A case of xanthinuria type I with a novel mutation in xanthine dehydrogenase

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Abstract Hereditary hypouricemia is generally caused by renal hypouricemia, an autosomal recessive disorder that is characterized by impaired renal tubular uric acid transport, or by xanthinuria, a rare autosomal recessive disorder caused by a deficiency of xanthine dehydrogenase (XDH; xanthinuria type I) or by a deficiency of both XDH and aldehyde oxidase (xanthinuria type II). In contrast to renal hypouricemia, which sometimes leads to exercise-induced acute kidney injury (EIAKI), xanthinuria has not been associated with this disorder. We report here a case of xanthinuria type I due to a compound heterozygous mutation. A 46-year-old woman was found to have undetectable plasma and urinary levels of uric acid. She had no symptoms and no history of EIAKI. Xanthinuria type I was diagnosed following the allopurinol loading test. Mutation analysis revealed a compound heterozygous mutation [c.305A>G (p.Gln102Arg) and c.2567delC (p.Thr856Lysfs*73)] in the XDH gene. Of these two mutations, the former is novel. The patient did not exhibit EIAKI. However, because

xanthinuria is a rare disease, the identification of additional cases is necessary to determine whether this disease is complicated with EIAKI.

Keywords Exercise-induced acute kidney injury · Hypouricemia · Xanthinuria · Xanthine oxidase

Introduction

Hypouricemia is a benign condition where plasma uric acid (UA) levels are lower than 2.0 mg/dL. Hereditary hypouricemia can be caused by either renal hypouricemia or xanthinuria. Renal hypouricemia is an autosomal recessive disorder characterized by impaired tubular UA transport. It is caused by mutations in *SLC22A12* encoding URAT1, and *SLC2A9* encoding GLUT9 [1, 2]. Although most cases of renal hypouricemia have no clinical symptoms, 7–10 % of patients have urolithiasis and 10 % have exercise-induced acute kidney injury (EIAKI) [3]. Xanthinuria is a rare autosomal recessive disorder caused by mutations in the xanthine dehydrogenase (XDH) gene leading to a deficiency or decreased activity of this enzyme [4]. It is characterized by hypouricemia, reductions in urinary UA excretion, and increases in the plasma and urinary levels of xanthine and hypoxanthine [5]. Xanthinuria is classified into two types: xanthinuria type I is characterized by a deficiency of XDH alone while xanthinuria type II is characterized by a deficiency of both XDH and aldehyde oxidase [6, 7].

Most cases of hereditary hypouricemia are reported as renal hypouricemia. Cases of xanthinuria are reported rarely. Here, we report a case of xanthinuria type I due to a compound heterozygous mutation in *XDH*.

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Case report

A 46-year-old woman was referred to our institution as an inpatient because of exacerbation of bronchial asthma. Her medical record at the age of 40 years, as well as other medical examinations performed at later points in her life, showed the blood level of UA as 0.0 mg/dL. There were no other abnormalities. At 44 years of age, she was diagnosed with bronchial asthma. Laboratory data at the time of admission for an acute bronchial asthma attack showed 0.0 mg/dL plasma UA and 0.0 mg/day urinary excretion of UA. Other tests showed a normal complete peripheral blood cell count, negative results for urinary protein, urinary red blood cells <1 per high powered field, and creatinine clearance of 112.6 mL/min/1.73 m². An abdominal computed tomography scan showed normal kidney size and shape, without nephrolithiasis or urinary tract stones. None of her family members had hypouricemia, although her younger sister had a urinary tract stone. The undetectable level of UA in the plasma and urine of this patient indicated the possibility of xanthinuria.

Allopurinol loading test

The allopurinol loading test was administered to discriminate between xanthinuria type I and II. Plasma and urinary levels of hypoxanthine and xanthine were measured by high-pressure liquid chromatography [4]. The plasma hypoxanthine level was elevated at 0.095 mg/dL (normal range 0.014–0.061 mg/dL [8]). The plasma xanthine level was also elevated at 0.390 mg/dL (normal range 0–0.038 mg/dL [8]). After allopurinol administration (300 mg/body) [9], her plasma oxypurinol levels were increased to 0.614 mg/dL (Table 1). Based on these results, the patient was diagnosed with xanthinuria type I.

Molecular genetic analysis

After approval for the genetic analysis had been granted by the ethics committee of the hospital, informed consent was

obtained from the patient. The patient was tested for *XDH* mutations by polymerase chain reaction amplification of genomic DNA and bidirectional automated DNA sequencing of *XDH*. She was found to be heterozygous for the c.305A>G (p.Gln102Arg) and c.2567delC (p.Thr856Lysfs*73) mutations (Fig. 1).

Discussion

We identified two mutations in *XDH* as the cause of xanthinuria type I in our case. *XDH* is a large molecule comprising 1333 amino acids with functions that have been mapped to three peptide domains [10]. The NH₂-terminal domain contains a 2Fe/2S nonheme iron-binding site (Fe/S domain). The adjacent site contains a flavin-binding domain (FAD domain), and the COOH-terminal domain contains a molybdenum cofactor binding site (Moco domain) [11].

To date, 17 mutations in *XDH* have been implicated in xanthinuria type I (Table 2) [6, 12–20]. More than 50 % of these mutations have been detected in the Moco domain, the largest domain in *XDH* where hypoxanthine and xanthine are hydrolyzed to uric acid. Although almost all Moco domain mutations are missense, c.2567delC leads to a frameshift at amino acid 856, creating a stop codon at position 928 [12, 20].

The Fe/S domain contains a cluster of two nonheme iron-binding sites (FeS I, FeS II) that function as reaction centers. Two mutations affecting each of these sites have been reported [11, 12]. The c.140_141insG mutation affects FeS I [12]. This is a frameshift mutation early in the DNA sequence and may disrupt *XDH* synthesis. In contrast, the c.445C>T mutation at the FeS I cluster motif likely disrupts cluster formation resulting in a loss of the electron transfer ability of the enzyme, despite correct folding and processing [5, 13].

We detected a novel mutation between FeS I and FeS II; c.305A>G (p.Gln102Arg). This missense mutation leads to substitution of arginine with glutamine. Because arginine

Table 1 Allopurinol loading test

Plasma	Cr (mg/dL)	UA (mg/dL)	HX (mg/dL)	X (mg/dL)	Allp (mg/dL)	Oxyp (mg/dL)
Pre-administration	0.54	0	0.095	0.39		
3 h	0.58	0	0.074	0.376	0.207	0.614
Urine	Cr (mg/dL)	UA (mg/dL)	HX (mg/mg Cr)	X (mg/mg Cr)	Allp (mg/mg Cr)	Oxyp (mg/mg Cr)
Pre-administration	77.3	0	0.07	0.277		
2–4 h	61.5	0	0.082	0.384	0.174	0.173

Cr creatinine, UA uric acid, HX hypoxanthine, X xanthine, Allp allopurinol, Oxyp oxypurinol

Fig. 1 DNA sequencing of the mutated regions of the XDH gene. The lower sequence from the patient showed the heterozygous mutations, c.305A>G and c.2567delC, as compared to the upper control sequence. The reverse sequence is shown on the right because the forward sequence was not clear

Table 2 Mutations causing type I xanthinuria

Domain	Codon change	Amino acid change	Codon number	Phenotype	References
Fe/S domain	c.140_141insG	p.Cys48Leufs*12	47	Xanthinuria type I	[11]
Fe/S domain	c.305A>G	p.Gln102Arg	102	Xanthinuria type I	Our case
Fe/S domain	c.445C>T	p.Arg149Cys	149	Xanthinuria type I	[12]
domain linker	c.641delC	p.Pro214Glnfs*4	214	Xanthinuria type I	[13, 14]
FAD domain	c.682C>T	p.Arg228*	228	Xanthinuria type I	[6]
domain linker	c.1664_1665insC	p.Ala556Serfs*15	555	Xanthinuria type I	[15]
domain linker	c.1663C>T	p.Pro555Ser	555	Decreased activity	[16]
Moco domain	c.1820G>A	p.Arg607Gln	607	Decreased activity	[16]
Moco domain	c.1868C>T	p.Thr623Ile	623	Decreased activity	[16]
Moco domain	c.2164A>T	p.Lys722*	722	Xanthinuria type I	[17]
Moco domain	c.2473C>T	p.Arg825*	825	Xanthinuria type I	[14]
Moco domain	c.2567delC	p.Thr856Lysfs*73	856	Xanthinuria type I	[6, 18], our case
Moco domain	c.2641C>T	p.Arg881*	881	Xanthinuria type I	[14]
Moco domain	c.2727C>A	p.Asn909Lys	909	Decreased activity	[16]
Moco domain	c.2729C>A	p.Thr910Lys	910	XDH deficiency	[16]
Moco domain	c.2729C>T	p.Thr910Met	910	Xanthinuria type I	[11, 19]
Moco domain	c.3449C>G	p.Pro1150Arg	1150	Decreased activity	[16]
Moco domain	c.3953G>A	p.Cys1318Tyr	1318	Decreased activity	[16]

has a stronger electrical charge than glutamine, the mutation might disrupt the structure of the cluster or lead to its misfolding. Therefore, although the resulting XDH has a normal structure, the FeS I or FeS II sites might not function appropriately. Moreover, because the glutamine residue is highly conserved across species (Fig. 2), it is possible that this mutation affects the activity of XDH. We also administered in silico analysis to estimate this mutation to function of the protein. sorting intolerant from tolerant (SIFT) (<http://sift.jcvi.org/>) showed DAMAGING, SIFT score 0.01, PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>) showed PROBABLY DAMAGING, score

0.986, and mutation taster (<http://www.mutationtaster.org/>) showed disease causing, score 0.99. From the above results, this mutation should affect the activity of XDH. However, we cannot confirm this assumption because we did not conduct mutational studies.

Clinically, patients with xanthinuria have not been reported to have EIAKI although renal hypouricemia is often associated with this disorder [21, 22]. Our patient did not have any history of acute kidney injury, macrohematuria, or lumbago after exercise, despite playing tennis as a student. If xanthinuria is never associated with EIAKI, it is an important point when we consider the pathogenesis of EIAKI. The

Fig. 2 Alignment of the amino acid sequences of xanthine dehydrogenase enzymes from different species. The arrow indicates the position of the novel missense mutation. The glutamine residue was highly conserved across species

pathogenesis of EIAKI in renal hypouricemia is unclear; however, two main hypotheses have been proposed. The UA precipitation hypothesis states that renal tubules are obstructed by the precipitation and crystallization of UA caused by prolonged or strenuous exercise [23]. However, UA crystallization in renal tubules has not been observed in most renal biopsies in cases of EIAKI. Therefore, another mechanism has been suggested. The vasospasm hypothesis suggests that EIAKI is caused by the absence of the powerful antioxidant activity of UA. Two cases of renal hypouricemia demonstrated patchy wedge-shaped high-contrast regions, indicative of patchy renal vasoconstriction, in contrast computed tomography [24]. Anaerobic exercise induces accumulation of oxygen free radicals, which are well-known vasoconstrictors, and their accumulation can result in reduced glomerular filtration rate. UA seems to play a protective role in the kidney, and the decreased antioxidant potential in renal hypouricemia might be related to kidney injury caused by ROS. On the other hand, there are no reports of EIAKI in patients with xanthinuria, despite the lack of UA. This could be because in xanthinuria, UA deficiency is accompanied by XDH deficiency or decreased XDH activity. Normally, XDH is converted to xanthine oxidase (XO), which is known to increase oxidative stress [4]. However, because there are few case reports of xanthinuria, the association between this disorder and EIAKI is not known. To elucidate the pathogenesis of EIAKI, the identification of more cases of xanthinuria is important.

Conclusion

We report here a case of xanthinuria type I due to the compound heterozygous mutations c.2567delC and c.305A>G. This is a novel compound mutation that did not lead to EIAKI in this patient, although additional xanthinuria cases are needed to confirm this lack of association.

Compliance with ethical standards

Conflict of interest The authors have declared that no conflict of interest exists.

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