# Identification of a New Single-Nucleotide Mutation on the Hypoxanthine-Guanine Phosphoribosyltransferase Gene from 983 Cases with Gout in Taiwan

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***ABSTRACT.*** ***Objective***. The frequency of hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency within the gout-affected population in Taiwan was unclear. We evaluated its frequency and sought to identify a new genetic variation in a case with HPRT deficiency.

***Methods***. From 2004 to 2005, a total of 983 patients with gout were followed among outpatients attending the Department of Rheumatology. Among these, 12 cases were suspected to have HPRT deficiency, and HPRT activity was examined by HPLC. In the index case found to have HPRT deficiency, genetic variation was analyzed by RT-PCR, direct sequencing, and SSCP.

***Results***. Only a single case proved to have partial HPRT deficiency among 12 suspicious cases. Both cDNA and genomic DNA analysis identified a new mutation on exon 2 with T to G transition at cDNA base 93, resulting in a change from aspartic acid to glutamic acid at position 31. It was designated as $HPRT_{Chia\text{-}Yi}$, from our case's residence at Chia-Yi Hsein, Taiwan.

***Conclusion***. According to this hospital-based survey, HPRT deficiency is a rare trait in the Taiwanese gouty population. However, our index case with HPRT deficiency provided the first proven HPRT mutation in non-aboriginal Taiwanese patients with gout, which was different from a mutation previously found in aboriginal Taiwanese. Hence, in non-aboriginal Taiwanese gouty patients with HPRT deficiency, exon 2, rather than just exon 3, should be analyzed. (First Release Feb 15 2007; J Rheumatol 2007;34:794–7)

*Key Indexing Terms:*
GOUT MUTATION HPRT TAIWAN

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Gout is a very common disease in Taiwan[1]. There is evident variation in prevalence of gout between different populations: prevalence is reported to be 15.2% in aboriginal men, and 0.3% in non-aboriginal men[2]. Hypoxanthine-guanine phosphoribosyltransferase (HPRT) is a key enzyme related to purine metabolism, which contributes to the regulation of production of uric acid. Therefore, it is important to investigate the *HPRT* gene in cases with gout. In 1999, we first found a new point mutation on exon 3 of the *HPRT* gene from an aboriginal Taiwanese with severe gout, and designated it $HPRT_{Tsou}$ due to his maternal origin from the aboriginal Tsou tribe of Taiwan and not from the non-aboriginal Taiwanese (also known as Han Taiwanese), who make up 98% of the population[3].

On clinic service, we care for hundreds of gouty patients every year. However, the frequency of HPRT mutation in our gouty population was unclear. We evaluated the frequency of HPRT deficiency among gouty patients by a hospital-based cohort study and investigated any possible new mutation of *HPRT* gene.

## MATERIALS AND METHODS

*Patients*. From 2004 to 2005, a total of 983 cases (868 men, 115 women) with gout were followed at the outpatient service of the Department of Rheumatology at Chang Gung Memorial Hospital at Chia-Yi. Among these, 12 cases with suspicious HPRT deficiency based on early-onset gout, numer-

ous tophi, familial aggregation with maternal inheritance, frequent attacks of gouty arthritis, or persistent hyperuricemia in spite of urate-lowering agents were enrolled for HPRT activity assay (Table 1). Only one case (index case) was proved to have HPRT deficiency.

The index case was age 52 years at time of diagnosis of HPRT deficiency. He had had recurrent attacks of gouty arthritis since age 32 years. Synovial fluid analysis revealed typical needle-shaped urate crystals with strong negative birefringence under polarized light. We had tried many kinds of urate-lowering drugs including allopurinol to control his gout, yet attacks of gouty arthritis remained frequent, with serum urate level always around 8–9 mg/dl or more. In addition, numerous tophi over elbows, wrists, and left first metatarsal joint were noted. For this unusual presentation, HPRT deficiency was suspected and finally confirmed by both HPRT activity assay and genetic study.

*Methods*. We detected HPRT activity using high-performance liquid chromatography (HPLC). Subsequently we used reverse-transcription polymerase chain reaction (RT-PCR) and direct sequencing on amplified cDNA to identify the mutation on *HPRT* gene, and finally confirmed the mutation by single-strand conformation polymorphism (SSCP).

*HPRT activity assay*. HPRT activity analysis with HPLC was performed according to the method by Sakuma, *et al* using erythrocyte lysates, which could detect 0.3% of normal HPRT activity[4].

*Reverse transcription amplification of HPRT cDNA by RT-PCR*. Total RNA was extracted from the peripheral leukocytes using the RNA RiboPure™-Blood Kit (Ambion, Austin, TX, USA). cDNA synthesis was performed with 2 $\mu$g of isolated total RNA per sample using MMLV Superscript III RT kit (Promega™, Madison, WI, USA). Subsequently, a 700 bp fragment containing an entire peptide-coding region was amplified from cDNA by PCR as described[3]. RT-PCR was done using a set of primers (the forward primer, JoyD: CGC GCC GGC CGG CTC CGT T and the reverse primer, JoyU: CCA AAC TCA ACT TGA ACT CTC ATC) as described[3].

*Direct sequencing of PCR products to identify a mutation of HPRT*. Direct sequencing of PCR products was performed by an automatic fluorescence DNA sequencer (ABI Prism MTS 373A; Perkin Elmer, Foster City, CA, USA).

*Amplified genomic DNA and SSCP*. SSCP was used to confirm the mutation found by direct sequencing. Genomic DNA was extracted from buffy coats using a QIAamp DNA Blood Kit (Qiagen, Valencia, CA, USA). Exon 2 was amplified according to Yamada, *et al*[5]. PCR products were mixed with an equal volume of formamide buffer (95% formamide, 10 mM EDTA, 0.1% bromophenol blue, 0.1% xylene cyanol). Denatured samples were electrophoresed on a GeneGel Excel 12.5/24 (Amersham Biosciences, Sunnyvale, CA, USA) for 4 h at 200 V and 4°C. After electrophoresis, gels were silver-stained and analyzed[6].

## RESULTS

*Frequency of HPRT deficiency*. Among 983 cases with gout, 12 cases suspected to have HPRT deficiency were analyzed by HPLC: only a single case was proved to have partial HPRT deficiency (Table 1). HPRT activity in this patient was found to be as low as only 0.12 $\mu$mole/min per g hemoglobin, about 5.4% of normal values. Therefore, the frequency of HPRT deficiency is rare in our gouty patients.

*Identification of HPRT mutation*. By direct sequencing, a single-nucleotide mutation in cDNA base 93 with T to G transition within Exon was found, resulting in a change from aspartic acid to glutamic acid at position 31 (Figure 1). This mutation was confirmed by SSCP with the appearance of an aberrant band after electrophoresis and silver staining.

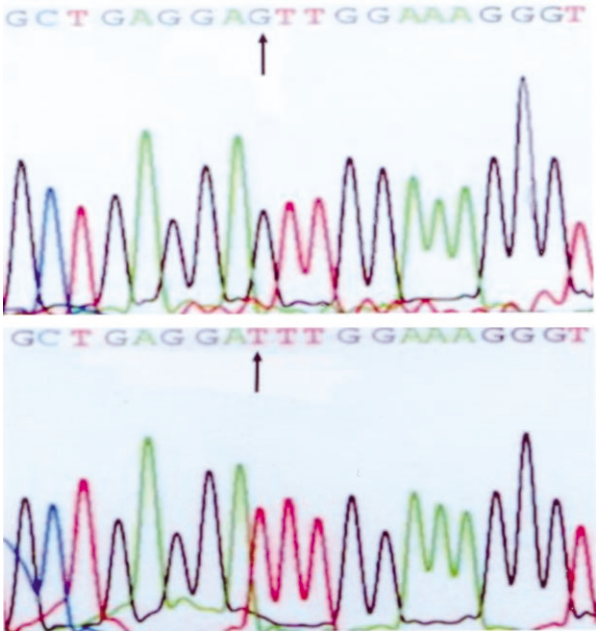


*Figure 1*. Direct sequencing of HPRT cDNA showed the mutation with T to G transition at cDNA base 93. The cDNA sequence in the index case is shown in the upper panel and a normal case is shown in the lower panel.

*Table 1*. The reasons for suspicion of HPRT deficiency in 12 gouty patients.

| | Suspicious Case | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| Selection criteria | | | | | | | | | | | | |
| Early onset* | | | | | | | + | | | | + | |
| Numerous tophi** | | | | | + | + | | | | | | + |
| Maternal inheritance*** | | + | | + | + | | | + | | + | + | |
| Frequent attacks† | + | | + | | + | | | | | | | |
| Persistent hyperuricemia†† | | | | | + | | | | + | + | | |

* The first attack of gouty arthritis before 30 years of age. ** More than 5 evident tophi around joint area. *** History showed family aggregation of gouty arthritis with maternal inheritance. † Under regular medication, there were at least 2 attacks per month for more than 1 year. †† Within the period taking regular medication and without attacks of gouty arthritis, serum urate level > 9 mg/dl.

*Detection of the HPRT mutation in family members.* HPRT activity in the index case's mother was 0.85 $\mu$mole/min per g hemoglobin and in his brother 2.97 $\mu$mole/min per g hemoglobin. Direct sequencing of cDNA showed a heterozygote in his mother and a normal pattern in his brother (Table 2).

*Designation of the new HPRT mutation.* After comparison with the existing database[7], we designated this new mutation $HPRT_{Chia\text{-}Yi}$ due to his residence at Chia-Yi Hsein in Taiwan.

## DISCUSSION

Our study revealed that the frequency of HPRT deficiency is rare in our gouty patients in Taiwan. This finding is compatible with the previous study from Yu, *et al*, who reported 7 cases including 5 cases in a family with HPRT deficiency among 425 gout cases in the USA[8]. Both studies suggested that the cases with HPRT deficiency were really the minority in patients with gouty arthritis.

Although HPRT deficiency is uncommon, the identification is very important in clinical practice, because the patient with HPRT deficiency usually needs aggressive therapy and develops gout at a much younger age. Although our index case with $HPRT_{Chia\text{-}Yi}$ was 52 years old at the time of diagnosis of HPRT deficiency, he had suffered a first attack of gouty arthritis at age 32 years. Because of his refractory response to therapy, HPRT deficiency was suspected and finally confirmed. To our knowledge, this is the first HPRT mutation in a non-aboriginal Taiwanese (also known as Han Taiwanese) with gout. Surprisingly, this unique mutation was located on exon 2, in contrast to the previously found mutation in aboriginal Taiwanese, located on exon 3. Therefore, besides the well known exon 3, exon 2 should also be examined in non-aboriginal Taiwanese patients with HPRT deficiency.

HPRT is an important enzyme in the purine metabolic pathway. Its complete deficiency (less than 1.5% of residual activity) can cause Lesch-Nyhan disease. Partial deficiency causes excessive purine production, resulting in severe gout. To date, more than 302 mutations have been reported for HPRT, with different clinical manifestations; these include deletions, insertions, duplications, abnormal splicing, and point mutations at different sites on the coding region[7]. In Taiwan, including this case, a total of 5 novel mutations in HPRT have been reported[3,9,10] (Table 3). However, different clinical pictures were found among these mutations of *HPRT* gene. Three cases presented as Lesch-Nyhan syndrome[9,10] and the patient with $HPRT_{Tsou}$ presented as early-onset gout[3]. However, the case with $HPRT_{Chia\text{-}Yi}$ presented as adult-onset gout, and HPRT deficiency was found when the subject was 52 years old, a relatively late onset age, which is unexpected for HPRT mutation.

Our case reminds us that in patients with gout, HPRT mutation can be found not only in young men but also in middle-aged men. In view of the number of patients with gouty arthritis, we suggest that HPRT activity assay be indicated in male patients with the following characteristics: maternal inheritance, urate overproduction, onset before middle-age, and poor therapeutic response to urate-lowering agents.

*Table 2.* HPRT activity in index case with HPRT deficiency and his family members.

| | HPRT Activity* ($\mu$mole/min per g Hb) | % of Normal HPRT Activity |
|---|---|---|
| Normal cases | 2.22 ± 0.34** | |
| Index case | 0.12 | 5.4 |
| Mother | 0.85 | 38.5 |
| Brother | 2.22 | 100.0 |
| Elder sister | 1.23 | 55.4 |
| Younger sister | 2.27 | 102.7 |

* HPRT activity was determined by HPLC using erythrocyte lysates.
** Mean ± SD, derived from 11 cases without HPRT mutation.


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*Table 3.* Review of HPRT mutation in patients with Lesch-Nyhan disease (LND) or gout in Taiwan.

| Patient | Exon/Intron | cDNA Base | position | Amino Acid | Disease |
|---|---|---|---|---|---|
| 1, present study | Exon 2 | 93 T > G | 31 | Asp > Glu | Gout |
| 2[3] | Exon 3 | 152 G > A | 51 | Arg > Gln | Gout |
| 3[9] | Exon 3 | 222C > G | 74 | Phe > Leu | LND |
| 4[10] | Exon 8 | 569G > A | 190 | Gly > Glu | LND |
| 5[10] | Intron 4 | 31617G > A* | Splicing error | | LND |

* This mutation is described according to NCBI, accession number M26434.

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