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Clinico-pathologic findings in medullary cystic kidney disease type 2

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Abstract Medullary cystic kidney disease type 2 is an uncommon autosomal dominant condition characterized by juvenile onset hyperuricemia, precocious gout and chronic renal failure progressing to end-stage renal disease in the 4th through 7th decades of life. A family suffering from this condition is described. The patient in the index case presented with renal insufficiency as a child. A renal biopsy revealed tubular atrophy, and immunohistochemical staining of the tissue for uromodulin (Tamm Horsfall protein) revealed dense deposits in renal tubular cells. Genetic testing revealed a single nucleotide mutation (c.899G>A) resulting in an exchange of a cysteine residue for tyrosine (C300Y). Medullary cystic kidney disease type 2 (also known as uromodulin-associated kidney disease) likely represents a form of endoplasmic reticulum storage disease, with deposition of the

abnormal uromodulin protein in the endoplasmic reticulum, leading to tubular cell atrophy and death.

Keywords Uromodulin-associated kidney disease · Familial juvenile hyperuricemic nephropathy · Tamm Horsfall protein · Uromodulin

Introduction

Medullary cystic kidney disease type 2 (MCKD 2) is characterized by the autosomal dominant inheritance of chronic tubulo-interstitial renal disease and the common occurrence of hyperuricemia and precocious gout [1]. Mutations in the uromodulin (Tamm Horsfall protein) gene [2, 3] have been found to be responsible for a number of cases of MCKD 2. Uromodulin is a 616 amino acid glycoprotein, which has an N-terminal signal peptide, three epidermal growth factor domains, a zona pellucida-like region and a glycosphosphatidylinositol anchor. Proteolytic cleavage of the glycosphosphatidylinositol anchor allows for secretion of uromodulin into the urine [4]. The function of uromodulin is unclear despite many years of study. It may be involved in the prevention of urinary tract infections, though families with uromodulin mutations do not appear to have an increased incidence of infection [5].

The following case describes a young child with a newly identified mutation in the uromodulin gene who underwent a renal biopsy early in the course of his disease. These findings expand the current insights into the pathophysiology and clinical presentation of individuals with a mutation in the uromodulin gene.

Case report

The child presented at age 7 1/2 years for evaluation of azotemia following treatment of an otitis media. The blood urea nitrogen level was 52 mg/dl (normal range 8–24 mg/dl) and the serum creatinine level 1.6 mg/dl (normal range 0.5–1.0 mg/dl). He had no gross hematuria, edema, rash, joint pains, fever or weight loss. The

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patient's two siblings and his father were in good health without any evidence of kidney disease. The patient's mother had hyperuricemia and a history of a renal biopsy revealing interstitial nephritis. In addition, the maternal grandfather had had gout and died of kidney failure at age 33 years.

Physical examination revealed a well-developed child with height 46.5 inches (118 cm; 15th percentile), weight 52 lbs (23.6 kg; 40th percentile) and blood pressure 108/78 mmHg. The estimated glomerular filtration rate (GFR) was 46 ml/min/1.73 m². A renal ultrasound showed two normal-sized kidneys without hydronephrosis or increased echogenicity. The urinalysis revealed a specific gravity of 1.012, no hematuria and no proteinuria. The serum uric acid was 9.3 mg/dl (normal range, 2.1–6.1 mg/dl). A kidney biopsy contained 40 glomeruli, 5 with global sclerosis, 3 with sclerosed crescents and the remaining glomeruli with normal cellularity. There were multiple foci of tubular atrophy with minimal interstitial fibrosis and no cellular infiltration. The blood vessels were normal. The fractional excretion of uric acid was 4.5% (normal, 6–20%) [6], and the patient was started on allopurinol, 100 mg per day. In July 2001, at the age of 19 years, the patient developed gout in his left hand and was started on colchicine, 0.6 mg per day. He continues to have attacks of gout every 2–3 months. At his most recent follow-up in March 2003, the patient was attending college and doing well. He was taking allopurinol (100 mg twice per day) and colchicine (0.6 mg orally each day). His blood urea nitrogen and serum creatinine levels were 57 mg/dl (normal range 8–24 mg/dl) and 2.4 mg/dl (normal range 0.5–1.0 mg/dl), respectively, with an estimated GFR of 48 ml/min/1.73 m². His serum uric acid was 6.1 mg/dl (normal range 4.6–7.8 mg/dl), and the fractional excretion of uric acid was 1.9% (normal range 6–20%).

The patient's mother was first noted to have mild azotemia and hyperuricemia at age 25 years. A renal biopsy demonstrated the presence of chronic interstitial nephritis. She has had persistent hyperuricemia, but has never suffered attacks of gout and has not been treated with allopurinol or colchicine. At her most recent evaluation at age 55 years, the BUN and serum creatinine were 77 (normal range 8–24 mg/dl) and 2.0 mg/dl (normal range 0.5–1.0 mg/dl), respectively, with an estimated GFR of 45 ml/min/1.73 m². Her serum uric acid was 8.3 mg/dl (normal range 2.6–5.4 mg/dl) and the fraction excretion of uric acid was 2.0% (normal range 6–20%).

One author (HT) identified the strong family history of hyperuricemia and renal failure and referred the family for genetic evaluation. After obtaining informed consent, a blood sample was obtained from the index case patient, as well as a paraffin-embedded block section from the patient's prior renal biopsy.

DNA purification, amplification and sequencing of the uromodulin gene were carried out as previously described [7]. Immunohistochemical staining was performed with antibody to uromodulin provided by Dr. John Hoyer from the Children's Hospital of Philadelphia, PA. This polyclonal antibody was obtained from rabbit anti-serum after a series of injections of purified Tamm Horsfall protein over a period of 7 months [8]. The epitopes that are recognized by this antibody have not been determined. Paraffin sections were dehydrated, treated for 10 min with 0.3% hydrogen peroxide and exposed to antigen retrieval using antigen unmasking solution (Vector Labs) for 1 h at 95°C. The sections were then washed and blocked with 1% bovine serum albumin-phosphate buffered saline (BSA-PBS), and the primary antibody (1:1,000 dilution) was incubated with the sections in this blocking solution for 30 min at 23°C. After further washing in PBS, the sections were incubated with goat anti-rabbit IgG conjugated to horseradish peroxidase (Jackson ImmunoResearch) (25 µg/ml) for 30 min in 1% BSA-PBS. After washing, the peroxidase was developed using diaminobenzidine-peroxide substrate solution, counterstained with hematoxylin, dehydrated and mounted in Permount.

Results

Sequence analysis of exons 4 and 5 of the uromodulin gene in the index case and his mother identified a single nucleotide change (c.899G>A) resulting in an exchange of a cysteine residue for tyrosine (C300Y). This uromodulin gene mutation was not present in the two unaffected siblings or in any of the 200 control chromosomes tested. Figure 1 demonstrates immunohistochemical staining for uromodulin. Figure 1A–C shows photomicrographs from the patient, with Fig. 1D demonstrating immunohistochemical staining in a control with normal renal function. Figure 1D demonstrates diffuse staining of the thick ascending limb cells for uromodulin. In contrast, in Fig. 1A–C, irregular staining of tubules is noted, with dense deposits noted in tubular cells.

Discussion

This patient demonstrates the four characteristic clinical findings found in individuals with a mutation in the uromodulin gene (see Table 1). He presented at a young age with renal insufficiency that was slowly progressive. The urinalysis revealed an inactive sediment—indicating the patient did not suffer from active glomerulonephritis. There was a strong history of renal failure transmitted in an autosomal dominant fashion. Other affected family members also had an inactive urinary sediment, and they developed end-stage renal disease in the 4th or 5th decade. Hyperuricemia and precocious gout (resulting from a reduced fractional excretion of uric acid) are frequent in this condition and were the key factors leading to consideration of this diagnosis. Of note, the renal ultrasound did not reveal medullary cysts.

Medullary cystic kidney disease is an unfortunate misnomer for this condition, as many affected individuals and families have no evidence of medullary cysts. Moreover, these cysts, if present, frequently do not develop until late in the course of disease [1, 9]. The renal biopsy is also frequently unhelpful, as it was initially in this case. Careful pathologic examination of affected kidneys will reveal tubular atrophy and tubular dilatation with secondary glomerular changes. However, in many cases histopathologic examination of the renal tissue focuses on the glomeruli, where secondary findings often lead to diagnoses such as focal glomerulosclerosis or hypertensive nephrosclerosis. The presence of three sclerotic glomerular crescents is likely the result of global glomerulosclerosis. Active glomerular crescents are not a feature of MCKD2.

Mutations in the uromodulin gene have also been found in some, but not all, families suffering from familial juvenile hyperuricemic nephropathy (FJHN) [10]. This disease has very similar clinical manifestations to medullary cystic kidney disease type 2. Given the fact that medullary cysts are frequently not present in this disorder, and given the fact that uromodulin mutations are responsible for some, but not all, cases of medullary cystic kidney disease and FJHN [3], we believe the term uro-

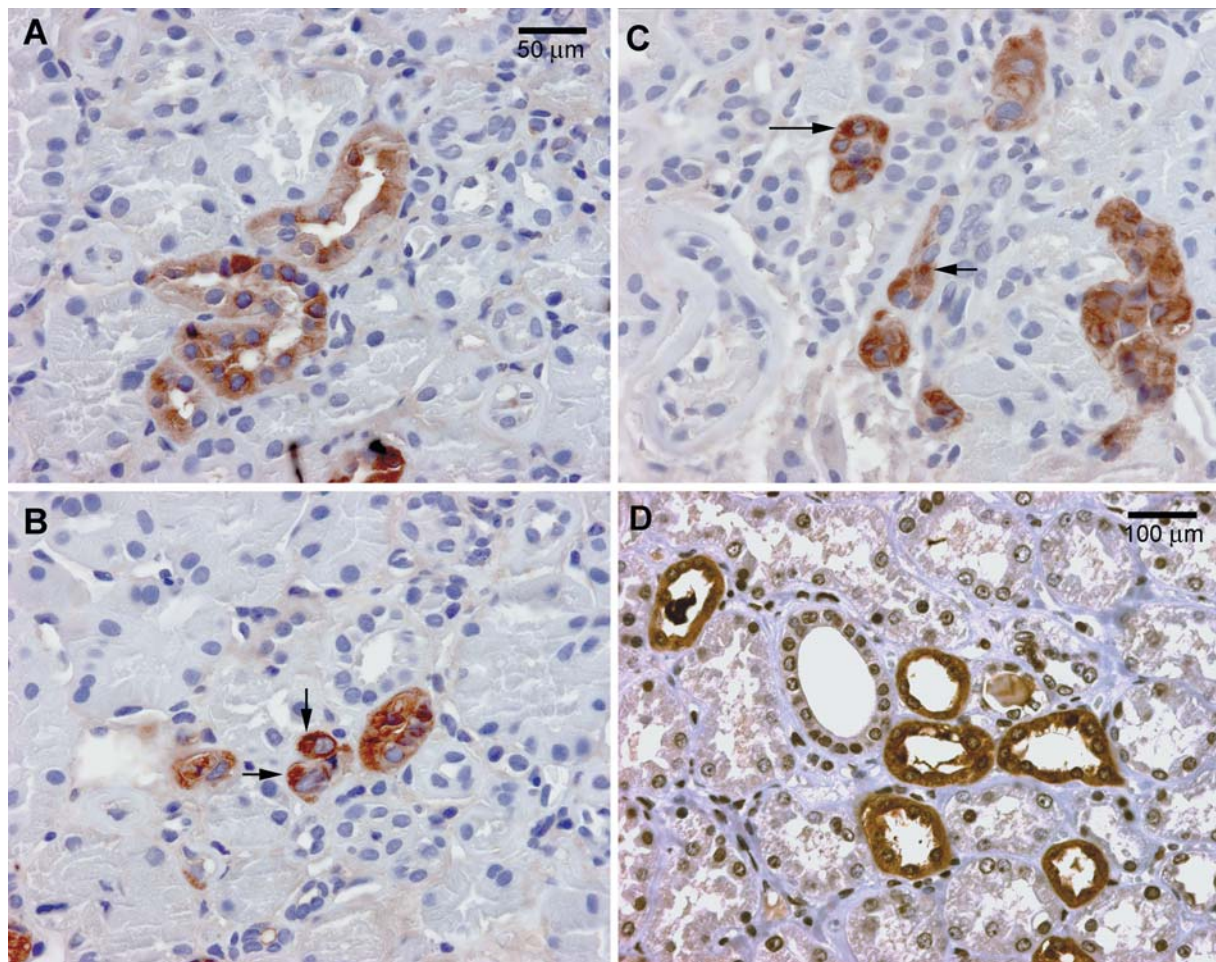


Fig. 1 Immunohistochemical staining with antibody to uromodulin in the index case (A – C) and control (D). A, B and C demonstrates irregular staining with dense deposits noted (see arrows) in the

cytoplasm of tubular cells. D demonstrates diffuse staining for uromodulin in the tubular cells of a healthy control

Table 1 Characteristics of uromodulin-associated kidney disease

Autosomal dominant transmission
Slow progressive decline in GFR beginning in early teens and progressing to ESRD in 40s to 60s
Low grade or absent proteinuria
Inactive urinary sediment
Frequent hyperuricemia (serum uric acid 2 SD above the mean for age and gender)
Fractional excretion of uric acid usually less than 5% prior to decline in GFR
Medullary cysts sporadically present with renal imaging
Renal pathology non-specific with tubular dilatation and atrophy; antibody staining for Tamm Horsfall protein reveals dense deposits in tubular cells

modulin-associated kidney disease (UMAK) is a more specific term when describing individuals suffering from a mutation in the uromodulin gene.

Uromodulin, also known as Tamm-Horsfall protein, is produced exclusively in the thick ascending limb of Henle [4]. It is secreted into the tubular lumen and coats the luminal surface of this segment of the tubule. However, its function is unknown [2]. Reported mutations in the uro-

modulin gene have occurred in exons 4 or 5, usually resulting in the loss or gain of a cysteine residue [1, 2, 3, 10, 11]. The uromodulin molecule has a very high cysteine content, and the cysteine residues are believed to be important in inter-chain disulfide bonding that results in the molecule's tertiary structure [4]. The cysteine mutations may lead to improper folding of the uromodulin molecule with deleterious consequences (see below). In the current case, a mutation in exon 5 (c.899G>A) of the molecule was found to be responsible for the patient's condition, resulting in an exchange of a cysteine residue for tyrosine (C300Y). In a family previously reported [10], mutation at an adjacent nucleotide resulted in the same cysteine residue being exchanged for a glycine (C300G), implying the importance of this particular residue (C300) in maintaining the conformation of uromodulin.

The histologic findings in this case are unique and provide further insight into this condition. This is one of the first reports to provide immunohistochemical staining in an individual with UMAK. In two previous reports, antibody staining of kidney tissue for uromodulin revealed abnormal cellular aggregates of uromodulin pres-

ent in the endoplasmic reticulum [11, 12]. We believe this patient is the first adolescent whose biopsy specimen has undergone immunohistochemical evaluation for uromodulin when the disease was relatively mild. The young age of the patient and the mild renal insufficiency present at the time of the renal biopsy allow us to conclude that the abnormal uromodulin inclusions are not merely a consequence of advanced renal failure, but are an integral feature early in the disease course.

The findings in this case increase our knowledge regarding the pathophysiology of this condition. These findings support the hypothesis that the presence of abnormal uromodulin is responsible for the development of renal failure in these patients. UMAK is an example of a group of disorders known as endoplasmic reticulum storage disease [13]. In these disorders, mutations result in structural alterations of proteins. The inability of these proteins to fold properly or undergo further modification results in their deposition in the endoplasmic reticulum. Overload of the endoplasmic reticulum with non-functional proteins activates the nuclear transcription factor NF κ B [13], which leads to an inflammatory response. Eventual death of renal tubular cells likely leads to progressive renal failure.

At present, the definitive diagnosis of UMAK requires genetic testing. Such testing is currently available through Athena Diagnostics, Worcester, Mass. Genetic testing is helpful in these patients to secure a diagnosis, to prevent the development of tophaceous gout and to determine appropriate family members for kidney donation. This is the second case identified by one of the authors (HT) in his pediatric nephrology practice, implying that improved knowledge of this disorder might lead to more frequent diagnosis and appropriate therapy.

In summary, the current case demonstrates the abnormal deposition of uromodulin in renal tubular cells at a young age. This finding provides evidence that UMAK is likely an endoplasmic reticulum storage disease, with abnormal deposition possibly leading to renal failure. Further studies are needed to elucidate the pathophysiologic changes involved in this process.

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