10 minutes at 95°C, and then digestion samples (3 μL) were amplified with the forward (TCCAGAATCTGTCCA-GAGCGTGC) and reverse (GCTGTGAAGGTTGCTGTTCCTCAT) primers, as described previously.[10] Amplified DNA products were electrophoresed on 8% polyacrylamide gels as described previously.[10] In addition, they were sequenced using a dye-termination method with a DNA sequencing kit (Perkin-Elmer, Foster, CA) using the same primers as for the PCR, and then analyzed with an ABI PRISM 310 (Applied Biosystems, Foster, CA).

Reagents

*Rsa*I, *Hpa*II, and buffer 1were purchased from Roche. Other products were purchased from Wako Pure Chemicals.

RESULTS

Laboratory Data

The plasma concentration of uric acid was 583 μmol/L, that of hypoxanthine 4.2 μmol/L (reference range, 1.1 to 3.0 μmol/L), and that of plasma xanthine 1.5 μmol/L (reference range, 0.7 to 1.2 μmol/L). The urinary excretion of hypoxanthine was 9.1 μmol/h (reference range, 4.0 to5.7 μmol/h), that of xanthine 6.2 μmol/h (reference range, 3.5 to 4.6 μmol/h), and that of uric acid 5.67 mmol/d.

The activity of HPRT in erythrocyte samples from the patient was 0.52 μmol/g hemoglobin (Hb)/min (reference value, 2.00 ± 0.39 μmol/g Hb/min), PRPP synthetase activity in erythrocyte was 1.10 μmol/g Hb/min (reference value, 1.15 ± 0.13 μmol/g Hb/min), and APRT activity in erythrocyte was 0.72 μmol/g Hb/min (reference value, 0.41 ± 0.17 μmol/g Hb/min). From these findings, the patient was diagnosed with a partial HPRT deficiency.

Sequence of HPRT cDNA and AR cDNA From Mononuclear Cells

A DNA sequence analysis of HPRT cDNA from mononuclear cells demonstrated both normal and mutant HPRT genes. A point mutation of G to A in nucleotide 40, which changed codon 14 from GAA (Glu) to AAA (Lys), was shown.

To examine methylation in the AR locus, a 280-bp strand including the flanking *Hpa*II and *Hha*I sites and the trinucleotide repeat element, was developed for the AR locus on the X-chromosome. When using this technique, amplification will occur in the methylated restriction site. However, when the restriction sites are not methylated, digestion will occur between the flanking oligonucleotides and amplification will not occur. Since methylation of the flanking oligonucleotides correlates with X-chromosome inactivation, amplification occurs only when the X-chromosome is inactive. In the present patient, amplification without AR gene digestion showed 2 bands (Fig 1). However, amplification following AR gene digestion showed a single band, suggesting nonrandom X-chromosome inactivation. In her father, amplification without AR gene digestion showed a single band, whereas amplification following AR gene digestion showed no visible band. On the other hand, in her mother amplification revealed 1 band following AR gene digestion as well as following amplification without AR gene digestion (Fig 1). Sequence analysis of both amplified PCR products with and without AR digestion showed that 1 PCR product was 3 bases longer than the other (data not shown), suggesting that 2 amplified PCR products of the AR gene were not separated, because the 2 PCR products were similar in size.

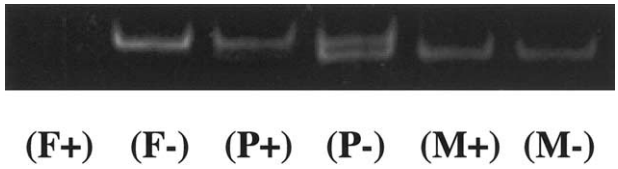

Fig 1. Amplification following AR gene digestion. In the patient, amplification with and without AR gene digestion showed 1 and 2 bands, respectively, suggesting nonrandom X-chromosome inactivation. In her father, amplification with and without AR gene digestion shows no and 1 band. In her mother, amplification with and without AR gene digestion both showed 1 band because the 2 amplified genes were different in size by only 3 bases. (P+) and (P-), (F+) and (F-), and (M+) and (M-) denote results of amplification with and without AR gene digestion in the patient, her father, and her mother respectively.

Sequence of Genomic DNA Including the Mutation Site

The present patient showed a partial HPRT deficiency and a point mutation of G to A in exon 2 of HPRT genomic DNA, indicating that she was heterozygous for this mutation, while no mutation of the XIST minimal promoter region was found. In her parents, no point mutation was found in exon 2 of HPRT genomic DNA, indicating that the mutation was not inherited.

DISCUSSION

We examined a female patient with gout, but without a family history of hyperuricemia and diagnosed her with a partial deficiency of HPRT. Previously, 5 female cases with complete HPRT deficiency were reported.[2-4,12] However, there is no known report of a female patient with partial HPRT deficiency (Kelley-Seegmiller syndrome). Both mutant and normal HPRT genes on the X-chromosome are expressed randomly in heterozygote females. Further, a selection against HPRT-deficient blood cells skews this population toward normal cells and results in normal HPRT activity, although both mutant and normal HPRT genes are expressed in fibroblasts due to random inactivation of the X-chromosome. In 5 previously described heterozygote females with a complete deficiency of HPRT,[12] only the mutant HPRT gene was selected and expressed. In heterozygote females, a mutant HPRT gene is not usually expressed in blood cells since a normal HPRT gene is selected. However, in the present patient, a selection against HPRT-deficient blood cells did not skew toward normal cells. Further, amplification following AR gene digestion showed nonrandom X-chromosome inactivation, suggesting that the mutant HPRT gene was expressed more often in this present patient. Therefore, the mutant HPRT gene was speculated to be the cause of gout. Although the nature of the mutation provides a rough guide for determining phenotypic severity, it is considered that similar mutations may sometimes produce dissimilar enzyme activity and phenotypic severity, based on the current database of many HPRT mutations. In addition, since nonrandom X-chromosome inactivation occurred in the present female case, it is very difficult to interpret the relationship

between the mutation and phenotypic severity. In a recent study of 271 cases with 218 different mutations,[12] 200 cases were associated with the Lesch-Nyhan disease phenotype and 63 with less severe forms of the disease (Kelly-Seegmiller syndrome or mild Lesch-Nyhan disease), while 8 could not be categorized from the clinical information available. However, genotype–phenotype correlations provide no indication that specific disease features are associated with specific mutation locations. Accordingly, identification of this point mutation with concurrent measurement of HPRT activity can confirm the diagnosis of HPRT deficiency. However, it does not predict phenotypic severity in male patients with this mutation.

In conclusion, a female patient suffering from gouty arthritis was diagnosed with a partial deficiency of HPRT by measurement of erythrocyte HPRT as well as the analysis of HPRT and AR genes. She was found to possess a single HPRT base substitution of G to A in nucleotide 40, which changed codon 14 from GAA (Glu) to AAA (Lys). This mutation has not been previously reported. In addition, nonrandom X-chromosome inactivation was suggested from results of investigation of methylation at the AR locus. This heterozygote with a partial deficiency of HPRT is the first known female case to be reported.

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