Hints to the diagnosis of uromodulin kidney disease

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Abstract

Background: Uromodulin kidney disease (UKD) is an inherited kidney disease caused by a uromodulin (UMOD) gene mutation. The UMOD gene encodes the Tamm–Horsfall protein (THP), which is the most abundant protein in healthy human urine. Because of its rarity, the incidence of UKD has not been fully elucidated. The purpose of the present study is to clarify the frequency of UKD among patients who underwent renal biopsy.

Methods: Immunostaining for THP was performed for patients <50 years of age with renal insufficiency and hyperuricemia without overt urinalysis abnormality from renal biopsy databases. Serum and urinary THP concentrations were evaluated in available individuals.

Results: Fifteen patients were selected for immunostaining from a total of 3787 patients. In three independent patients, abnormal THP accumulation in renal tubular cells was observed. A novel missense A247P UMOD mutation was detected in two of the three patients, including one having a typical family history of familial juvenile hyperuricemic nephropathy. Serum and urinary THP concentrations of all available patients with UMOD A247P mutation were significantly lower than those of controls.

Conclusions: In the present study, UKD was detected in <1 in 1000 subjects who underwent renal biopsy. However, in subjects meeting all of the above criteria, abnormal THP accumulation was detected in 20% (3/15), suggesting that renal biopsy with immunostaining for THP is a good tool for diagnosing UKD. Also, low serum THP concentration detected in the present subjects might be a good diagnostic marker or important in understanding the pathogenesis of UKD.

Key words: CKD, familial juvenile 2, hyperuricemic nephropathy, kidney biopsy, Tamm–Horsfall protein, uromodulin
Introduction

Familial juvenile hyperuricemic nephropathy (FJHN) is characterized by urinary concentration defect, hyperuricemia, gout and progressive renal failure. Affected patients usually reach end-stage kidney disease (ESKD) between the fourth and seventh decade [1]. Uromodulin (UMOD) gene mutations have been found in FJHN, medullary cystic kidney disease type 2 (MCKD2) and glomerulocystic kidney disease (GCKD). These diseases share some clinical features and are called uromodulin kidney disease (UKD) [2, 3]. Recently a new disease category, autosomal dominant tubulointerstitial kidney disease (ADTKD), was proposed for the purpose of avoiding confusion arising from the use of multiple names for the same condition [4]. UKD is also called ADTKD-UMOD accordingly. UMOD gene encodes the Tamm–Horsfall protein (THP), which is the most abundant protein in human urine. Urinary THP is reported to protect against urinary tract infection [5], prevent urolithiasis [6], ensure water impermeability and create the countercurrent gradient [7].

Recently a number of genome-wide association studies have revealed that particular variants of the UMOD gene are associated with the risk of developing chronic kidney disease (CKD) or hypertension [8, 9]. However, the exact function of THP and the mechanism by which mutated THP triggers UKD are still elusive. Although >100 UKD families have been reported, the exact epidemiology of this condition is still unclear [10]. Also, awareness of this disease is not sufficient among clinicians, meaning that a considerable number of UKD patients have likely reached end-stage kidney disease (ESKD) with only ambiguous diagnosis of CKD, despite undergoing renal biopsy. The aim of the present study is to elucidate the frequency of UKD among patients who underwent renal biopsy.

Subjects and methods

Subjects

Clinical data of 3787 patients who underwent renal biopsy at the Kanazawa University hospital and affiliated medical facilities from 1992 to 2013 were analyzed. Immunostaining for THP was performed in renal sections of patients who met all of the following criteria: (i) renal insufficiency [serum creatinine (sCr) > 1.0 mg/dL] at <50 years of age, (ii) hyperuricemia [serum uric acid > 7 mg/dL] or under treatment for hyperuricemia, (iii) no or only very mild abnormalities in urinalysis and (iv) no other apparent renal disease present clinically or histopathologically.

The DNA of patients who had an abnormal THP staining pattern were analyzed for sequence analysis of the UMOD gene after written informed consent was obtained. Serum and urinary THP concentrations were measured in individuals who were diagnosed with UKD and 135 individuals (62% men, mean age 64.4 ± 16.6 years, mean eGFR 66.4 ± 34.0 mL/min) with glomerulonephritis, nephrosclerosis, interstitial nephritis, diabetes mellitus or controls without kidney disease.

Methods

Immunostaining

THP was stained with anti-THP rabbit polyclonal antibody (Santa Cruz Biotechnology, Dallas, TX, USA). Anti-PDI (protein disulfide isomerase) mouse monoclonal antibody (Enzo Life Sciences, Farmingdale, NY, USA) was used for endoplasmic reticulum (ER) staining together with THP staining. Fluorescence-labeled antibody Alexa Flour 488 donkey anti-rabbit IgG and Alexa Flour 594 donkey anti-rabbit IgG (Invitrogen, Carlsbad, CA, USA; dilution 1:200) were used for detection with Biorevo BZ-9000 (KEYENCE, Osaka, Japan). Abnormal accumulation of THP was positive when clusters of THP were seen in >90% of THP-positive tubules and negative when most THP showed a granular or linear appearance.

Mutation analysis

Genetic DNAs were extracted from peripheral blood leukocytes. AmpliTaq gold DNA polymerase (Applied Biosystems, Foster City, CA, USA) and a universal touchdown thermal cycling program with GeneAtlas PC 320 (Astec, Fukuoka, Japan) were applied as described [11]. For some GC-rich regions, LTAq (Takara Bio, Otsu, Japan) was used as DNA polymerase for PCR as recommended by the manufacturer. The specific flanking primers for the mutation analysis of UMOD were referred to Hart et al. [2]. After purification using a BigDye X Terminator Purification Kit (Applied Biosystems), the amplified PCR products containing exons were sequenced in both directions on an automatic fluorescence sequencer (ABI Prism 310 DNA sequencer, Applied Biosystems) using a dye terminator thermal cycle sequencing kit (Amersham Biosciences, Piscataway, NJ, USA).

Haplotype type

Linkage analysis to the UMOD gene was performed with six polymorphic microsatellite markers: cen – D16S3046 – D16S773 – D16S3217 – D16S749 – D16S3036 – D16S3041 – tel. Fluorescently labeled PCR products were detected with an ABI Prism 310 genetic analyzer (Applied Biosystems) and were analyzed using Genemapper software (Applied Biosystems).

Quantitative analysis of THP concentration by ELISA

Serum and urinary THP concentrations were measured with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (BioVendor, Brno, Slovenia). Fresh urine and serum samples were kept frozen after collection until the measurement. Urinary THP concentration was corrected according to the urinary creatinine level. The results are expressed as mean ± SD unless stated otherwise.

Potential differences of urinary and serum THP concentrations between groups were evaluated by non-parametric Mann–Whitney test. A P-value <0.05 was regarded as statistically significant. Correlations were tested using the Spearman’s test.

Results

Immunostaining

Fifteen of 3787 patients met all of the criteria and immunostaining for THP was performed for them. Clinical characteristics of the 15 patients are shown in Table 1. Since the presence or absence of a family history was not a required item on the renal biopsy request form, ‘positive’ refers only to cases in which comments regarding a positive family history were noted in the free space of the form. Three of the 15 patients listed in Table 1 had abnormal THP staining in tubular cells of their renal kidney sections (Figure 1). Their THP staining was colocalized with PDI, which is an ER resident chaperone protein in double fluorescent immune staining (Figure 2). We could not find abnormal THP staining in 10 individuals between 50 and 60 years old who met other criteria (data not shown).

Case 6 stopped visiting the hospital after she underwent renal biopsy, and since we were unable to contact her or her family, her
DNA could not be obtained. This meant that the UMOD sequencing analysis could be performed in only cases 12 and 14. Heterozygosity for a novel missense mutation was identified in exon 3 in the two patients. There was a base exchange (c. 739G>C) in position 739, which led to replacement of the amino acid alanine with proline in the protein in codon 247 (p.Ala247Pro). This

Table 1. Clinical characteristics of 15 patients with renal insufficiency and hyperuricemia without urinalysis abnormality at <50 years of age

| Case number | Year | FH | Age (years) | Sex | sCr (mg/dL) | S-UA (mg/dL) | U-prot | U-ob | Renal pathology |
|-------------|------|----|-------------|-----|-------------|--------------|--------|------|----------------|
| 1           | 1992 | −  | 44          | M   | 1.3         | 7.3          | ±      | ±    | Nephrosclerosis |
| 2           | 1992 | −  | 19          | M   | 1.0         | 8.5          | −      | ±    | ESKD           |
| 3           | 1993 | −  | 28          | M   | 3.1         | T            | −      | −    | TIN, nephrosclerosis |
| 4           | 1994 | −  | 36          | M   | 2.0         | 7.9          | ±      | −    | TIN            |
| 5           | 1994 | −  | 50          | F   | 2.6         | 7.2          | −      | −    | TIN, nephrosclerosis |
| 6           | 1996 | −  | 47          | F   | 1.5         | 7.8          | −      | −    | TIN            |
| 7           | 1996 | −  | 35          | M   | 2.5         | 13.2         | −      | −    | TIN            |
| 8           | 1998 | −  | 28          | F   | 2.1         | 8.4          | −      | ±    | Nephrosclerosis |
| 9           | 1999 | −  | 26          | M   | 1.0         | 7.7          | −      | −    | Nephrosclerosis |
| 10          | 2000 | −  | 37          | M   | 8.2         | 9.4          | −      | −    | TIN            |
| 11          | 2004 | −  | 49          | M   | 1.5         | T            | −      | −    | TIN, nephrosclerosis |
| 12          | 2005 | −  | 31          | M   | 1.31        | 7.8          | −      | −    | TIN            |
| 13          | 2005 | −  | 48          | M   | 1.26        | 8.5          | −      | −    | TIN            |
| 14          | 2007 | +  | 29          | M   | 1.4         | 9            | −      | −    | TIN, nephrosclerosis |
| 15          | 2009 | −  | 34          | M   | 2.64        | 12.8         | −      | −    | Nephrosclerosis |

FH, family history of kidney disease; sCr, serum creatinine; S-UA, ; U-prot, ; U-ob; M, male; F, female; T, treated for hyperuricemia; TIN, tubulointerstitial nephritis; ESKD, end-stage kidney disease.

Fig. 1. THP immunostaining images of 15 patients (1–15) with renal insufficiency and hyperuricemia but abnormal urinalysis at <50 years of age and a control (C). In the control kidney, THP shows diffuse cytoplasmic staining and most abundant signals in the apical membrane. In the kidneys of cases 6, 12 and 14, massive clusters of THP accumulate intracellularly.
mutation did not exist in the DNA of 50 normal persons or the unaffected father of case 14. Affected available family members of case 14 (II-2, II-5) had the same A247P mutation (Figure 3).

**Haplotype analysis**

Haplotype analysis with six polymorphic microsatellites was performed in all available individuals in family 14 and case 12. Although in case 12 their alleles could not clearly be assigned to one haplotype because DNA from his family was not available, under the assumption of a shared haplotype, the haplotype colored in pink showed cosegregation with the phenotype (Figure 3).

**Clinical data**

**Case 14 (proband, III-4 of family 14)**

At a medical checkup at the age of 22 years, sCr (1.4 mg/dL) and uric acid (9.0 mg/dL) were noted. Renal biopsy was performed and focal tubulointerstitial nephropathy was detected. No cysts were detected in his kidneys on ultrasound. There was a small stone in his left kidney. He is currently 32 years old and his sCr is 2.41 mg/dL. His mother (II-2) experienced a gout attack at the age of 40 years and started uric acid-lowering and antihypertensive therapy at that time. At the age of 62 years her sCr level increased to 2.5 mg/dL. Her sister (II-5) is currently 71 years old with an sCr of 2.26 mg/dL. Their father (I-3) experienced gout attacks from a young age and started hemodialysis (HD) therapy at the age of 60 years. He died of ileus caused by dialysis-related amyloidosis at the age of 73 years.

**Case 12**

Hyperuricemia was noted out when the patient was 26 years old. At 30 years, he experienced a gout attack. Renal biopsy was performed because of gradually worsening renal insufficiency at 31 years. An obvious family history of renal disease was absent. His urinalysis was normal. His current age is 42 years and his sCr is 1.57 mg/dL.
Case 6
When she was 47 years old, renal biopsy was performed for renal insufficiency without any abnormalities on urinalysis. She had no family history of kidney disease. Subsequent clinical information is not available.

Serum and urinary THP concentrations
In individuals with various kidney diseases and controls, serum and urinary THP concentrations were positively correlated with eGFR (Supplementary Figure S1), as already reported \[12, 13\]. However, serum THP concentrations showed a stronger correlation \(r = 0.604\) with eGFR than did urinary THP concentrations \(r = 0.325\).

Serum and urinary THP concentrations of affected patients with an A247P UMOD mutation were compared with those of kidney disease patients whose GFR showed similar values (Figure 4). Urinary THP concentrations of patients with A247P UMOD mutations were significantly lower than those of controls with an eGFR <44 mL/min \(P = 0.003172\).

Serum THP concentrations of A247P patients were significantly lower compared with controls with similar eGFR values \(P < 0.00001\) but were not different from serum THP concentrations of ESRD patients on HD \(P = 0.1127\).

Discussion
UKD is a rare inherited disease. Based on renal replacement therapy (RRT) patient surveys, the calculated prevalence of UKD is reported to be 1.67 cases per million population and 0.73 per 1000 RRT patients in Austria \[14\] and 1.52 patients per million population and 0.58 per 1000 RRT patients in the Czech Republic \[7\].

It is suggested that hereditary tubulointerstitial nephritis may be underrecognized, as the hallmarks of this disease are also common in patients with other CKD, and a family history may be absent or unknown \[15\].

In the present study, two UKD patients were detected among 3787 patients who underwent renal biopsies. Interestingly, both independent patients had the same A247P UMOD mutation. Haplotype analysis of their DNA indicated that they have a high possibility of sharing one ancestor.

Although the DNA of case 6 was not available, this patient is also assumed to have UKD, because of the abnormal THP accumulation in her kidney samples. Case 14 had been surmised to have FJHN before renal biopsy, because of his family history of kidney disease. Meanwhile, case 12 had no such obvious history. This suggests a wide spectrum of phenotypes among patients with the same A247P UMOD mutation.

The C744G UMOD mutation in which the 248th cysteine is substituted by tryptophan (Cys248Trp) is frequently found in Europe or Turkey, with this assumed to be due to a founder effect \[16\]. This mutation causes both FJHN and MCKD. The A247P mutation detected in the present study was the amino acid next to this Cys248Trp mutation. They are located in D8C domains that consist of eight conserved cysteine residues that are probably involved in disulfide bond formation. Mutations of cysteine
residues in D8C domains lead to misfolding and mistransporting of THP, resulting in abnormal deposition of THP in the ER. Mutation of 247Ala is assumed to have the same effect.

THP is exclusively synthesized in the epithelial cells lining the thick ascending limb (TAL) of Henle’s loop and predominantly targeted apically and secreted in urine. However, a little THP is basolaterally targeted and released in the serum [17]. Although the significance and function of serum THP are not fully understood, its concentration is reported to be 45–490 ng/mL in the serum of healthy individuals and associated with kidney function [13]. Serum THP concentrations in UKD patients are still elusive. Very high or very low serum THP concentrations were reported in UKD patients with an A247P mutation [18]. Prajczer et al. [19] reported that serum THP concentrations in UKD patients did not differ as compared with healthy controls and showed a very broad range.

THP extracted from the urine in UKD patients has been reported to be significantly low in various reports based on the results of impaired THP trafficking in TAL cells [20]. In the present report, both the serum and urinary THP concentrations of patients with an A247P UMOD mutation were statistically lower than those of controls with similar eGFRs. However, the decrease of serum THP concentrations was more prominent than that of urinary THP concentrations.

In conclusion, the number of UKD patients is very small (<1 in 1000 patients) in renal biopsy based populations. However, 2 definitive and 1 probable UKD patients were detected among 15 patients with renal insufficiency, and hyperuricemia without urinalysis abnormality at <50 years of age. All available A247P UMOD patients showed significantly low serum THP
concentrations, which may be an important and specific finding in UKD. Serum THP might be a better marker for UKD than urinary THP and also may be a good target for UKD treatment in the future.

Supplementary data
Supplementary data are available online at http://ckj.oxfordjournals.org.

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Conflict of interest statement
None declared.

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