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Carrier Detection of Partial Hypoxanthine-Guanine Phosphoribosyltransferase Deficiency by Analysis with *Bam*HI Restriction Fragment Length Polymorphisms and Oligonucleotide Probes

TAKASHI IGARASHI AND SHIGEHIKO KAMOSHITA

Department of Pediatrics, Faculty of Medicine, The University of Tokyo, Tokyo 113, Japan

ABSTRACT. Hyperuricemic nephropathy can progress to the permanent renal damage even in infancy in partial hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency. We have encountered two unrelated patients with partial HPRT deficiency, and found that early detection of the disease and long-term management for hyperuricemia were necessary to prevent renal impairment. The HPRT gene is situated in the q26-27 region of the long arm of the X-chromosome, and females with mutant HPRT alleles are heterozygous for the disease, and they develop gout after menopause. We undertook the investigation of carriers in the two patients' families, using *Bam*HI restriction fragment length polymorphisms and oligonucleotide probes that recognized the specific mutations within the HPRT gene. We also demonstrated that the allele frequencies of *Bam*HI restriction fragment length polymorphisms in 62 Japanese females were 0.36 for the 22-kb/25-kb allele, 0.41 for the 12-kb/25-kb allele, and 0.23 for the 22-kb/18-kb allele, resulting in a heterozygous state in 66% of females. (*Pediatr Res* 27: 417–421, 1990)

Abbreviations

HPRT, hypoxanthine-guanine phosphoribosyltransferase
RFLP, restriction fragment length polymorphisms
LN, Lesch-Nyhan

Deficiency of the enzyme HPRT (E.C.2.4.2.8) is associated with two clinical syndromes (1, 2). Complete absence of enzyme activity is responsible for LN syndrome, which is characterized by hyperuricemia, choreoathetosis, muscle spasticity, mental retardation, and compulsive self-mutilation (3). Patients with a partial deficiency of the enzyme activity are spared the devastating neurologic and behavioral abnormalities of LN syndrome (4). However, they present with hyperuricemia and a severe form of gout at an early age (5). Several recent reports have indicated that patients with partial HPRT deficiency or suspected LN syndrome can develop acute renal failure due to uric acid nephropathy in early infancy (6–8). Therefore, evaluation of the serum level of uric acid is necessary in male babies born to mothers who are the carriers of partial HPRT deficiency. If the male infant has hyperuricemia, fluid supplements and administration of allopurinol are necessary to prevent neonatal acute uric acid nephropathy. Carrier women have an overproduction of uric acid throughout their lives. After menopause, the endogenous production of uricosuric estrogens declines, and the serum uric acid level increases resulting in gout (9). Thus, a reliable method to detect the carrier state is needed to help both these patients groups.

HPRT enzyme assay in erythrocytes or lymphoblasts is not able to discriminate the carrier state from normal, as erythrocytes or lymphoblasts from carrier women often show normal HPRT enzyme activity (10). Carrier state detection in LN syndrome has been performed by the demonstration of mosaicism for $HPRT^+$ and $HPRT^-$ clones of fibroblasts or lymphocytes and by the demonstration of two populations of hair roots, those with normal and those with either zero or subnormal levels of HPRT activity (11–16). The use of cells from hair follicles imposes logistical problems, and there is also evidence that each hair follicle is not truly clonal but is derived from two or three different cells (13, 17). Furthermore, both of these methods are time-consuming and although carriers can be identified, it is difficult to be sure that a negative result is absolutely reliable. These methods have not yet been applied to detecting the carrier state of partial HPRT deficiency. The clones derived from 8-azaguanine- or 3-thioguanine-resistant cells from LN syndrome heterozygotes had the phenotype of HPRT-deficient cells since they did not incorporate hypoxanthine and had almost no HPRT activity. However, it was often impossible to demonstrate mosaicism with respect to $HPRT^+$ and partial $HPRT^+$ clones in partial HPRT deficiency; the partial $HPRT^+$ clone was often not resistant to either 8-azaguanine or 3-thio-guanine. Gibbs *et al.* (18) recently demonstrated the usefulness of *Bam*HI RFLP in diagnosis of the carriage of LN syndrome. Twenty of 28 women, whose carrier state could not be determined by pedigree analysis or who were apparently normal according to the biochemical tests, were diagnosed as definite carriers on the basis of the RFLP analysis.

We have recently shown molecular abnormalities in two unrelated patients with partial HPRT deficiency. *1*) Two single-base substitutions were detected at codon 179 (from GTT to GGT) and codon 180 (from GGA to AGA), resulting in the putative replacement of valine with glycine and glycine with arginine. *2*) The deletion of 51 nucleotides between nucleotides 747 and 797 was also found, resulting in the formation of a shorter HPRT mRNA and a putative two amino acid-deleted HPRT protein (19). In this study, we applied both oligonucleotide hybridization techniques and *Bam*HI RFLP analysis to identify carriers in two unrelated families with partial HPRT deficiency.

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Correspondence: Dr. T. Igarashi, Department of Pediatrics, Faculty of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan.
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Table 1. *Hypoxanthine-guanine phosphoribosyltransferase activity in erythrocytes**

Subject	HPRT activity (nmol/mg protein/min)
Controls	1.94 ± 0.24 (11)
Family A	
I.1	1.86
II.1	2.27
II.2	1.98
II.3	2.04
III.1	0.01
III.2	0.00
Family B	
II.3	2.05
II.4	1.97
III.1	0.19
III.2	2.24
III.3	2.11

* Control activity is the mean ± 1 SD for the number of individuals given in parentheses. Family member numbers are as in Figure 1.

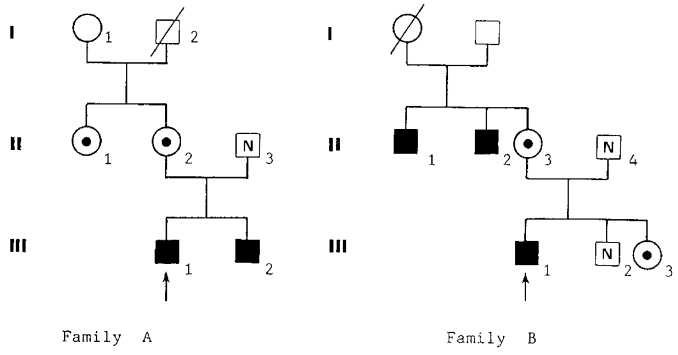

Fig. 1. Genealogic trees of two families affected with partial HPRT deficiency. *Diagonal lines* indicate dead family members. *Dark boxes* represent males affected with partial HPRT deficiency, *circles with dots* represent female carriers, and *N* represents family members with normal erythrocyte HPRT enzyme activity. The propositus is identified by an *arrow* in each family.

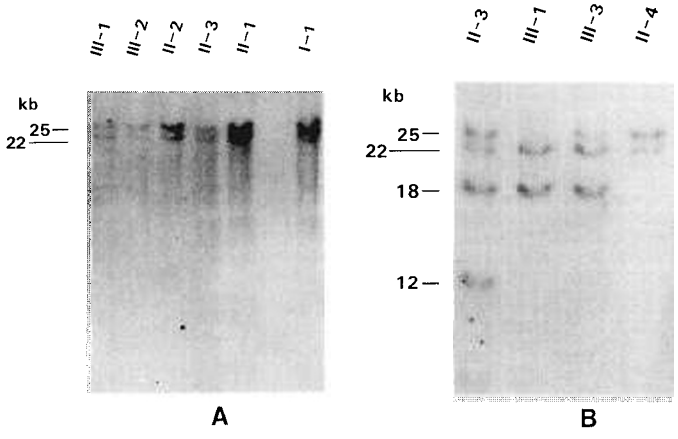

Fig. 2. Southern blot analysis of peripheral WBC HPRT DNA in two families A and B. *Numbers* on each *track* indicate the same family members as in Figure 1.

MATERIALS AND METHODS

Subjects. Nine subjects belonging to two families with partial HPRT deficiency were studied. In addition, 62 normal females were analyzed by Southern blotting to determine the average level of heterozygosity for *Bam*HI RFLP.

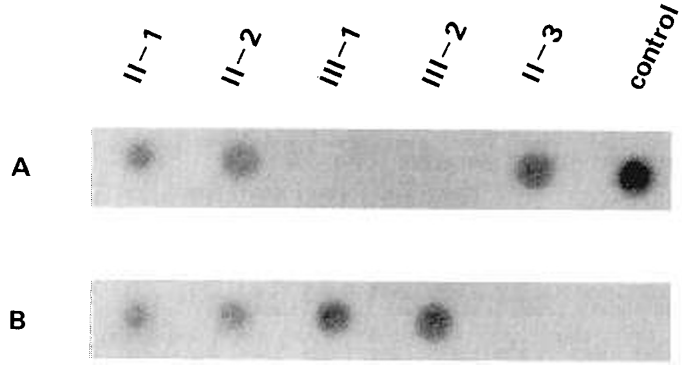

Fig. 3. Oligonucleotide hybridization analysis of genomic DNA from a normal individual, carriers, and patients in family A. *A* and *B* are autoradiographs of the membranes after hybridization with normal oligonucleotide probe A and mutant oligonucleotide probe B, respectively. *Numbers* on each *dot* indicate members of family A as in Figure 1. *Control* represents a normal female.

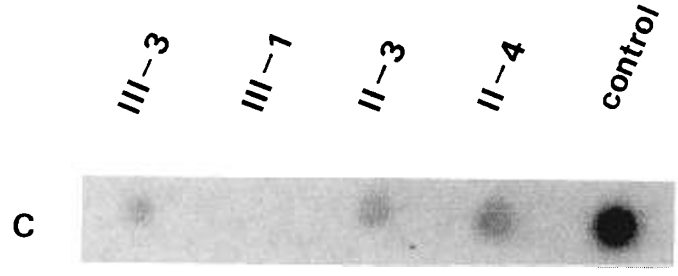

Fig. 4. Oligonucleotide hybridization analysis of genomic DNA from a normal individual, carriers, and the patient in family B. *C* is an autoradiograph of the membrane after hybridization with normal oligonucleotide probe C. *Numbers* on each *dot* indicate the members of family B as Figure 1. *Control* represents a normal female.

Red cell lysates and enzyme assays. Red cell lysates were prepared from heparinized venous blood by centrifugation at 70 × *g* for 10 min followed by removal of plasma and the buffy coat. Red cells were washed four times for an equal volume of PBS at 4°C. Packed red cells were suspended in an equal volume of 10 mmol phosphate (pH 7.3) and lysed by sonication. Lysates were centrifuged at 7000 × *g* for 30 min and the supernatants were recovered for assay at −70°C.

Sensitive radiochemical techniques were used to quantify HPRT enzyme activity in 50 mmol Tris-HCl (pH 7.4) containing 5 mmol $HgCl_2$, 1 mmol 5-phospho-D-ribose-1-pyrophosphate (Sigma Chemical Company, St. Louis, MO), 0.1 mmol [^{14}C]-hypoxanthine (sp act 50–55 mCi/mmol), and 1.5/2.5 mg/mL protein. Incubation was performed for 10–15 min at 37°C (20).

DNA isolation and Southern blot analysis. High mol wt DNA was isolated from peripheral blood samples as described in the literature (21). DNA samples of 7.5 μg were digested to completion with restriction endonuclease *Bam*HI (Boehringer Mannheim GmbH, Mannheim, FRG) at a concentration of 5 U/μL, and fractionated in 0.8% agarose gels (Ultrapure agarose, Bethesda Research Laboratories, Gaithersburg, MD) treated according to the method of Whal *et al.* (22). Samples were then transferred into nitrocellulose filters and hybridized with ^{32}P-deoxycytidine triphosphate labeled full-length HPRT cDNA (pHP31; provided by Dr. W. N. Kelley) (sp act of more than 3000 Ci/mmol, Amersham Japan, Tokyo, Japan). Probes were labeled to a sp act of 1-3 × 10^8 cpm/μg using a multiprime DNA labeling system (Amersham Japan). Hybridization was carried out at 42°C in a mixture containing 50% formamide. Hybridization patterns were visualized by autoradiography.

Oligonucleotide synthesis and dot-blot analysis of genomic

Table 2. *Survey of phenotypes and X chromosomes with BamHI RFLPs in unrelated Japanese females*

	Homozygous			Heterozygous			
A. Phenotypes of *Bam*HI RFLP							
RFLP	22/25	12/25	22/18	22/25	12/25	22/25	
Phenotype	22/25	12/25	22/18	12/25	22/18	22/18	Total
No. of females	7	11 (34%)	3	14	10 (66%)	12	62 (100%)
B. X chromosomes with *Bam*HI RFLP							
RFLP							
Phenotype		22/25		12/25	22/18		Total
No. of X chromosomes		45		51	28		124

DNA. Three 18 mer oligonucleotide probes were synthesized. Probe A (5′-TTCAAATCCAACAAAGTC-3′) was complementary to normal HPRT cDNA and ranged between nucleotides 628 and 645 [nucleotide numbers were those assigned by Jolly *et al.* (23)]. Mutant probe B(5′-TTCAAATCTACCAAAGTC-3′) was complementary to the putative mutant HPRT cDNA and ranged between nucleotides 628 and 645 from patient T.A. Probe C (5′-TCAACTTGAACTCTCATC-3′) was complementary to normal HPRT cDNA and ranged between nucleotides 757 and 774, the region that was considered to possibly be deleted from the HPRT cDNA of patient M.T. Genomic DNA samples of 20 μg were applied directly by the dot-blot method onto nitrocellulose filters and analyzed by hybridization with oligonucleotide probes. Probes were labeled by ^{32}P-adenosine triphosphate (sp act of more than 3000 Ci/mmol, Amersham Japan) using T4 polynucleotide kinase (New England BioLabs). The hybridization temperature was specific to each probe, being 43°C for probe A, 41°C for probe B, and 45°C for probe C (24). Hybridization patterns were visualized by autoradiography.

CASE REPORTS

Family A. T.A. was born without complications on 8/7/1977. The birth wt was 3100 g. Both parents were healthy and there was no history of gout in either parent's family. Large amounts of orange colored crystals were seen in the diapers when the patient was 3 mo old. Hyperuricemia (serum uric acid 6.8 nmol/L) was found, and administration of allopurinol was started by the local hospital. The erythrocyte HPRT activity was determined at Tokyo University Hospital. This was less than 0.7% of the normal value, and LN syndrome was suspected initially. With careful control, the serum uric acid level was kept less than 3.8 nmol/L and the renal function remained within normal limits. The patient showed normal development without any of the neurologic symptoms that are characteristic of LN syndrome. HPRT activity in B cell lymphoblasts was determined in 1979 to be 3.9% of the normal value. The low HPRT activity in his lymphoblasts, together with the clinical course, made the diagnosis partial HPRT deficiency. A brother was born normally on 9/21/1980. He also showed hyperuricemia (serum uric acid 7.1 nmol/L) and negative erythrocyte HPRT activity (less than 0.7% of the normal value), and was started on allopurinol. Both of them are currently healthy, without any attacks of gout, urolithiasis, renal impairment, or neurologic symptoms.

Family B. M.T. was born healthy on 12/19/1972. The mother's brothers had gout and were being treated with allopurinol. The patient had a urinary calculus in 1982, and several episodes of swelling of the right big toe joint in 1983 and 1984. He presented to Tokyo University Hospital in 1984. Hyperuricemia (serum uric acid 7.6 nmol/L) was found, and administration of allopurinol was started. Radiographic examination demonstrated periarticular bone resorption in the joints of the big toes. The creatinine clearance was 48 mL/min/1.73 m^2 and urinary β-2 microglobulin excretion was increased (4574 μg/day). The arterial concentration of HCO_3^- was 24 mmol/L. The erythrocyte HPRT activity was 9.8% of the control level, and therefore partial HPRT deficiency was diagnosed. He did not have any of the neurologic symptoms that are seen in LN syndrome. With treatment, the serum uric acid level was kept less than 3.8 nmol/L. However, the impairment of creatinine clearance and the hyper-β-2 microglobulinuria (3470 μg/day) persisted.

RESULTS

Erythrocyte HPRT enzyme activity (Table 1, Fig. 1). The erythrocyte HPRT enzyme activity levels are summarized in Table 1. HPRT enzyme activity was undetectable in erythrocytes (less than 0.7% of the control) from patients III.1 and III.2 of family A. It was decreased to 9.8% of the control level in patient III.1 from family B. Carrier mothers II.2 from family A and II.3 from family B showed normal HPRT activity. Members II.1 from family A (the sister of carrier mother) and III.3 from family B (the daughter of carrier mother) also showed normal HPRT activity.

Southern blot analysis of the HPRT gene (Fig. 2). *Bam*HII RFLP were expressed phenotypically on Southern blots as three distinct pairs of fragments in normal Caucasian and Japanese population: a 22-kb/25-kb pair (frequency in the Caucasian population 0.77); a 12-kb/25-kb pair (0.16); and a 22-kb/18-kb pair (0.07) (25).

In family A, members III.1 (the propositus) and III.2 (his brother) had the 22-kb/25-kb allele, and members I.1 (his grandmother, a suspected carrier), II.2 (the carrier mother) and II.1 (the sister) also showed the double 22-kb/25-kb pattern. Therefore, it was not possible to identify which of their X chromosomes carried the mutant gene, the *Bam*HI polymorphism analysis was not helpful.

In family B, member III.1 (the propositus) had the 22-kb/18-kb allele that therefore marked the X-chromosome carrying the mutant HPRT gene. Member II.4, the father of the propositus, had the 22-kb/25-kb allele, whereas member II.-3 (the carrier mother) showed the combination of a 22-kb/18-kb allele and a 12-kb/25-kb allele. Member III.3, the sister of the propositus, had the combination of 22-kb/25-kb and 22-kb/18-kb, and she was thus considered to be a carrier. The *Bam*HI polymorphism analysis was therefore quite informative in this family.

Dot-blot analysis with oligonucleotide probes (Figs. 3 and 4). Genomic DNA from family members II.1 (the sister of the carrier mother), II.2 (the carrier mother), and II.3 (the father) of family A and a family control hybridized to normal probe A, which was complementary to normal HPRT cDNA ranging between nucleotides 628 and 645. The control dot appeared to be twice the intensity of the dots from members II.2 and II.3 However, genomic DNA from family members III.1 (the propositus) and III.2 did not hybridize to the probe A. However, genomic DNA from members II.1, II.2, III.1, and III.2 of family A hybridized to the mutant probe B, which was complementary to a putative mutant HPRT cDNA ranging between nucleotides 628 and 645

in the propositus. Genomic DNA from members II.3 and a female control did not hybridize to the probe B. These results implied that member II.1 was a carrier. Although *Bam*HI RFLP analysis had not been informative in this family, analysis with patient-specific and normal oligonucleotide probes was able to diagnose a carrier.

Genomic DNA from members II.3 (the carrier mother), II.4 (the father), and III.3 (the daughter) in family B hybridized to the probe C, which was complementary to normal HPRT cDNA ranging between nucleotides 757 and 774. Genomic DNA from member III.1 (the propositus) did not hybridize to probe C, because the region complementary to the probe was included in the 51 nucleotide deletion in the HPRT gene of the patient. The dot intensity of the carrier mother was almost equal to that of the daughter (III.3), and the intensity of the dot from the control female was almost twice as strong implying that III.3 was a carrier. This agreed with the result of the *Bam*HI RFLP analysis.

Population study (Table 2). A survey of the *Bam*HI RFLP phenotypes from 62 unrelated Japanese females is summarized in Table 2. Overall, 36% of X chromosomes carried the 22-kb/25-kb allele, 41% carried the 12-kb/25-kb allele, and 23% had the 22-kb/18-kb allele. Given these allele frequencies, 66% of Japanese females were heterozygous with respect to *Bam*HI RFLP.

DISCUSSION

Hyperuricemia is known to cause hyperuricemic nephropathy in which deposition of uric acid crystals in the tubules leads to rupture of the tubular basement membrane and thus to more severe interstitial and glomerular involvement. Recurrent episodes of nephropathy, either symptomatic or nonsymptomatic, can progress to permanent renal impairment (26). We encountered two patients with partial HPRT deficiency, and found that early detection of the disease and long-term management of hyperuricemia is mandatory to prevent renal impairment in this disease.

*Bam*HI RFLP analysis was informative to detect carriers of partial HPRT deficiency in family B, but was not helpful in family A. An additional *Taq*I RFLP, which has been reported for an anonymous sequence (DXS-10) closely linked to HPRT locus, can be applied to detect carriers in family A (27).

Hybridization analysis of amplified or unamplified genomic DNA with mutant oligonucleotide probes is also known to be feasible for carrier detection (28, 29). When *Bam*HI RFLP analysis was noninformative, genotypes could still be partially distinguished by direct analysis of mutation sites within the HPRT gene. Dot blot analysis with normal and mutant oligonucleotide probes clearly separated the normal members, the carrier, and the patient in family A. Moreover, dot-blot analysis with normal oligonucleotide probe confirmed the result shown by *Bam*HI RFLP analysis in family B.

For determination of carrier status in the at-risk daughter of a carrier mother using *Bam*HI RFLP analysis, it is necessary to identify which chromosome carries the HPRT mutant gene in the mother. Thus, she must be heterozygous with respect to the *Bam*HI RFLP phenotype. Nussbaum *et al.* (25) surveyed 83 independent X chromosomes from 30 families for *Bam*HI RFLP, and reported that the allele frequencies in the Caucasian population were 0.77 for the 22-kb/25-kb allele, 0.16 for the 12-kb/25-kb allele, and 0.07 for the 22-kb/18-kb allele, resulting in an average heterozygosity of 38% in females. However, our study showed that the allele frequencies in the Japanese female population were 0.36 for the 22-kb/25-kb allele, 0.41 for the 12-kb/25-kb allele, and 0.23 for the 22-kb/18-kb allele, indicating that 66% of Japanese females were heterozygous for *Bam*HI RFLP, a higher rate than in Caucasian females. In this respect, we cannot exclude the possibility that racial factors contributed to the different results of the two studies. No difference has been found between the frequencies of the *Bam*HI RFLP in normal X chromosomes, X chromosomes bearing HPRT mutations, and X chromosomes in individuals with a variety of X-linked diseases (25).

Accordingly, *Bam*HI RFLP analysis could be applied to the families with the disease more effectively in Japan than in countries with a predominantly Caucasian population.

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