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Genetic Analysis of Human Urate Transporter 1 (hURAT1) and Glucose Transporter 9 (GLUT9) in Taiwanese Patients with Idiopathic Renal Hypouricemia

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Background: The phenotypic characteristics of idiopathic renal hypouricemia (IRH) include impaired renal uric acid reabsorption, low serum uric acid levels, and severe complications such as exercise-induced acute renal failure (EIARF) and nephrolithiasis. IRH is increasingly reported to be associated with inactivating mutations in either *SLC22A12* gene encoding the human urate acid transporter 1 (hURAT1) or *SLC2A9* gene encoding glucose transporter 9 (GLUT9) in proximal tubules. Mutations in hURAT1 or GLUT9 have not been reported in Taiwanese patients with IRH. **Purpose:** To investigate the phenotypic and genetic characteristics in Taiwanese patients with IRH. **Patients:** Six Taiwanese patients (5 males and 1 female, mean age 30±23 years) with hypouricemia (serum uric acid concentration < 2.3 mg/dL) and concomitant excessive renal uric acid excretion (FE_{UA} > 10%) from five unrelated families were enrolled. Their clinical symptoms and biochemical studies were recorded. **Methods:** Molecular analysis of both *SLC22A12* and *SLC2A9* genes was performed by polymerase chain reaction (PCR)-single strand conformation polymorphism (SSCP) and direct sequencing. **Results:** Serum uric acid concentration was 1.16±0.23 mg/dl with a fractional excretion of uric acid of 61±12 %. Two male patients experienced exercise-induced acute renal failure requiring hemodialysis. None of them had nephrocalcinosis or uric acid stone. Despite a negative mutation in *SLC2A9*, four novel *SLC22A12* mutations (W120R, M215L, T217M and T467M) and one recurrent homozygous (R90H) mutations were identified in five patients. **Conclusion:** EIARF can develop in Taiwanese patients with IRH, and most of them are males. Mutations in *SLC22A12* rather than *SLC2A9* cause IRH in patients with FE_{UA} < 100%. Other candidate genes need to be identified for IRH patients without *SLC22A12* and SLC2A9 gene mutations.

Key words: acute renal failure, Glucose transporter 9 (GLUT9), human urate transporter 1 (hURAT1), idiopathic renal hypouricemia

INTRODUCTION

Idiopathic renal hypouricemia (IRH) characterized by impaired renal tubular uric acid reabsorption was first reported in 1972.[1] The incidence of IRH ranged from 0.12 to 0.72[2,3] with higher frequency in Japan and among non-Ashkenazi Jews.[4-6] With better understanding of renal urate handling, the reported number of IRH cases has steadily increased in the past decade. Patients with IRH are usually asymptomatic and often incidentally discovered after an episode of EIARF without rhabdomyolysis.[7-11] However, a long-term follow-up of patients with IRH showed decreased ability of urine concentration, uric acid nephrolithiasis, hematuria and progressive renal interstitial fibrosis.[6]

With the advent of advanced molecular analysis, IRH resulted from mutation in hURAT1 in the apical membrane of proximal tubules was first identified in 2002 and coded by the gene SL*C22A12* on chromosome 11q13.[12] The hURAT1 transporter reabsorbs the filtered urate in exchange for monovalent organic anions and loss of hURAT1 function produces a partial uric acid (UA) absorption defect.[13] Moreover, mutation in GLUT9 encoded by *SLC2A9* has recently been found to cause IRH.[14] The

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Table1 Clinical data and molecular studies of patients with renal hypouricemia and five family members

Family	Patient	Sex	Age	Exercise-induced acute renal failure	Location	Mutation	Laboratory Findings		
							UA (mg/dL)	Cr (mg/dL)	FE_{UA} (%)
A	1*	M	76	No	Exon 1	W120R	1.5	1.2	44
					Exon 3	M215I			
B	2	F	18	No	No mutation		1.2	1.0	59
C	3	M	14	Yes	Exon 3	R90H	0.9	1.1	76
	4	M	18	No	Exon 3	R90H	0.9	0.8	51
D	5	M	22	Yes	Exon 9	T467M	1.3	0.8	65
E	6	M	36	No	Exon 3	T217M	1.2	0.9	71

UA: uric acid; FE_{UA}: fraction excretion of uric acid; *the patient has membrous glomerulonephritis with nephrotic syndrome

loss of GLUT9 function on the basolateral membrane precludes UA absorption by all of the apical transporters (including hURAT1) through complete blocking of UA efflux, resulting in total UA reabsorption defect.[24] Therefore, IRH can be caused by inactivating mutations in either hURAT1 or GLUT9 in proximal tubules. To the best of our knowledge, there is no genetic report on hURAT1 or GLUT9 in Taiwanese patients with IRH. In this study, we performed the molecular analysis of *SLC22A12* and *SLC2A9* in six Taiwanese patients with IRH. Results obtained indicated that despite the absence of mutation in *SLC2A9*, we have identified five different hURAT1 mutations including four novels (W120R, M215L, T217M, T467M) and one recurrent mutations (R90H) in these patients. Of note, two males had EIARF.

SUBJECTS AND METHODS

The study protocol was approved by the Ethics Committee on Human Studies at Tri-Service General Hospital, National Defense Medical Center (Taiwan, R.O.C.). The subjects were given a detailed description of the study before they provided informed consent.

Study Subjects

We enrolled six patients with IRH (Table 1). Renal hypouricemia was diagnosed by the presence of hypourisemia (serum uric acid concentration < 2.3 mg/dL) and concomitant excessive renal uric acid excretion (FE_{UA} > 10%). IRH was confirmed after excluding other causes of renal hypouricemia including volume expansion, pregnancy, SIADH, effects of uricosuric drugs, and dysfunction of proximal tubules (Fanconi's syndrome or Hartup syndrome). These patients are unrelated and provided informed consent for various analysis procedures at the beginning of the study.

Methods

Genomic DNA isolation and polymerase chain reactions (PCR): Genomic DNA was isolated and purified from peripheral blood of the patients and family members and used for PCR amplification of individual exons of hURAT1 and GLUT9. PCR was performed in a 25 μl volume containing 100 ng genomic DNA, 0.4 μM primers, 0.25 mM deoxynucleoside triphosphates (dNTPs), and 2.5 μl 10 X Taq buffer (100 mmol/l Tris-HCl, pH 8.4, 100 mmol/l $(NH_4)_2SO_4$, 100 mmol/l KCl, 22.5 mmol/l $MgCl_2$, 0.1% gelatin, 1% Triton X-100, and 2 U Taq polymerase). PCR was performed using standard conditions with an initial denaturation step at 94°C for 5 minutes, followed subsequently by 30 cycles with denaturation at 94°C for 40 seconds, annealing at 60°C for 40 seconds, and elongation at 72°C for 40 seconds.

Single strand conformation polymorphism (SSCP) analysis and sequencing: DNA samples were amplified by PCR for SSCP as described above. The PCR products were mixed with 10 ml PCR product and 50 ml loading dye (95% formamide, 20 mM EDTA, 0.05% bromophenyl blue, and 0.05% xylene cyanol). The samples were denatured in boiling water for 10 minutes and placed on ice immediately. Electrophoresis was performed on 15% polyacrylamide gel (29:1 acrylamide:bisacrylamide) and 0.5X Tris-borate-EDTA electrophoresis (TBE) buffer (54 mM Tris, 54 mM boric acid, 1.2 mmol/l EDTA, pH 8.3) at 5 mA for 24 hours at 4°C. The gels were then stained by ethidium bromide. hURAT1 and GLUT9 exons resulting in SSCP shifts were purified for direct sequence analysis by PCR reactions with identical oligonucleotides as used in PCR-SSCP analysis. Amplified DNA fragments were isolated from agarose gels, using the "freeze-

squeeze" method and subsequently used as template in PCR-cycle-sequence reactions in the presence of dye-dideoxy terminators. The reaction conditions were those supplied by the manufacturer (Perkin-Elmer ABI, Foster City, CA, USA) and analyzed on an ABI 377 automated DNA sequencer (Perkin-Elmer ABI).

RESULTS

Index case

A 22-year-old male (patient 5) presented to our emergency department with nausea, dizziness, bilateral loin pain and progressive decrease in amount of urine for 3 days after an 800-meter training program. There was no history of renal stone. His family history was non-contributory. His body temperature was 36.1°C, pulse rate 79/min, and blood pressure 120/70 mmHg. His physical examination was unrevealing. Pertinent laboratory investigations revealed blood urea nitrogen 85 mg/dL, creatinine 10.7 mg/dL, UA 6.2 mg/dL and creatine phosphokinase 176 IU/L. Fractional excretion (FE) of sodium and UA were 1.13 and 62.4 %, respectively. Urinalysis was normal except for red blood cell 10-18/HPF and protein (1+). Serologic tests including antineutrophil antibody, rheumatoid factor, antistreptolysin O titer, cryoglobulin, complement, immunoglobin, and antineutrophil cytoplasmic antibodies were all unremarkable. Abdominal ultrasonography revealed normal kidney size without hydronephrosis. He did not have identifiable causes of acute renal failure. Renal biopsy revealed acute tubular necrosis. His renal function improved after five courses of hemodialysis in 2 weeks, while serum UA was very low (1.3 mg/dL) with FE_{UA} 65%. Idiopathic renal hypouricemia was diagnosed according to the absence of other causes of hyperuricosuric hypouricemia. Without any strenuous exercise in the following 3 years, he did not experience recurrent acute renal failure.

Clinical symptoms and biochemical features

As shown in Table 1, most of the subjects were male, and none of them had nephrocalcinosis or uric acid stone. Two male patients (patients 3 and 5) experienced exercise-induced acute renal failure (EIARF) requiring hemodialysis with a recovery of renal function. Their serum uric acid concentration was 1.16 ± 0.23 mg/dl with a fractional excretion of uric acid 61 ± 12 %.

Mutation analysis

Amplification, SSCP screening and direct sequencing of *SLC22A12* and *SLC2A9* genes revealed that these six patients had a negative mutation in *SLC2A9* but four novel *SLC22A12* mutations (W120R, M215L, T217M and T467M) and one recurrent homozygous (R90H) mutation were identified in five patients except patient 2 (Table 1). Individual mutations in these six patients are shown below.

Family A (patient 1)

Two different novel point mutations were identified. A cytosine to thymine single-base substitution at nucleotide 358 (TGG to CGG) and an adenosine to guanine single-base substitution at nucleotide 645 (ATG to ATA) were found in exon 1 and exon 3, respectively. This resulted in a novel mutation from tryptophan to arginine at codon 120 (W120R) and another novel mutation from methonine to isoleucine at codon 215 (M215I) (Figure 1A).

Family C (patients 3 and 4)

A homozygous adenosine to guanine single-base substitution at nucleotide 269 (CGC to CAC) was found. This resulted in a homozygous missense mutation from arginine to histidine at codon 90 (R90H) in exon 1 of URAT1. R90H mutation, a previously reported pathogenic mutation, was inherited from their parents who are both asymptomatic (Figure 1B).

Family D (patient 5)

A homogeneous thymine to cytosine single-base substitution at nucleotide 1400 (ACG to ATG) was found in exon 9, resulting in a novel mutation from threonine to methothyline at codon 467 (T467M) (Figure 1C).

Family E (patient 6)

A homogeneous thymine to cytosine single-base substitution at nucleotide 650 (ACG to ATG) was found in exon 3, resulting in a novel mutation from threonine to methothyline at codon 217 (T217M) (Figure 1D).

DISCUSSION

This study describes the clinical, biochemical and genetic characteristics of six Taiwanese patients from five unrelated families. They had clinical diagnosis of IRH. All of them, except one, were found to have *SCL22A12* mutations including four novels and one already reported recurrent mutation despite undetected mutation in *SLC2A9*. All of these *SCL22A12* mutations were indentified in male patients. Furthermore, none of them had nephrocalcinosis or uric acid stone. Only two patients had exercise-induced acute renal failure (EIARF) requiring hemodialysis. These findings demonstrated pheno-

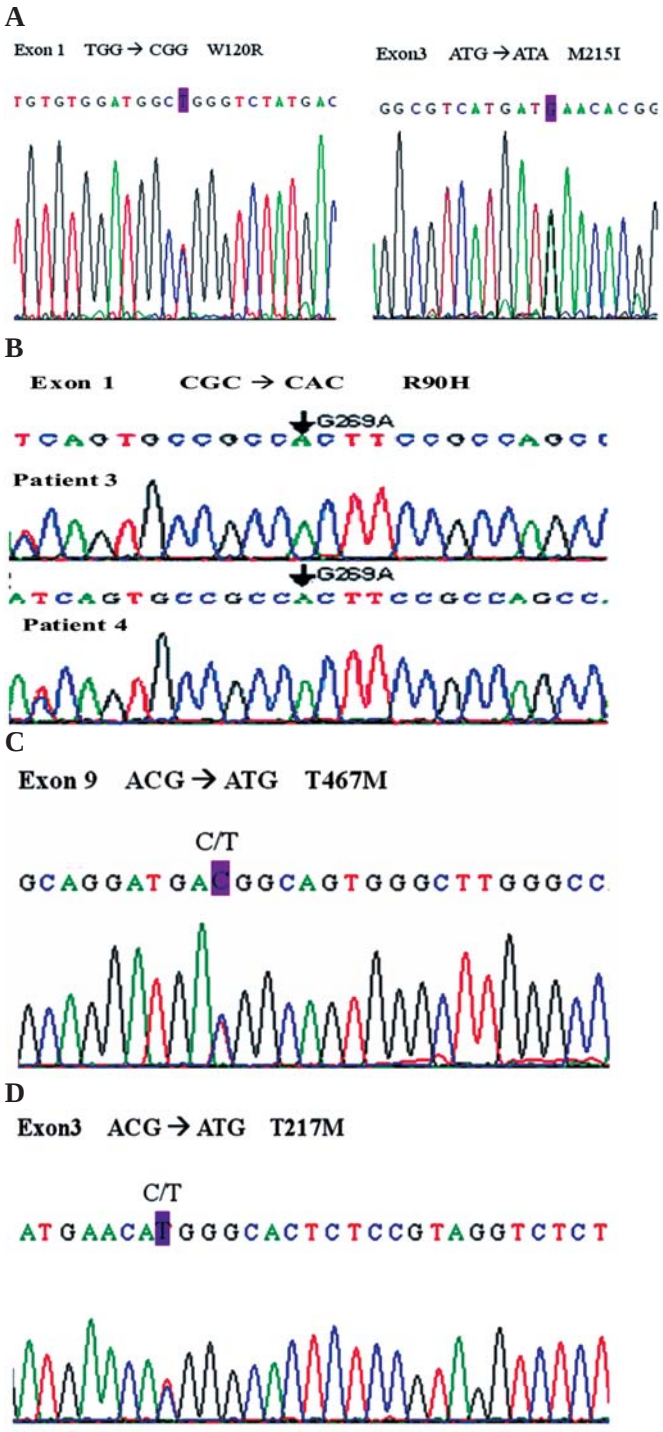

Fig. 1 Direct sequencing of *SLC22A12* in patient 1, 3, 4, 5 and 6. Patient 1 has compound heterozygous W120R and M215L mutation in exon 1 and exon 3, respectively (A). Family C (patient 3 and patient 4) has homozygous R90H mutation in exon 1 (B). Patient 5 and 6 have a heterozygous T467M (C) and T217M (D) mutation in exon 9 and exon 3, respectively.

typic heterogeneity of SLC2A9 mutations in Taiwanese patients.

IRH is an autosomal recessive disorder characterized by hypouricemia with high renal UA clearance but normal daily UA excretion. In human kidney, filtrated UA is reabsorbed at proximal tubules primarily through hURAT1 on the apical membrane and newly identified GLUT9 on both apical and basolateral membranes. Most patients with IRH have loss-of-function mutations in hURAT1.[15-19] The definition of hereditary renal hypouricemia type 2 (RHUC2; OMIM 61276), in addition to hereditary renal hypouricemia type 1 ((RHUC1; OMIM 220150), had been reported.[14] Mutations in canin *SLC2A9* have been recently found to affect UA handled by kidney and liver of Dalmatian dogs, which exhibit hyperuricosuria and relative hyperuricemia.[20] GLUT9 encoded by *SLC2A9* was also found to cause IRH in human.[14] Therefore, UA reabsorption from the tubular lumen is carried out not only by hURAT1 but also by GLUT9 and possibly other apical transporters.

UA reabsorption depends on apical and basolateral transporters. GLUT9 mediates renal UA reabsorption on both sides of proximal tubular cells. hURAT1 is expressed only on the apical side. The loss of hURAT1 function on the apical membrane produces a partial UA absorption defect (fractional excretion of UA of 40 to 90%) due to the function of GLUT9 being preserved. Pathogenic mutations in GLUT9 on both sides of proximal tubules result in total UA reabsorption defect.[14,21] In this study, the serum uric acid concentration was 1.16±0.23 mg/dl with a fractional excretion of uric acid (FE_{UA}) 61±12% in homogeneous *SCL22A12* gene mutations only. It had been reported that both families had homozygous *SLC2A9* mutations resulting in severe renal hypouricemia with FE_{UA} > 150 %.[22] Herein, our finding may support that mutations in *SLC22A12* rather than *SLC2A9* cause IRH in patients with FE_{UA} < 100%.

The most common recurrent mutations identified in Japan and Korea are W258X and R90H, respectively.[23,24] In this study, we found only one recurrent R90H mutation of *SLC22A12*. Since the mode of inheritance in IRH is autosomal recessive, there may be an unidentified gene abnormality on the other allele. The other explanation for the failure to identify the mutation in the second allele is a possible concurrent heterozygous mutation in the genes that regulate or interact with gene *SLC22A12* or *SLC2A9*. A more extensive and sensitive sequencing strategy and screening of other possible candidate genes are warranted for IRH patients without *SLC22A12* and *SLC2A9* gene mutations.

Patients with IRH had high risk of EIARF, and urolithiasis due to hyperuricosuria and hypercalciuria. The pathogenesis of EIARF in IRH remains unclear. Two mechanisms have been initially proposed. One is acute UA nephropathy due to increased UA production from ATP degradation during exercise. The other is oxidative stress from oxygen free radicals produced during exercise.[6] A third mechanism had been proposed subsequently for EIARF. That is, reduced clearance of urate-coupled anions by hURAT1 due to loss-of-function mutations of either hURAT1 or GLUT9 may exert toxic effects on renal proximal tubules, leading to toxic acute tubular necrosis.[21]

EIARF occurs predominantly in young male and is characterized by nausea/vomiting and loin pain within 6-12 hours following acute anaerobic exercise. CPK may be normal or slightly elevated. Of note, UA is often "inappropriately" normal. Depending on the severity and complications, medical treatment including hydration with saline solution or diuretics may apply for IRH patients. The use of non-steroidal anti-inflammatory drugs for groin pain may worsen EIARF via increased vasoconstriction. However, approximately 20% of patients requiring dialysis and renal function usually recover within 2-3 weeks.[8,25] EIARF can be recurrent if IRH goes unrecognized or without appropriate management. The repetitive recurrence of ARF may cause irreversible renal damage. Avoidance of strenuous exercise helps prevent recurrence. Urolithiasis is a known complication of IRH attributed to hURAT1 mutations with a prevalence of 8.5% compared with 2.0-3.0% in the general population.[26] The renal UA absorption defect is more severe in *SLC2A9*-associated hypouricemia than in hURAT1-associated disease, thus explaining why none of the *SCL22A12* mutations had nephrocalcinosis or uric acid stone.

In conclusion, we report that EIARF can develop in Taiwanese patients with asymptomatic IRH, and most of them are males. Mutations in *SLC22A12* rather than *SLC2A9* may cause IRH in patients with $FE_{UA} < 100\%$. Unexpectedly "normal" serum UA in ARF is an important clue to renal hypouricemia. In order to elucidate the genetic defect in this disorder, other candidate genes need to be identified for IRH patients without *SLC22A12* and *SLC2A9* gene mutations.

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