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A Putative Mutation Hotspot of the *AGXT* Gene Associated with Primary Hyperoxaluria Type 1 in the Chinese Population

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Primary hyperoxaluria type 1 (PH1), a rare autosomal recessive disorder, is characterized by renal stones, nephrocalcinosis, and chronic kidney disease. PH1 is caused by defects in alanine glyoxylate aminotransferase (AGT, 392 amino-acid residues), which is encoded by the alanine-glyoxylate and serine-pyruvate aminotransferase (*AGXT*) gene. This study aimed to determine the clinical, biochemical, and mutation spectrum of patients with PH1 from mainland China. Four patients (two adults and two children, age range: 1 to 34 years) from four unrelated families were admitted because of kidney stones. The adult patients had chronic kidney disease, while the pediatric patients retained the normal kidney function. Four mutations of the *AGXT* gene were detected, including one novel mutation, c.1015delG (p.V339Sfs*2). One adult male with late-onset PH1 is a compound heterozygote of the c.815_816insGA (p.S275Rfs*38) and c.1015delG (p.V339Sfs*2) mutations. These frame-shift mutations could result in the production of truncated AGT proteins. Other patients include an adult female who is heterozygous for c.473C>T (p.S158L) and c.815_816insGA mutations and two boys that are respectively homozygous for the c.815_816insGA mutation and for the c.614C>T (p.S205L) mutation. Thus, the c.815_816insGA mutation accounts for 4/8 alleles in the present study; importantly, the position c.815 represents the 5′-end of the consecutive wild-type sequence of GAGAGAGA. In conclusion, we describe one novel mutation, c.1015delG, and a common mutation, c.815_816insGA, of the *AGXT* gene among four unrelated families with PH1. Moreover, we suggest that the short repeat of the GA dinucleotide may represent a mutation hotspot in the Chinese population.

Keywords: alanine glyoxylate aminotransferase; alanine-glyoxylate and serine-pyruvate aminotransferase; kidney stones; plasma and urine oxalate; primary hyperoxaluria type 1
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Introduction

Primary hyperoxaluria type 1 (PH1; MIM 259900), a rare autosomal recessive disorder that affects glyoxylate metabolism, is caused by defects in the alanine-glyoxylate and serine-pyruvate aminotransferase (*AGXT*) gene (MIM 604285), a 10-kb gene located on 2q37.3 with 11 exons (Purdue et al. 1991). PH1 causes about 1% of all cases of end-stage renal failure (ESRD) due to severe chronic renal dysfunction in children (Hoppe and Langman 2003). The incidence of PH1 varies, depending on geographic regions. The overall incidence of PH1 is 1-3/120,000 in Europe and 1-32/1,000,000 in the Middle East (Kopp and Leumann 1995; Cochat et al. 2006). PH1 is more common in Mediterranean countries (Kamoun and Lakhoua 1996) than other countries.

The *AGXT* gene encodes alanine glyoxylate aminotransferase (AGT, EC 2.6.1.44), a pyridoxal 5′-phosphate

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(PLP)-dependent enzyme of 392 amino-acid residues, that catalyzes the transamination of glyoxylate to glycine (Milliner et al. 1993). Defects in AGT lead to elevated levels of endogenous oxalate, which could form insoluble calcium oxalate crystals and contribute to oxalate precipitation throughout the body, particularly in the kidneys, bladder, eyes, heart, bones, and sometimes lungs, thereby causing organ dysfunction. Thus, typical PH1 patients present with renal stones, urolithiasis, nephrocalcinosis, and chronic kidney disease, and eventually develop into ESRD (Milliner et al. 1993). Treatment with vitamin B6, a co-factor of AGT, is recommended to enhance the enzymatic activity and reduce urine oxalate levels in some PH1 patients (Cochat et al. 2012).

In Tunisia, PH1 accounts for 13.5% of all cases of ESRD in children, and 0.7% of all such cases in North America (Kamoun and Lakhoua 1996). To the best of our knowledge, however, only 19 patients with PH1 have been reported in Chinese population (Coulter-Mackie et al. 2001, 2004; Yuen et al. 2004; Li et al. 2014; Chen et al. 2015; Wang et al. 2016; Gao et al. 2017; Cui et al. 2017; Du et al. 2018). These PH1 patients were found in PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) by searching the keywords: primary hyperoxaluria type 1, China, Chinese, and *AGXT*. The PH1 variant spectrum in China has not been established, and no mutation hotspot was detected. However, the incidence of kidney stones increased dramatically among adolescents in the general population (Dwyer et al. 2012), and PH1 has also been reported in adult patients (Wang et al. 2016). These results suggest that PH1 may have been misdiagnosed in Chinese patients with kidney stones especially in adolescents and adults.

Here, we report the clinical, biochemical, and potentially causative mutations in four Chinese PH1 patients, including a novel mutation and a common mutation at a putative hotspot of the *AGXT* gene, thereby expanding the variant spectrum of disease. We also provide biochemical and molecular diagnostic methods for detecting PH1 that are suitable for Chinese hospitals. We are now performing these methods on additional patients who develop kidney stones early in their lives.

Materials and Methods

Patients

Four patients (one adult male, one adult female, and two male children; ages range: 1 to 34 years) from four unrelated Chinese families were diagnosed at the Department of Pediatrics, Peking University First Hospital, Beijing, China between January 2016 and March 2018. They had surgery at Department of Urology, Beijing Friendship Hospital, Capital Medical University. The clinical onset of the symptoms was between 1 and 28 years of age. The patients were admitted with indications of vomiting, hematuria, hematuria, and dysuria (Table 1). The parents of all patients were healthy and non-consanguineous. This study was approved by the institutional review board of the General Hospital of Tianjin Medical University and the Peking University First Hospital, and it was conducted by the Declaration of Helsinki.

Routine tests and metabolic studies

Routine blood and urine laboratory tests were conducted to assess liver and renal functions, glucose, ammonia, ketones, thyroid hormone, creatine kinase, creatine kinase isoenzymes, and rheumatic antibody. We also performed transabdominal ultrasonography, urinary system ultrasonography, abdominal computed tomography (CT), 24-h proteinuria estimation, and blood gas analysis. Fundoscopic eye examinations were performed because oxalate is easily accumulated in the retina of affected individuals.

Blood and 24-h urine were collected, and Agilent 1100 (Agilent, CA, United States), a reversed-phase high-performance liquid chromatography (RP-HPLC), was used to analyze plasma and urinary oxalate concentrations, which are presented as mg per liter (mg/L) (Fry and Starkey 1991).

AGXT analysis

We obtained informed consent from the adult patients, the parents of pediatric patients, and an adult healthy control individual for genetic analysis. Peripheral blood samples were collected from all patients and their parents, and lymphocytes genomic DNA was extracted using a TIANamp blood DNA kit (Tiangen Biotech, Beijing, China). We performed exome sequencing (Agilent V5, Agilent) involving the genes related to PH1. The exons and flanking intronic regions of the variants detected in *AGXT* were amplified by polymerase chain reaction (PCR) and then sequenced. The sequence data were compared with the reference sequence of *AGXT* (NM_000030) to detect the variations. Variants were compared to HGMD data sets to identify the previously reported ones (http://www.hgmd.cf.ac.uk). Then variants were compared to 1000 Genomes Project (http://www.1000genomes.org), and ExAC browser (http://exac.broadinstitute.org) to determine the Minor Allele Frequency (MAF) and later rule out the ones with high MAF (the MAF of most pathogenic variation in rare disease < 1%).

Prediction of the effects of AGXT sequence variants

PolyPhen-2 (http://genetics.bwh.harvard.edu/pph) and MutationTaster (http://www.mutationtaster.org) were used to predict the impact of variations alterations on protein function.

Results

The clinical data and the results of the laboratory examinations are presented in Table 1. All patients were born at term. Elevated plasma and urinary oxalic acid levels as well as kidney stones were detected in all four patients. Fundoscopic eye examinations of all four patients showed normal results. The two adult patients, Patient 1 and Patient 2, presented with deteriorating kidney function, whereas the two pediatric patients, Patient 3 and Patient 4, responded well to vitamin B6 and urine alkalization treatment with stable kidney function.

Patient 1

Patient 1 is a 30-year-old male who presented with vomiting and anorexia at the age of 28 years (Table 1). Laboratory tests showed an elevated blood creatinine level (455.2 μmol/L), which indicated damaged kidney function. Increased plasma and urine oxalic acid levels and hyperthy-

roidism were later detected. Ultrasonography of the urinary system indicated hydronephrosis and kidney and bladder stones. This patient showed normal eGFR level when he was 22 years old. The patient was diagnosed with chronic kidney disease at 4th stage [CKD4, with eGFR 22.54 ml/(min*1.73 m^2)], kidney stones, nephrocalcinosis, cystolith, hydronephrosis, hyperthyroidism, and PH1. Patient 1 carries the heterozygous mutations, c.815_816insGA (p.S275Rfs*38) and c.1015delG (p.V339Sfs*2), inherited from each parent (Fig. 1a, Table 2). The insertion and deletion variants (p.S275Rfs*38 and p.V339Sfs*2) are predicted to be "disease causing" by MutationTaster. The patient was given a high dose of vitamin B6 (20 mg/kg/day), regular fluid intake, and oral potassium citrate according to treatment guidelines. Allopurinol was used to reduce the high levels of lithic acid, which could be a consequence of reduced kidney function. The patient underwent ultrasonic lithotripsy and received peritoneal dialysis. The disease slowly progressed, but the eGFR remained relatively stable.

Patient 2

Patient 2 is a 35-year-old female who first showed PH1 symptoms (specifically kidney stones) at the age of 5 years (Table 1). She underwent three rounds of ultrasonic lithotripsy and was diagnosed with renal tubular acidosis at age 18. Hyperthyroidism and elevated creatinine levels (600 μmol/L) were observed during her pregnancy at age 29. The pregnancy ended in an emergency cesarean section to restore the deteriorating renal function. Later, renal replacement treatment (peritoneal dialysis and hemodialysis) was administered. The patient also suffered from recurrent urinary infections because of kidney stone obstruction, and was diagnosed with uremia [eGFR 9.53 ml/(min*1.73 m^2)], hyperthyroidism, kidney stones, renal tubular acidosis, and nephrocalcinosis. At age 34, after plasma and urine oxalic acid estimation with genetic testing, she was diagnosed with PH1. Patient 2 carries the two heterozygous mutations, c.473C>T (p.S158L) and c.815_816insGA (p.S275Rfs*38), inherited from each parent (Fig. 1b, Table 2). The patient received a high dose of vitamin B6, normal fluid intake (relatively high fluid intake before hemodialysis), and oral potassium citrate treatment. As of this study, she was regularly undergoing hemodialysis three times per week. However, the creatinine levels (600-900 μmol/L) continued to increase. As of this writing, she was planning to undergo a combined liver/kidney transplantation.

Table 1. Clinical and laboratory data of four Chinese patients with PH1.

Patients	**1**	**2**	**3**	**4**	**Normal range**	**Units**
Sex	M	F	M	M		
Age of onset	28y	5y	8m	6y		
Age of diagnosis	28y	34y	1y	7y		
Present Age	30y	35y	1y5m	8y		
Positive family history	–	–	–	–		
Symptoms and signs						
renal tubule acidosis	–	+	–	–		
nephrocalcinosis	+	+	–	–		
kidney stones	+	+	+	+		
multiple urolithiasis	–	+	+	–		
repeated urinary infection	–	+	–	–		
hydronephrosis	+	+	–	–		
dysuria	+	–	+	+		
hematuria	–	+	–	+		
chronic renal failure	+	+	–	–		
systemic oxalosis	+	–	–	–		
laboratory finding						
urine oxalate	73.90	105.98	86.84	68.52	< 50	mg/L
plasma oxalate	6.78	7.64	8.13	6.91	< 5.4	mg/L
plasma creatinine	455.2	895.22	36.21	51.81	14.9-42.9 (1-3y) 27.1-58.3(6-12y) 44-133 (> 18y)	mg/L
urea nitrogen	36.68	33.85	3.1	4.22	1.8-6.4	mmol/L
Outcome	CKD4	uremia	normal eGFR	normal eGFR		

M, male; F, female; y, years; m, months; d, days; CKD4, chronic kidney disease 4th stage; eGFR, estimated Glomerular Filtration Rate.

Patient 3 and Patient 4

Patients 3 and 4 are male children, 1 year and 5 months old and 8 years old, respectively, as of this writing (Table 1). These patients have similar medical histories. Patient 3 visited our hospital because of dysuria, and Patient 4 visited the clinic with a chief complaint of hematuria, dysuria and backache. Cystolith and kidney stones were detected in Patient 3 at age 8 months, and Patient 4 was diagnosed with kidney stones at age 7 years. Both patients presented with normal serum creatinine levels, but high levels of plasma and urine oxalic acid. Patient 3 is a homozygote for the mutation, c.815_816insGA (p.S275Rfs*38) (Fig. 1c, Table 2), while Patient 4 is a homozygote for the c.614C>T (p.S205L) mutation (Fig. 1d, Table 2). After genetic diagnosis, both patients were placed on high doses of vitamin B6, high fluid intake, and oral potassium citrate. As of this writing, the eGFR levels of both children were normal, and their kidney stones had not progressed; namely, these pediatric patients responded well to vitamin B6 and urine alkalization treatment.

Features of the identified mutations

Four mutations were identified from all four patients (Table 2, Fig. 1), and the changes in codons and amino acids are listed in Table 3. The mutations include c.473C>T (p.S158L), c.614C>T (p.S205L), c.815_816insGA (p.S275Rfs*38), and c.1015delG (p.V339Sfs*2). Among them, only one mutation, c.1015delG (p.V339Sfs*2), is

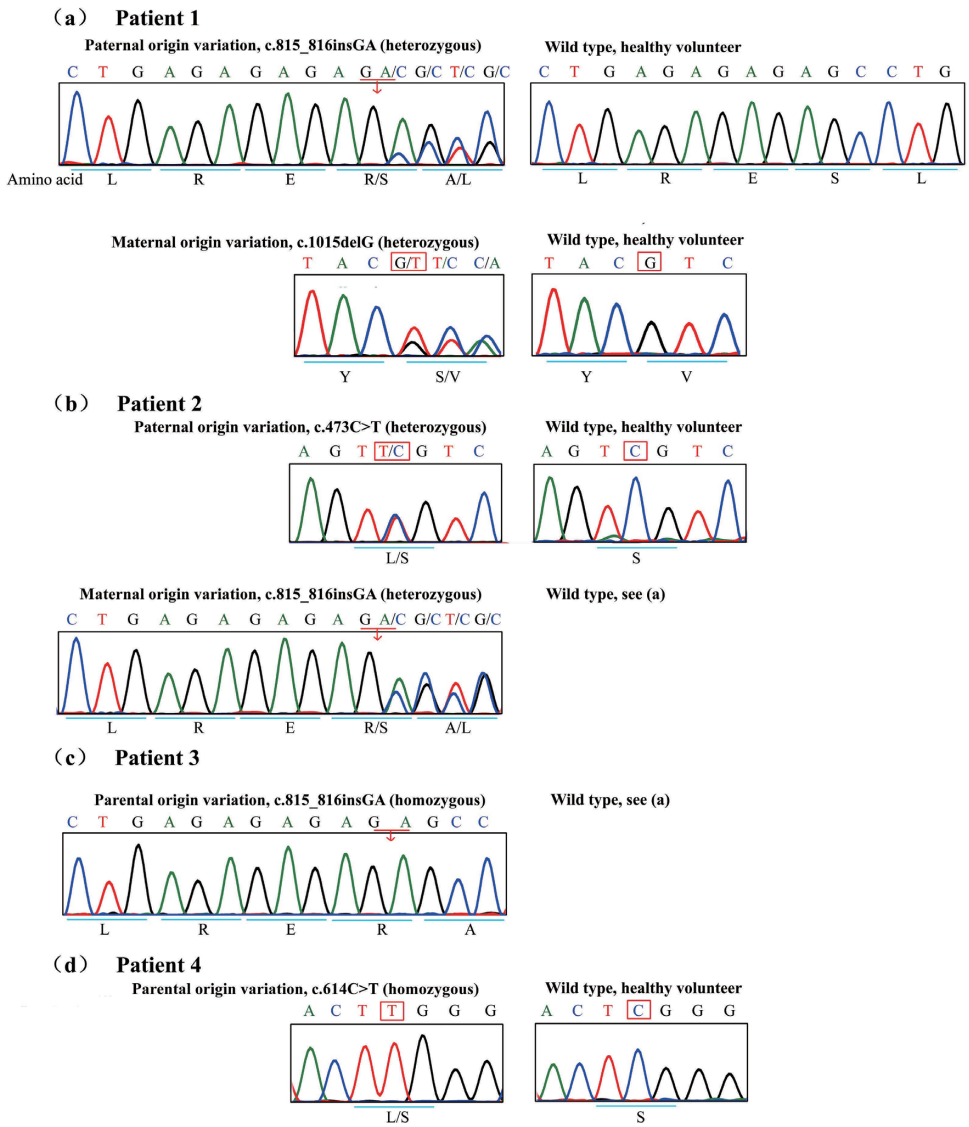

Fig. 1. *AGXT* variants analyses of our four PH1 patients, and one healthy volunteer (wild-type).
(a) compound heterozygous c.815_816insGA (p.S275Rfs*38) and c.1015delG (p.V339Sfs*2) variants on Patient 1; (b) homozygous c.815_816insGA (p.S275Rfs*38) variant on Patient 2; (c) compound heterozygous c.473C>T (p.S158L) and c.815_816insGA (p.S275Rfs*38) variants on Patient 3; (d) homozygous c.614C>T (p.S205L) on Patient 4; all the parents of our patients are carriers.
Red square frame: missence variation changes; red arrow: bases insertion position.

Table 2. Variants detected in *AGXT* gene among four Chinese patients with PH1.

Patients	Variant at nucleotide level	Variant type	Variant at protein level	PolyPhen-2.0 prediction and score	MutationTaster prediction and score	Conservation	Allele frequency *	Origin of the variant
1	c.815_816insGA rs180177273	heterozygous	p.S275Rfs*38	N/A	Disease Causing (1)	Conserved in vertebrates	4/8	Paternal
	c.1015delG	heterozygous	p.V339Sfs*2	N/A	Disease Causing (1)	Not conserved	1/8	Maternal
2	c.473C>T rs180177225	heterozygous	p.S158L	Probably damaging (1)	Disease Causing (0.99)	Yes	1/8	Paternal
	c.815_816insGA rs180177273	heterozygous	p.S275Rfs*38	N/A	Disease Causing (0.99)	Conserved in vertebrates	4/8	Maternal
3	c.815_816insGA rs180177273	homozygous	p.S275Rfs*38	N/A	Disease Causing (0.99)	Conserved in vertebrates	4/8	Both parents
4	c.614C>T rs180177248	homozygous	p.S205L	Probably damaging (1)	Disease Causing (1)	Conserved in mammals	2/8	Both parents

N/A, not applicable.
*Allele frequency: in this study, allele frequency represent the mutated alleles frequency among 8 alleles.

Table 3. Comparison of codons and amino acids sequence of four *AGXT* variations.

Variant	Codons and amino acids sequence of mutant	Codons and amino acids sequence of wild-type
c.473C>T	codon: GGG GAG T**T**G TCC ACC GGC	codon: GGG GAG T**C**G TCC ACC GGC
(p.S158L)	aa[1]: G E **L** S T G	aa: G E **S** S T G
c.614C>T	codon: ATC CTG TAC T**T**G GGC TCC	codon: ATC CTG TAC T**C**G GGC TCC
(p.S205L)	aa: I L Y **L** G S	aa: I L Y **S** G S
c.815_816insGA	codon: CT[2]**G A**GA GAG AGA GCC TGG	codon: CT[2]G AGA GAG AGC CTG GCC
(p.S275Rfs*38)	aa: L R E **R A W**	aa: L R E **S L A**
c.1015delG	codon: GTC AGC TAC [3]TCA TAG ACC	codon: GTC AGC TAC [3]**G**TC ATA GAC
(p.V339Sfs*2)	aa: V S Y **S** # N/A	aa: V S Y **V I D**

[1]amino acid; [2]insertion position; [3]deletion position.
#, stop codon; N/A, not applicable.
The base and amino acid differences between wild-type and mutant are highlighted in bold.

novel, and this variation was neither found in 1000 Genomes Project nor the ExAC program. The mutation, c.1015delG (p.V339Sfs*2), was identified from a late-onset male patient (Patient 1), and this mutation is expected to result in the production of a truncated AGT protein lacking the carboxy-terminal 54 amino acids. The V339 is not well conserved among species (Table 4).

The other three pathogenic variants were previously reported: c.473C>T (p.S158L) (Alkhunaizi et al. 2012), c.614C>T (p.S205L) (Williams et al. 2009), and p.S275Rfs*38 (Yuen et al. 2004; Li et al. 2014; Chen et al. 2015; Cui et al. 2017). The two missense variants (p.S158L and p.S205L) are predicted to be "probably damaging" and "disease causing," respectively, by PolyPhen-2.0 and MutationTaster. The c.473C>T (p.S158L) mutation was found in a late-onset Saudi PH1 patient who is homozygous for this allele (Alkhunaizi et al. 2012); the patient developed ESRD and underwent combined liver-kidney transplantation. In addition, the p.S205L variant was shown to have 3% in vitro enzyme activity (% normal control)

Table 4. Amino acid alignment in AGT residues (highlighted in bold).

Species	AGT Sequences (the 339th amino acid of AGT)	AGT Sequences (the 275th amino acid of AGT)
Humans	RDIVSY**V**I DHFDI	SLYSLRE**S**LALIAEQG
Pan troglodytes	RDIVSY**V**MDHFDI	N/A
Mus musculus	RDIVSY**V**LDHFDI	SLYCLRE**S**LALIAEQG
Trubripes	REMLAY**I**MKHHQM	GFFALRE**S**LAILAEKG
Danio rerio	KELLAY **I** MKHHQ	GFFALRE**S**LAILAETG
Drosophila melanogaster	KKVAEY**A**MRKYSV	LLYGLREALAHFCAVG
Xenopus tropicalis	RD I TTF **I** MNHAI	NFFSLRE**G**LALIAEQG

N/A, not applicable.

(Williams et al. 2009).

The mutation, c.815_816insGA, has been described in different ways of notation, such as c.817insAG (Yuen et al. 2004), c.823_824dupAG (Li et al. 2014), c.823_824dupGA (Isiyel et al. 2016), and c.815_816insGA (Cui et al. 2017), although the mutation results in the same frame shit. Such a difference in the notation is probably due to the presence of GA repeats (Fig. 1); namely, the wild-type allele carries the GAGAGAGA sequence (4x GA), whereas the mutant allele carries an additional GA dinucleotide between the positions 815 and 816, yielding the GAGAGAGAGA sequence (5x GA). In the present study, we use the notation of c.815_816insGA, because the position c.816 represents the 5′-end of the wild-type sequence of GAGAGAGA. The c.815_816insGA (p.S275Rfs*38) causes a frame shift that changes the amino-acid residues (positions 275-311) of "SLALIAEQGLENSWRQHREAAAYLHGRLQALGLQ LFV" to "RAWPSLRNRAWRTAGASTARPRRICMGA CRHWGCSSS," eventually generating a truncated AGT protein lacking the carboxy-terminal 81 amino-acid residues. In addition, S275 is conserved among vertebrates (Table 4).

Discussion

In this study, we present four Chinese patients with PH1 and describe their symptoms, clinical findings, treatment, and treatment response. We also describe the four mutations in the *AGXT* gene, including one novel mutation and a common mutation at a putative hotspot.

PH1 is initially diagnosed based on clinical features, biomarkers, liver enzyme analysis, and genetic testing. Elevated urinary oxalate excretion and elevated levels of plasma and urinary glycolic acid contribute to the biochemical diagnosis (Milliner et al. 1993). An elevated rate of urinary oxalate excretion (> 0.7 mmol/1.73 m^2/day) is the most typical biochemical marker, increased plasma oxalate concentrations (> 50 μmol/L) are highly suggestive of PH1, and elevated urinary glycolic acid (glycolate) levels are found in approximately 75% of all PH1 patients (Milliner et al. 1993). Liver biopsy combined with an AGT enzyme activity assay was previously considered the gold standard of PH1 diagnosis, but this invasive assay has been replaced by genetic testing. Here, in our patients, AGT enzyme analysis was not performed, because the two adult patients and the parents of the two pediatric patients refused.

The combined liver-kidney transplantation is suitable for most PH1 patients and serves as the first-line treatment. Patients are also advised to avoid oxalate-rich or oxalate-precursor-rich foods (such as spinach, tomatoes, and beet-roots) (Cochat et al. 2012). However, because PH1 patients have a relatively low intestinal oxalate absorption ability, the effect of low oxalate intake therapy remains uncertain. Other recommendations include a low-salt and high-fiber diet, and relatively low vitamin C and D intake because these vitamins promote stone formation. Excessive quantities of vitamin C may result in precipitation of calcium oxalate, and vitamin D excess may cause hypercalciuria, a risk factor for kidney stones (Cochat et al. 2012). Thus, our four patients were advised to avoid excessive vitamin C and D intake. Other treatments under development include small-molecule chaperones and probiotics (Sidhu et al. 2001; Danpure 2005a, b), and genetic therapy (Castello et al. 2016; Springer and Dowdy 2018).

The clinical features of PH1 are heterogeneous, with specific symptoms including systemic oxalosis, nephrocalcinosis, nephrolithiasis, and urinary tract infection. Additional atypical symptoms were reported, including failure to thrive and neonatal early death, even in the absence of typical phenotypes (Milliner et al. 1993). In the present study, the two adult patients had hyperthyroidism, which was also reported (Martin et al. 2011). By contrast, calcium oxalate accumulation may cause thyroid tissue damage, leading to hypothyroidism (Frishberg et al. 2000). Thus, the thyroid dysfunction is not rare in PH1 patients. For cor-

rect diagnosis of PH1, measurement of plasma and urine oxalate levels should be performed in patients with kidney stones.

Among our four patients, two patients showed symptoms during early childhood, while two adult patients showed renal failure on admission (see Table 1). In Europe, about 10% of all PH1 patients showed symptoms before the age of 6 months, 15% before the age of 1 year old, and 50% before the age of 5 years; the remaining patients presented with symptoms in late childhood or early adolescence (Cochat et al. 1999; Kemper 2005; Mandrile et al. 2014). About 33% of all PH1 patients presented with ESRD before diagnosis (van Woerden et al. 2003). Thus, PH1 patients require lifelong treatment to retard calcium oxalate precipitation, including urine alkalization and hyperhydration. Urine alkalization could prevent kidney stone formation by enhancing the solubility of uric acid in the urine; for example, citrate (an element of urine alkalization medicine) inhibits the growth of calcium oxalate. Hyperhydration is the most effective method to slow down kidney stone formation. High fluid intake and oral potassium citrate treatment are recommended especially for patients with normal renal function (Milliner et al. 1993).

The AGT crystal structure is represented as a compact dimer with PLP binding site in each subunit. PLP is covalently bound to the AGT via a Schiff base linkage with Lys209 (Cellini et al. 2012). Every AGT subunit consists of a long unstructured N-terminal tail (residues 1-21), a large domain (resides 22-282), and a small domain (residues 283-392). The small domain carries the peroxisome targeting information (Cellini et al. 2012). However, the small domain is not the only determinant for peroxisome targeting; namely, the amino-terminal domain (the residues 1-21 and 22-282) may be required for peroxisome targeting. For instance, the polymorphism c.32C>T (p.P11L) was reported to be responsible for reducing AGT enzymatic activity, because p.P11L variant causes the peroxisome mistargeting (Lumb and Danpure 2000). Moreover, p.G170R and p.F152I variants were responsible for peroxisome mistargeting (Danpure 1993; Monico et al. 2005). According to the previous study of phenotype-genotype relationships, vitamin B6 responsiveness does not depend on the level of residual AGT activity, but probably related to the degree of peroxisome targeting (Monico et al. 2005). The variant, p.V339Sfs*2, was newly identified from a late-onset male patient (Patient 1). The V399 is located in a small domain (residues 283-392) that has the peroxisome targeting information (Huber et al. 2005). However, we do not know whether each of our four variants could influence the peroxisome targeting of AGT.

To date, over 200 variants have been reported in *AGXT* (http://www.hgmd.cf.ac.uk/ac/index.php). According to previous reports, four sequence variants (p.F152I, p.G170R, p.I244T, and c.33_34insC) account for more than 50% of all PH1 alleles (Rumsby et al. 2004). The p.S205P variant is common in Japanese patients (Kawai et al. 2012). However, none of the four patients examined here carries these common variants, although the c.614C>T (p.S205L) mutation was found in Patient 4.

Total 19 genetically diagnosed patients with PH1 were reported in Chinese population (Coulter-Mackie et al. 2001, 2004; Yuen et al. 2004; Li et al. 2014; Chen et al. 2015; Wang et al. 2016; Gao et al. 2017; Cui et al. 2017; Du et al. 2018), including one Chinese mixed-Canadian patient (Coulter-Mackie et al. 2001). Among the 19 patients, three patients are family members (Wang et al. 2016). Thus, there are 17 unrelated patients reported in the previous studies and 4 patients in the present study; namely, there are 41 mutant alleles of the *AGXT* gene in Chinese population, excluding one allele inherited from a Canadian mother (Coulter-Mackie et al. 2001). Incidentally, the mutation, c.815_816insGA (p.S275Rfs*38), was found in the four patients of the present study (4/8 alleles, see Table 2). This mutation was also found in the four patients reported in the previous studies; each of them is a compound heterozygote of c.815_816insGA (p.S275Rfs*38) and either c.33_34insC (p.K12Qfs*156) (Yuen et al. 2004), c.242C>A (p.S81X) (Li et al. 2014), c.346G>A (p.G116R) (Chen et al. 2015), or c.364C>T(p.R122X) (Cui et al. 2017). Thus, among the 41 mutant alleles, the c.815_816insGA (p.S275Rfs*38) accounts for 8 alleles (8/41 alleles), suggesting that the c.815_816insGA mutation could be a common variant in Chinese population. In this context, the c.815_816insGA mutation results in 5x GA repeats, instead of 4x GA repeats in the wild-type sequence (see Fig. 1). The varying numbers of the GA repeat is reminiscent of the repeating pattern of 2-6 bases, known as microsatellite or tandem simple sequence repeat (SSR). A pathogenic SSR causes various inherited diseases, such as Fragile X syndrome, Huntington's disease, and myotonic dystrophy (Li et al. 2002). The short tandem repeat of the GA dinucleotide may represent a mutation hotspot, although the frequency of GA repeats (4 or 5 times) may be too low to generate a microsatellite instability.

On the other hand, the mutation, 679-(IVS6+2) delAAgt, has been proposed as a specific mutation in Chinese population (Coulter-Mackie et al. 2004). There are two Chinese patients homozygous for the 679-(IVS6+2) delAAgt mutation (Coulter-Mackie et al. 2004; Yuen et al. 2004). In addition, this mutation was found in one Chinese mixed-Canadian patient, a compound heterozygote of 679-(IVS6+2) delAAgt and c.454T>A (p.F152I) mutations, inherited from a Chinese father and a Canadian mother, respectively (Coulter-Mackie et al. 2001). However, the 679-(IVS6+2) delAAgt mutation was not detected in our four patients. Thus, this mutation accounts for 5/41 alleles in Chinese population.

The genotype-phenotype relationship remains unclear. For example, Mbarek et al. (2017) found that children and adults carrying the same c.33_34insC variant might have completely different outcomes with unknown reasons. Patient 2 is heterozygous for the c.473C>T (p.S158L) and

c.815_816insGA mutations, and her condition became very serious. In fact, the severity of patients harboring the c.815_816insGA are various (Yuen et al. 2004; Li et al. 2014; Chen et al. 2015; Cui et al. 2017), and a Saudi PH1 patient who is homozygous for the c.473C>T (p.S158L) allele developed ESRD and underwent combined liver-kidney transplantation (Alkhunaizi et al. 2012). Patient 3 is homozygous for the c.815_816insGA mutation, and Patient 4 is homozygous for the c.614C>T (p.S205L) mutation. These pediatric patients show relatively stable conditions at present, but the future prognosis of each patient is hard to predict.

In summary, our pediatric patients have been treated with vitamin B6, alkalization, and high fluid intake, while two adult patients with abnormal eGFR received similar treatment with the addition of high normal fluid intake. All of them were advised to avoid excessive vitamin C and D intake. Overall, our patients, except for Patient 2, responded well to the treatment, as judged by stable eGFR levels and reduced disease progression rate. However, as the follow-up time is limited in our study, the treatment responses still need long-time observation.

In conclusion, we report one common mutation, c.815_816insGA (p.S275Rfs*38), in the Chinese population and one novel mutation, c.1015delG (p.V339Sfs*2). Moreover, we suggest that the short repeat of the GA dinucleotide may represent a mutation hotspot of the *AGXT* gene. These results will provide a framework for future genetic diagnosis of PH1. A large-scale study of PH1 in China should be performed.

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Conflict of Interest

The authors declare no conflict of interest.

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