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CASE REPORT

A novel mutation in the uromodulin gene in a Japanese family with a mild phenotype of familial juvenile hyperuricemic nephropathy

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Abstract Familial juvenile hyperuricemic nephropathy (FJHN) is an autosomal-dominant disorder that is characterized by hyperuricemia and chronic renal failure and results in end-stage renal failure. FJHN is caused by mutations in the *UMOD* gene, which encodes uromodulin. Uromodulin contains three epidermal growth factor (EGF)-like domains, a domain of eight cysteine residues (D8C), and a zona pellucid-like domain. Over 90 % of *UMOD* mutations are missense mutations, and over 80 % exist in exon 4, which encodes both D8C and the EGF-like domains. A 56-year-old woman was diagnosed with hyperuricemia with a serum uric acid level of 7.5 mg/dL, and stage III chronic kidney disease (CKD) with a serum creatinine level of 1.12 mg/dL and an estimated glomerular filtration rate of 39.9 mL/(min 1.73 m^2). The patient had a family history of hyperuricemia and stage IV CKD; both the patient and her affected family members had a novel mutation in the *UMOD* gene: c.C518G (p.P173R), located between the EGF-like domains and D8C. This mutation, along with previously reported nearby mutations, causes a clinically mild phenotype of FJHN. It is important that physicians consider the diagnosis of FJHN in patients with a family history of hyperuricemia associated with renal dysfunction, even if the patient has only mild renal impairment.

Keywords FJHN · Uromodulin · Hyperuricemia · Mutation · *UMOD*

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Introduction

Familial juvenile hyperuricemic nephropathy (FJHN) is an autosomal-dominant disorder characterized by hyperuricemia due to decreased urinary excretion of uric acid. In such cases, chronic interstitial nephritis typically develops and most often leads to progressive renal failure; there is minimal or no proteinuria and inactive urine sediment without hyaline casts. The fractional excretion of uric acid is less than 5 % in the setting of a normal glomerular filtration rate. When renal insufficiency progresses, the urine concentrating ability is affected. Renal impairment usually develops between 15 and 40 years of age, leading to end-stage renal disease within 10–20 years. Histological analysis of kidneys demonstrates interstitial fibrosis and tubular atrophy. Genetic linkage studies have localized FJHN genes to chromosome 16p12 [1]. In 2002, Hart et al. [2] identified three mutations in the *UMOD* gene in three families with FJHN. Since then, over 46 different heterozygous *UMOD* mutations have been detected in patients with FJHN. *UMOD* mutations are also associated with medullary cystic kidney disease type 2 (MCKD2), a

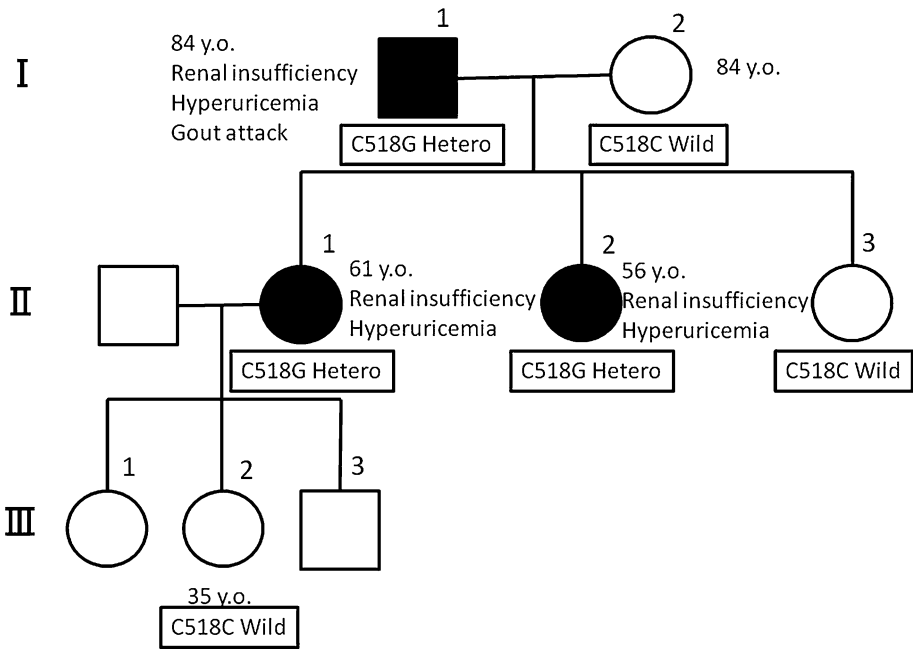

Fig. 1 Family pedigree, showing individuals with familial juvenile hyperuricemic nephropathy. Hyperuricemia was defined as a serum uric acid level of over 7.0 mg/dL. Renal insufficiency was defined as serum creatinine level of over 1.5 mg/dL. *UMOD* mutations are shown in *boxes*

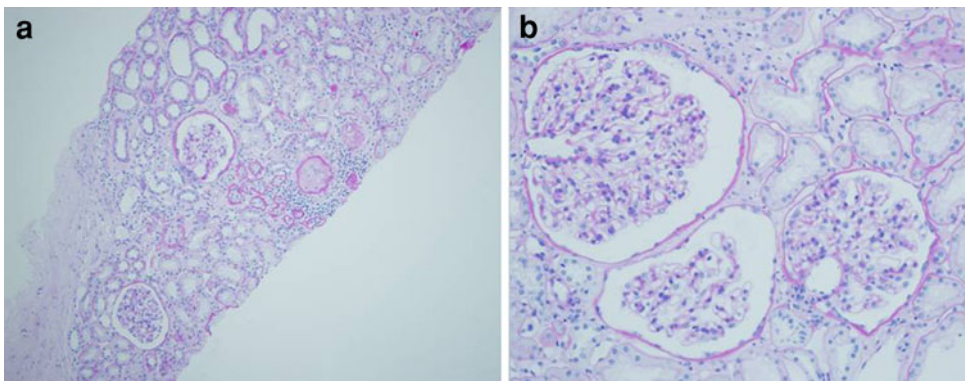

Fig. 2 Light microscopic findings with periodic acid Schiff (PAS) stain ×100 reveal tubular atrophy and surrounding lesions leading to global sclerosis (**a**). At higher magnification with PAS stain ×200, other glomeruli show minor abnormalities (**b**)

disease characterized by corticomedullary and medullary cysts, tubulointerstitial inflammation, progressive renal failure, and variable hyperuricemia. MCKD2 and FJHN are considered to be allelic variants [3]. We present a patient with a novel heterozygous missense mutation (c.C518G in exon 4) and a clinically mild phenotype of FJHN.

Case report

A 56-year-old woman was referred to our institution as an inpatient because of renal dysfunction and hyperuricemia. A medical examination performed when she was 51 years old showed the presence of hypertension, urinary protein, and mild renal insufficiency. At the age of 54 years, she started taking antihypertensive drug therapy. At 55 years of age, she was diagnosed with hyperuricemia, with a plasma uric acid level of 7.3 mg/dL and a fractional excretion of uric acid of 5.8 %. She was administered benzbromarone and eventually referred to our hospital for further examination. Laboratory tests showed a normal complete blood count, a serum creatinine level of 1.12 mg/dL, an estimated glomerular filtration rate (eGFR) of 39.9 mL/(min 1.73 m^2), a creatinine clearance of 68.5 mL/(min 1.73 m^2), a serum uric acid level of 7.5 mg/dL, fractional excretion of uric acid of 7.5 %, a serum albumin level of 3.9 g/dL, urinary protein excretion of 0.096 g/d, and negative results for urinary sedimentation. Renal ultrasonography showed normally sized kidneys without cysts. The patient's 84-year-old father had stage IV chronic kidney disease (CKD) with a history of gout; his serum creatinine level was 2.38 mg/dL and his eGFR was 21.1 mL/(min 1.73 m^2). He had hyperuricemia with a serum uric acid of 7.1 mg/dL and had been administered benzbromarone and allopurinol. The patient's elder sister, aged 61 years, had stage IV CKD with a serum creatinine level of 1.72 mg/dL and an eGFR of

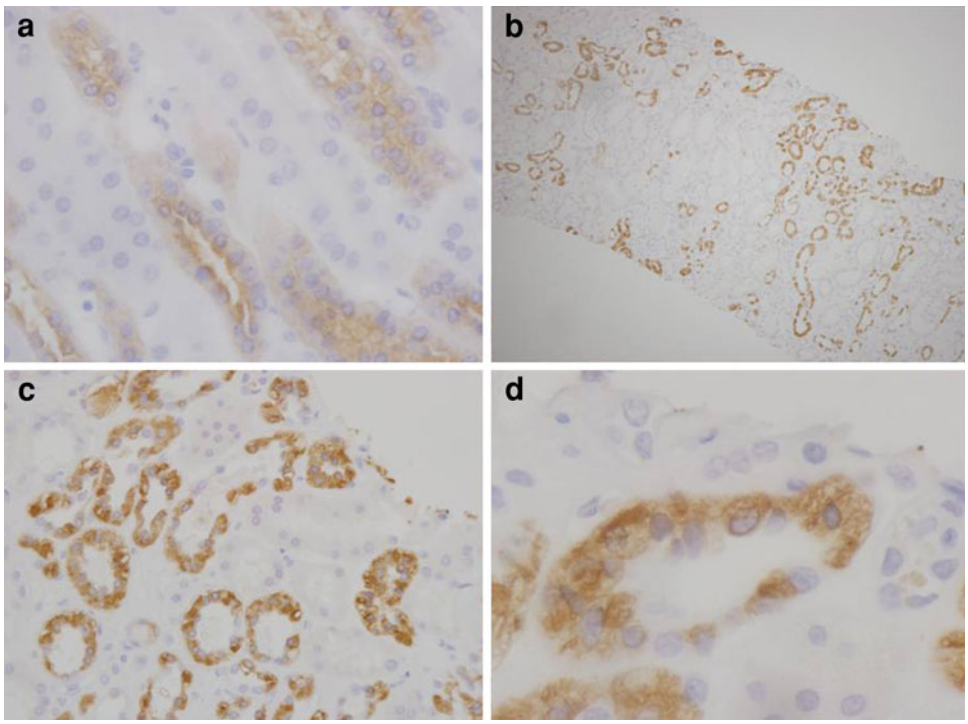

Fig. 3 Expression pattern and distribution of uromodulin in the kidney. Immunostaining for uromodulin in a control kidney shows staining in the thick ascending limb of the loop of Henle and the distal convoluted tubules, with a staining pattern characteristic of apical membrane reactivity (**a**). Immunostaining for uromodulin in our patient demonstrated more intense, diffuse staining, with increased density in the cytoplasm of the tubular cells (**b**, **c**, **d**)

24.3 mL/(min 1.73 m^2). She had hyperuricemia with a serum uric acid level of 7.1 mg/dL and a fractional excretion of uric acid of 7.9 % with benzbromarone and allopurinol. None of the family members were undergoing dialysis treatment (Fig. 1).

Light microscopy of the patient's renal biopsy specimens showed a total of 22 glomeruli, 10 of which had global sclerosis; the others had minor glomerular abnormalities. Approximately 30 % of the renal cortex showed patchy tubular atrophy and interstitial fibrosis (Fig. 2a, b). Immunofluorescence staining was negative for immunoglobulin, complement, and fibrinogen. In the normal human kidney, uromodulin is distributed in the thick ascending limb of the loop of Henle (TALH) and the distal convoluted tubules (DCTs), with an immunohistochemical staining pattern characteristic of apical membrane reactivity (Fig. 3a). In contrast, this patient's specimens exhibited more intense diffuse staining without the characteristic pattern but with dense staining in the tubular cytoplasm (Fig. 3b, c). Denser staining was also recognized in a part of the cytoplasm that may have represented the endoplasmic reticulum (ER) (Fig. 3d).

The patient's family members were tested for *UMOD* mutations by polymerase chain reaction amplification of genomic DNA and bidirectional automated DNA sequencing of the *UMOD* gene. After approval for the genetic analysis had been granted by the ethical committee of the hospital, informed consent was obtained from the patient and her family members. A novel heterozygous missense mutation, c.C518G, was detected. This mutation resulted in an exchange of proline for arginine at codon position 173 (p.P173R) (Fig. 4) and was present in the patient, her father, and her elder sister. The patient's mother, younger sister, and her affected elder sister's daughter did not have this mutation; they had normal renal function, normouricemia, and no history of gout. This mutation was not detected in any of the 100 control chromosomes tested, and the proline residue was highly conserved across species (Fig. 5). We consider that it is highly possible that this mutation causes FJHN.

Discussion

The *UMOD* gene encodes uromodulin, an 85-kD glycoprotein that is also known as Tamm–Horsfall protein. Uromodulin is likely to contain three epidermal growth factor (EGF)-like domains; the second and third domains contain a calcium-binding motif, a domain of eight cysteine residues (D8C) within a cysteine-rich region, and a zona pellucida (ZP)-like domain, which is responsible for the polymerization of extracellular proteins into helical filaments [2, 4].

The majority of the previously reported *UMOD* mutations are clustered in exon 4, which encodes D8C and the EGF-like domains; most are missense mutations, affecting the cysteine residues that allow the formation of the interchain disulfide bonds essential for maintaining the protein's

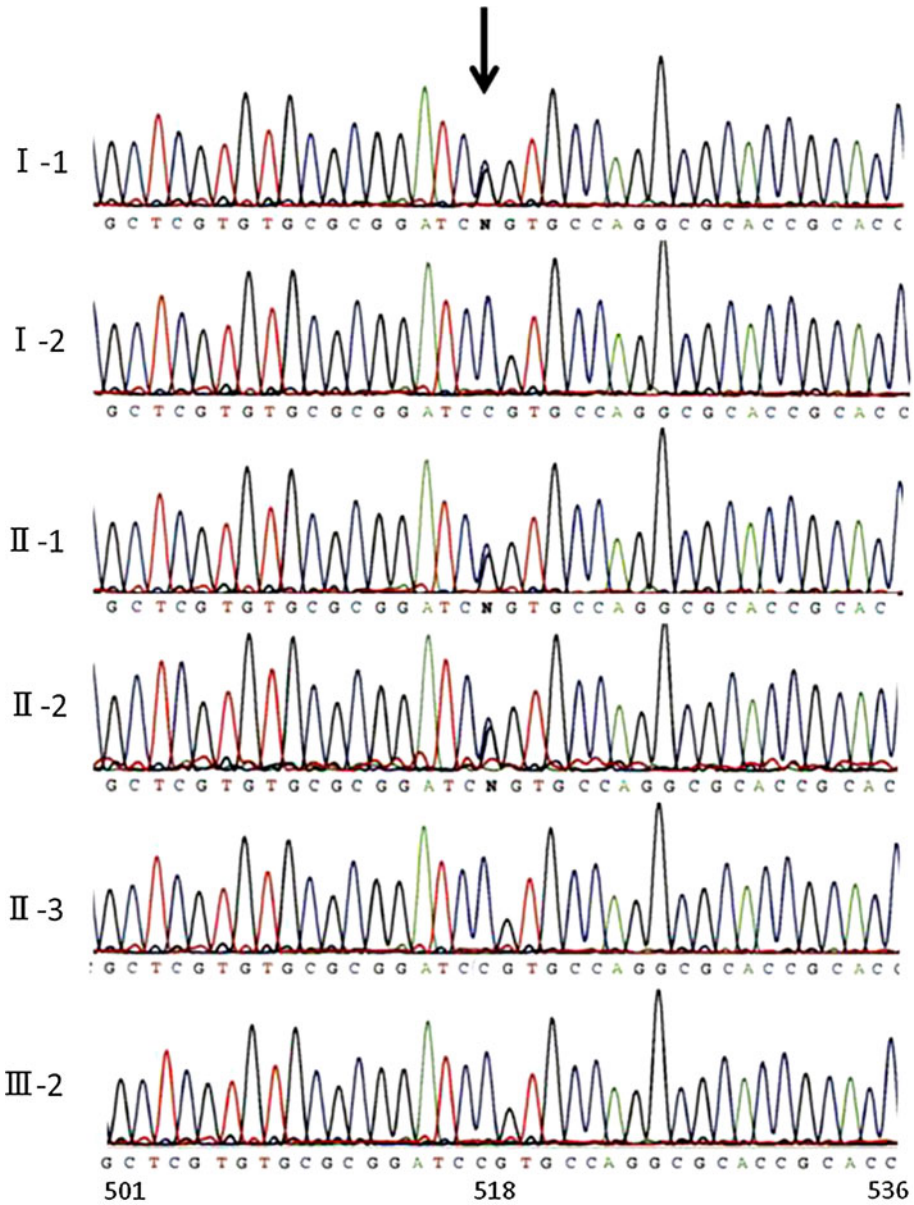

Fig. 4 Partial sequence data for the *UMOD* gene. A heterozygous C to G transition at nucleotide 518 in exon 4 results in the codon change of P173R

tertiary structure. This patient's novel mutation was located between the EGF-like domains and D8C.

In previous research, clinical findings have not been clearly correlated with *UMOD* mutations, probably because of the small number of such case reports. However, Bollée et al. reported that a slight trend toward higher renal survival in patients with polar residue substitutions than in those with cysteine substitutions was observed, despite high intrafamilial variability in renal survival. This suggests that modifier genes, in addition to environmental factors, have an effect on clinical findings [5]. Moreover, Williams et al. [4] reported that *UMOD* mutations may be classified based on the mature:precursor ratio of uromodulin. In their study, the group of patients with C32W, D196N, and G488R mutations had a higher mature:precursor ratio of uromodulin than the group with C126R, N128S, and C223R mutations. Both C126R and N128S are located in the third EGF-like domain, which is strongly recognized by ER chaperones. Thus, these mutations, which potentially disrupt the structure of EGF-like domain 3, are likely to result in ER retention. C223R is located within D8C, a highly structured domain that would be disrupted by substitutions in cysteine residues. C32W, D196N, and G488R are located at EGF-like domain 1, between the ZP-like domain and the EGF-like domains and D8C. These mutations have a high mature:precursor ratio of uromodulin. The novel mutation P173R is located between D8C and the EGF-like domains (Fig. 6). As P173R is located near D196N, it is logical to presume that the novel mutation probably has a high mature:precursor ratio of uromodulin.

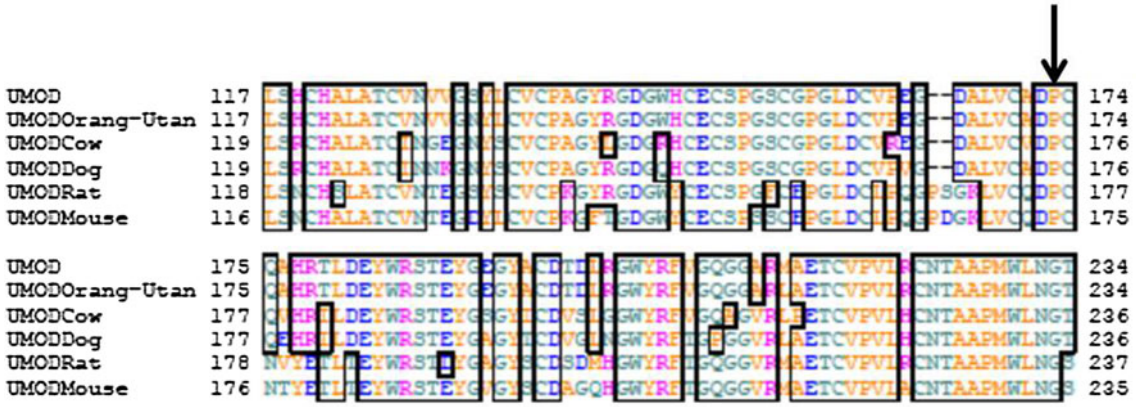

Fig. 5 Alignment of the amino acid sequence of human uromodulin with those of the orangutan, cow, dog, rat, and mouse. The *arrow* indicates the position of the novel missense mutation

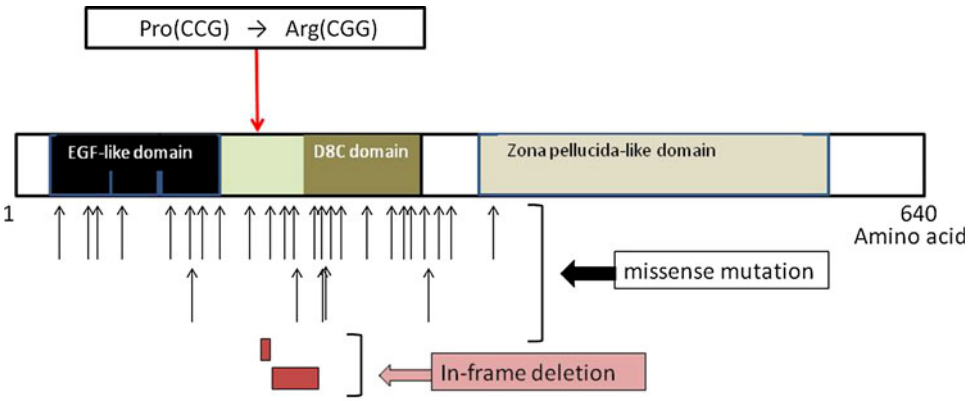

Fig. 6 Schematic representation of the uromodulin protein. The mutation described in this report, P173R, is shown above the protein. The previously described mutations are shown below the protein

Dahan et al. [6] reported the cases of two female patients with the mutation c.614G>A, which resulted in a C170Y mutation located very near to our patient's mutation. Despite their respective ages of 72 and 73 years, these two patients had not required dialysis therapy. One of the patient's 33-year-old daughter, who had the same mutation, had normal renal function and no history of gout. Our patient had mild renal insufficiency, and her affected family members have not required dialysis, although the median renal survival of FJHN patients is 54 years. Therefore, P173R and the neighboring mutations most likely confer clinically mild phenotypes wherein patients exhibit slight proteinuria and mild renal insufficiency. Because uromodulin's structure has not yet been clarified, we cannot analyze the clinical findings based solely on the protein's amino acid sequence. However, the location and type of mutation are probably related to the clinical effects.

In the normal kidney, uromodulin expression is restricted to the TALH and DCTs, with distinct apical membrane reactivity [1, 7]. In our patient, uromodulin was detected in the cytoplasm of tubular cells, and more intense staining was detected in part of the cytoplasm, like a mosaic pattern. This pattern of uromodulin expression was the same as that seen in an FJHN patient described in a study by Nasr et al. [8]. They reported that electron microscopy revealed intracytoplasmic inclusions composed of stacked lamellar structures that appeared to represent hyperplastic bundles of ER in distal tubules and TALH. Therefore, this mosaic pattern in immunohistochemical staining may represent the retention of uromodulin in the ER through gain of resistance to proteolytic cleavage, as *UMOD* mutation causes disruption of the molecule's stable tertiary structure.

FJHN patients are diagnosed at a mean age of 31 years (range 21–44 years), with a mean eGFR of 42 mL/(min 1.73 m^2) [range 30–60 mL/(min 1.73 m^2)], a mean uric acid level of 9.2 mg/dL (range 8.3–10.7 mg/dL) in men and 8.5 mg/dL (range 7.7–9.0 mg/dL) in women, and a mean fractional uric acid excretion of 4.6 % (range 4.0–6.0 %). Renal cysts are present in 34.3 % of patients. The median renal survival is 54 years (range 25 to >70 years). It is difficult to determine whether the uric acid level is really out of proportion to the GFR because uric acid increases with renal dysfunction. Moreover, the diagnostic value of fractional uric acid excretion is unclear. Fractional uric acid excretion has been reported to be above

the 25th percentile value of all CKD patients in only 30 % of patients with FJHN. This implies that a normal value for fractional uric acid excretion should not exclude a diagnosis of FJHN [5]. Therefore, the family history is important for diagnosis, especially for patients with mild disease, as in this case. Physicians should collect information from patients regarding any family history of renal dysfunction, hyperuricemia, gout, or renal cysts. Although our patient had only mild renal dysfunction and hyperuricemia, her family history led to the diagnosis.

In conclusion, we have identified a novel mutation in the *UMOD* gene. In previous reports, the location and type of *UMOD* mutation seemed to influence the clinical findings of FJHN. The P173R mutation and its neighboring mutations may confer clinically mild phenotypes. Physicians should suspect FJHN in patients with hyperuricemia and CKD, although the family history may be required to diagnose patients with mild presentations.

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

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