IDIOPATHIC RENAL HYPOURICEMIA: IDENTIFICATION AND CHARACTERIZATION OF *SLC22A12* MUTATIONS IN SPANISH PATIENTS

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Introduction

Idiopathic renal hypouricemia (RHUC) is a rare inherited disorder characterized by impaired uric acid (UA) reabsorption at the apical membrane of proximal tubule cells[1]. Patients present low serum levels of UA associated with excessive urinary wasting of UA, and some have severe complications like exercise-induced acute renal failure or nephrolithiasis[2,3]. Mutations in the *SLC22A12* gene, encoding the renal tubular UA transporter 1 (URAT1), are the major cause of this disorder (RHUC Type 1)[2,4]. A few patients present mutations in GLUT9, another UA transporter encoded by *SLC2A9* (RHUC Type 2)[5,6]. URAT1 is involved in the reabsortion of UA across the apical membrane of proximal tubule cells[4] (Fig. 1). Most *SLC22A12* mutations have been identified in Japanese patients, and only a few have been detected in Europeans[7-9]. Here, we report clinical and molecular data of five Spanish patients diagnosed with RHUC.

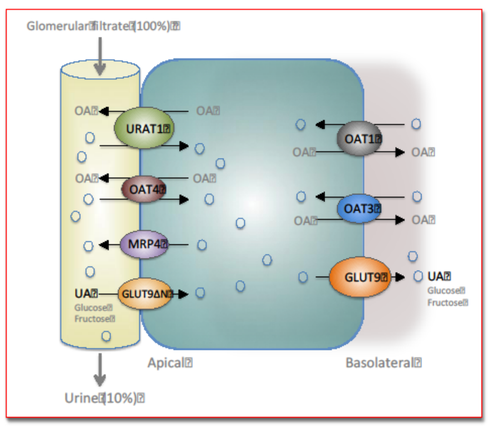


Fig. 1. Model of UA transport in the proximal tubule.
(adapted from ref. 10)

Methods

Blood and urinary samples were collected for measurement of UA and creatinine levels and for genetic analysis. Patients were evaluated for renal stones or other renal diseases. Genomic DNA was isolated from peripheral blood using a commercial kit, and *SLC22A12* exons were amplified by PCR. Mutational analysis was performed by direct DNA sequencing. The new *SLC22A12* allelic variant was prepared using site-directed mutagenesis. Urate uptake and subcellular localization studies were carried out using a *Xenopus* oocytes expression system.

Results

The patients had persistently low serum UA levels and elevated fractional excretion of UA (Table 1). One had nephrolithiasis and was also diagnosed with polycystic kidney disease. The other patients were asymptomatic.

Table 1. Clinical and genetic characteristics of patients with RHUC.

Family Patient	F1 P520	F2 P612	F2 P613	F3 P651	F4 P301
Sex	Female	Female	Male	Male	Male
Age (years)	40	8	10	21	16
Serum uric acid (mg/dl)	1.04	1.32	1.35	0.95	1.00
FE uric acid (%)	35%	22.5%	27.5%	60%	28%
Serum creatinine (mg/dl)	0.95	0.50	0.60	0.55	0.50
Renal symptoms	NL, CKD	None	None	None	None
Comorbidity	ADPKD	-	-	NDI	-
Ethnic group	Gypsy	Caucasian	Caucasian	Gypsy	Gypsy
Mutation	p.L415_G417 - -	p.A476D - -	p.A476D - -	p.T467M p.T467M	p.T467M p.T467M

NL, nephrolithiasis; CDK, chronic kidney disease; NDI, neprogenic diabetes insipidus; ADPKD, autosomal dominant polycystic kidney disease

Sequence analysis of *SLC22A12* revealed mutations in each patient (Table 1, Fig. 2). A new missense mutation, p.A476D, affecting a conserved residue in URAT1 transmembrane domain 11, was identified (Figs. 3 and 4). Analysis with informatics tools suggests that p.A476D is a pathogenic mutation (Table 2). Two previously reported mutations, p.T467M and p.L415_G417del, associated with RHUC in families from the Czech Republic[8] were also detected.

Fig. 2. *SLC22A12* mutations identified in patients.

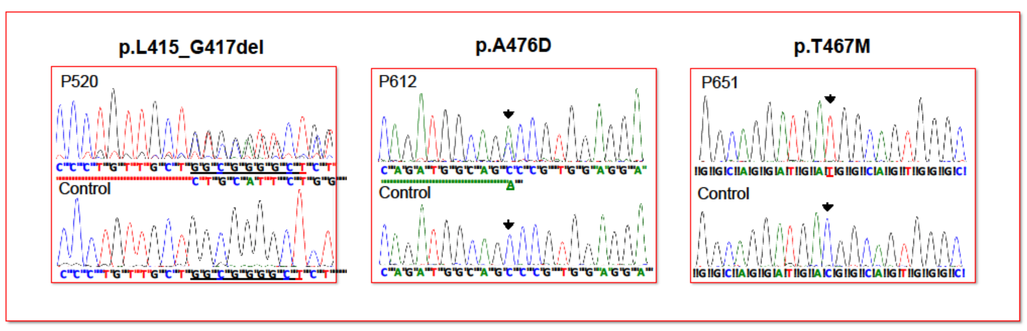


Fig. 3. Location of mutations in the URAT1 protein.

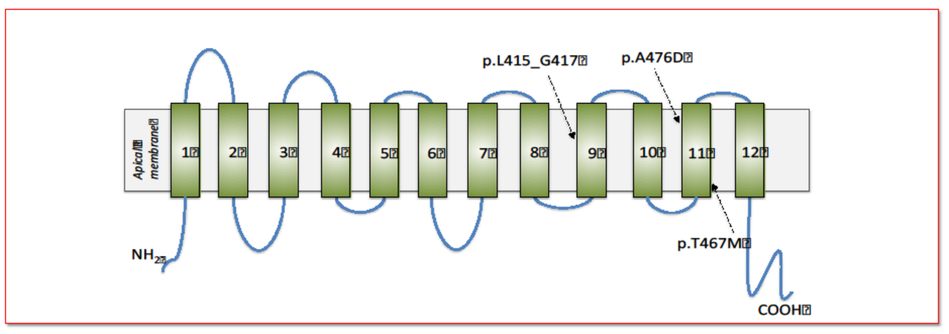


Fig. 4. Aminoacid residue A476 is conserve in evolution.

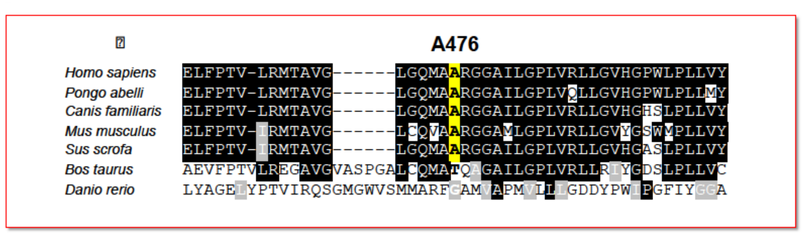


Table 2. Informatics analysis of missense mutations.

Mutation	Exon	PolyPhen-2	SIFT	Align GVGD
p.T467M	9	Probably damaging (1,00)	Affects function (0,09)	Highly pathogenic (C65)
p.A476D	9	Probably damaging (0,97)	Affects function (0,09)	Highly pathogenic (C65)

Functional studies showed that mutation p.A476D considerably diminished UA transport (Fig. 5). Oocytes expressing p.A476D showed a discontinuous URAT1 signal on the plasma membrane and intracytoplasmic staining was lower than in the normal control (Fig. 6).

Fig. 5 Urate transport in oocytes.

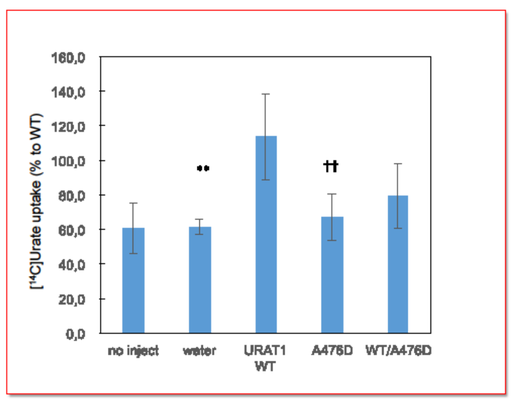
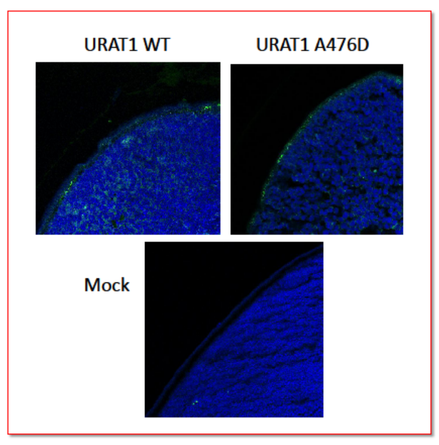


Fig. 6. Immunocytochemical analysis.



Conclusion

- Our study describes the clinical and molecular characteristics of the first Spanish patients diagnosed with RHUC.
- A new pathogenic *SLC22A12* mutation causing URAT1 loss of function was identified.
- Although RHUC has been described predominantly in patients from Asia it should be considered in European countries as well.

References

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