Brief Report

Functional consequences of a novel uromodulin mutation in a family with familial juvenile hyperuricaemic nephropathy

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Abstract

Background. Familial juvenile hyperuricaemic nephropathy (FJHN) is an autosomal-dominant disorder featuring hyperuricaemia, low fractional urate excretion, interstitial nephritis and chronic renal failure. The responsible gene UMOD was recently identified. UMOD encodes for uromodulin or Tamm–Horsfall glycoprotein, the most abundant protein in normal urine. We encountered a family with FJHN and identified a novel UMOD mutation in exon 6.

Methods. We sequenced the gene in all family members, identified the mutation, and verified its presence in the affected members. We next performed functional studies of the mutant protein by immunofluorescence and FACS analysis on transfected cells.

Results. The mutation p.C347G (c.1039T>G) results in a conserved cysteine to glycine amino acid substitution in the uromodulin zona pellucida (ZP) domain. The cell studies showed that the novel uromodulin mutation causes a delay in protein export to the plasma membrane due to its retention in the endoplasmic reticulum.

Conclusions. We describe the first reported mutation mapping in the ZP uromodulin domain. Our data provide further evidence showing why the excretion of uromodulin is reduced in this syndrome.

Keywords: familial juvenile hyperuricaemic nephropathy, genetics; interstitial nephritis, uric acid, uromodulin, Tamm–Horsfall glycoprotein

Introduction

Familial juvenile hyperuricaemic nephropathy (FJHN) is an autosomal-dominant disorder characterized by hyperuricaemia, a low fractional renal urate excretion, interstitial nephritis, and chronic renal failure [1–3]. The clinical presentation is heterogeneous. Affected women do not always have gout, but may also develop renal failure. We recently encountered a woman with chronic renal disease and a remarkable history of renal disease in her family. The family proved to have FJHN. When first assessed, the family tree was too small for a total genome scan. However, subsequent identification of the uromodulin (UMOD) gene and its role in FJHN led us to sequence this gene in our family. We found a hiterto fore not described UMOD mutation (C347G). A recent functional study showed that UMOD mutations lead to delayed protein trafficking to plasma membrane and ER retenion [4]. On the basis of this evidence, we assessed the effect of the newly identified mutation on protein maturation by transfecting wild-type and mutant uromodulin isoforms in HEK 293 cells.

Subjects and methods

Case report

The index patient is a 57-year-old woman, who was admitted to the HELIOS Klinikum-Berlin with sudden, severe headache. A cerebral aneurysm was found that was successfully clipped. Her physicians observed that her serum creatinine was elevated at 4.2 mg/dl (370 μmol/l) and a nephrological consultation was obtained. The patient stated that her renal function had been compromised for a long time. She had also been hypertensive for over 20 years, but the treatment had been sporadic. When asked, she indicated that quite a few people in her family had renal problems, although she could...
give no details on the nature of the problem. A renal ultrasound examination revealed small kidneys with decreased renal parenchyma. The urine showed scant proteinuria and no casts were observed in the urinary sediment. Her uric acid concentration was above the upper limits of normal, but she denied having had arthritis. At that time, we did not make a histological diagnosis. Instead, we prevailed on the patient to encourage younger members of her family to undergo examination.

The patient’s 33-year-old daughter was admitted to the nephrology service at Charité, Campus Mitte. She had had known renal disease for 17 years. Her blood pressure had been elevated for several years and she was aware of at least eight family members who also had an elevated blood pressure. Her physical examination was unremarkable; however, her laboratory evaluation disclosed mild anaemia, normal electrolytes, blood urea nitrogen 94 mg/dl (18 mmol/l), creatinine 2.59 mg/dl (230 μmol/l) and uric acid 11.0 mg/dl (650 μmol/l). The kidneys were small and had a decreased parenchyma. Medullary cysts were not identified. The urinalysis again showed only scant proteinuria and no urinary casts were seen. The renal biopsy showed no evidence of primary glomerular disease. Instead, there was copious interstitial fibrosis and vascular intimal thickening. A human genetic consultation was obtained.

**Molecular genetics and functional studies**

After due institutional review board approval with written informed consent, all known family members were contacted and underwent examination. Native DNA was extracted by routine methods and the entire *UMOD* gene was sequenced in all affected and non-affected family members. Once the responsible mutation was identified, cellular studies were done as outlined elsewhere [4]. Uromodulin full-length cDNA was mutated by using the QuikChange site-directed mutagenesis kit (Stratagene). We introduced the mutation 1039T>G by using the primer pair listed below (5' to 3'):

C347G for GTGTCCGTGGCCAGGCCACGCTGAA

GAGTC

G347G rev GACCTTCACTGGCCCTTGCCCAGC

GACAC

The construct was fully re-sequenced.

HEK293 cells were grown for 16 h prior to transfection on glass cover slips in 12 well plates (Corning Life Sciences). Transfections done using Metafectene (Biontex, Munich, Germany). We used 500 ng of plasmid DNA for 10⁶ cells. Transfection efficiency was determined by cotransfecting with enhanced green fluorescent protein (EGFP) expressing vector pcDNA3 × (+)-MyEGFP (Invitrogen). Fresh media was added 2 h after transfection. Cells were fixed at 6–8 h after transfection either in 4% PFA for 30 min at 37°C and washed with alpha MEM + FBS 2%, resuspended in 300 μl of alpha MEM + FBS 2% and incubated 10 min at room temperature with 7-amino-actinomycin D (7AAD) for the exclusion of non-viable cells. Cell fluorescence was measured by flow cytometry (FACSCalibur, BD Biosciences) and analysed by CellQUEST software (BD Biosciences).

**Results**

The family tree in Figure 1A was constructed. In the family tree, the index patient is subject III.2 (second person in the third generation), while her daughter who underwent biopsy is subject IV.2. The persons in the solid figures are those with clinical renal disease. These individuals had signs of decreased renal function and had increased uric acid values. In persons who had died (those scored), the status could only be obtained historically and no DNA was available. We were aware that the clinical picture of our family was consistent with FJHN. Our horizons were expanded substantially with the reports indicating that *UMOD* is mutated in FJHN [5,6]. We found a missense mutation in exon 6 (Figure 1B). The mutation p.C347G (c.1039T>G) results in a conserved cysteine to glycine amino acid substitution mapping in uromodulin ZP domain (Figure 1C). All affected persons and V:1 are mutated at this site. The mutation did not occur in any non-affected persons nor in 97 unrelated controls without FJHN.

We next tested whether or not the newly identified mutation C347G has the same delaying effect on protein trafficking to the plasma membrane that we previously reported for other uromodulin cysteine-affecting mutations [4]. To assess this possibility, we performed transient transfection experiments using wild type and mutant constructs in HEK293 cells that do not express uromodulin. Uromodulin maturation was evaluated by both measuring the number of uromodulin-positive cells by FACS and its intracellular distribution by immunofluorescence experiments.

When transfecting with equal amounts of constructs and with equal transfection efficiency, as determined by cotransfection with an EGFP-expressing plasmid, the number of transfected cells expressing uromodulin...
on the plasma membrane was significantly reduced in mutant C347G as compared to wild-type transfected cells at 14 h after transfection, as shown in Figure 2. To compare the effect of C347G mutation to other cysteine-affecting mutations, we carried out these experiments for mutant C148W that was previously shown to lead to a 40% reduction. The effect of mutation C347G seems to be more dramatic than those observed earlier. C347G led to a reduction of the number of uromodulin-positive cells by about 60%. The delaying effect of mutation C347G was also evident at early stages after transfection as shown in Figure 3.

To test whether or not mutation C347G could lead to retention of mutant uromodulin in the endoplasmic reticulum (ER), presumably due to protein misfolding, we performed immunolocalization of the transfected isoforms at 6 and 8 h after transfection. As shown in Figure 4, at 6 h after transfection intracellular wild-type uromodulin was mainly localized to the Golgi compartment, whereas C347 mutant signal was mainly confined to the ER. The different distribution was less evident at 8 h after transfection, probably reflecting the bias introduced by overexpressing the protein in transient transfection experiments [4]. The functional studies of C347G mutant uromodulin demonstrate that this mutation has a detrimental effect on protein maturation due to a delayed transit through the ER, likely due to the inability to establish the correct disulphide bonds.

**Discussion**

This single-family study sheds new light on FJHN. We identified a novel UMOD mutation (C347G) and conducted functional studies showing that the mutation has a major effect on uromodulin trafficking. Including the one described in this study, 33 mutations in the UMOD gene have been reported thus far [4–11]. Interestingly, all the previously reported mutations are clustered in the N-terminal half of the protein, in particular in the four EGF-like domains and in the region between amino acid 149 and 281 that has no sequence homology to any known domain. The
existence of a mutation hot-spot in exon 4 was already pointed out by Dahan et al. [7] and Wolf et al. [8]. Interestingly, the mutation we identified is the first one to be reported in the zona pellucida (ZP) domain, which spans most of the C-terminal half of the protein and is important for uromodulin polymerization [9]. We demonstrated by transfection experiments that trafficking to the plasma membrane of uromodulin carrying the C347G mutation is impaired because of its delayed transit through the endoplasmic reticulum, presumably due to protein misfolding. Our work confirms and extends our earlier studies indicating that UMOD mutations feature the inability to establish correct disulfide bonds, thereby contributing to faulty protein folding [4]. Retention in the endoplasmic reticulum may lead to the formation of the intracellular uromodulin aggregates observed in patient kidney biopsies and is likely a key step in the pathogenesis of FJHN and type 2 MCKD [3,4].

The clinical features of FJHN are hyperuricemia, decreased fractional excretion of uric acid, chronic interstitial disease and end-stage renal disease in the fourth to fifth through seventh decades [10]. Because we had biopsy evidence and serum uric acid values consistent with the diagnosis, we did not perform fractional urate excretion determinations in our affected subjects. When the creatinine clearance decreases below 80 ml/min, the fractional urate excretion increases in any event. Our affected family members all met the criteria for the diagnosis of FJHN except for one small child. Similar to descriptions of other families, our index patient, a woman, had uric acid values in the high normal range, but had not developed clinical gout. Notable is the indolent clinical progression of the disease. UMOD is also mutated in medullary cystic kidney disease type 2. This disease also features hyperuricaemia, defects in urinary concentrating ability, interstitial nephritis and end-stage renal disease. The mode of inheritance is autosomal-dominant and the condition is the same disease [11]. Interestingly, persons homozygous for UMOD mutations have been described in a consanguineous family [13]. These persons had particularly severe and early onset disease. The fact that the heterozygous state causes an autosomal-dominant disease is consistent with a dominant-negative effect.

UMOD codes for the Tamm–Horsfall (TH) glycoprotein or uromodulin [14]. The TH glycoprotein is the most abundant protein excreted in the urine of healthy persons. The TH glycoprotein forms the matrix of urinary casts. Hyaline and even granular casts can be found in the urinary sediments of normal
persons. We were therefore interested in the fact that no urinary casts were observed in the sediments of our affected patients. The function of the TH glycoprotein is still not clear. The protein may be an inhibitor of renal stone formation and may modulate renal immune responses [15]. The TH glycoprotein can be identified in the ascending limb of Henle’s loop, the distal tubule and the collecting duct [16,17]. The protein binds cytokines such as tumor necrosis factor-alpha and interleukin-1. The fact that human urate transporters have now been cloned may change this state of affairs [18]. Umod has since been disrupted in the mouse [19]. The mouse is reported as being susceptible to urinary tract colonization by fimbriated bacteria. The TH glycoprotein also appears to play a role in urinary concentration. Preliminary evidence on the Umod gene-disrupted mice from our laboratory (Bachmann et al., unpublished observations) show that the mice are polyuric compared to controls and cannot concentrate the urine to the same degree as control mice when deprived of water.

How TH glycoprotein might influence uric acid handling by the kidney is unknown. In contrast to man, the mouse has full expression and function of the gene encoding for hepatic uricase and metabolizes uric acid further to the more soluble allantoin. Deletion of the uricase gene causes diabetes insipidus in mice [20]. Perhaps the phenotype FJHN will first be elucidated when mice with gene deletions for UMOD and hepatic uricase are crossed, thereby producing a ‘double knockout’.

We believe that a specific genetic diagnosis is imperative in patients with a genetic disease whenever possible. Skeptics might argue that our family members have not directly profited from knowing that they harbor a UMOD mutation. We believe that early diagnosis may help our younger patients. They will be spared undergoing diagnostic renal biopsy. We are not certain that allopurinol alters the course of the disease. However, uric acid has been shown to play a role in the progression of chronic renal disease irrespective of the cause [21,22]. We are therefore prescribing allopurinol for our affected family members.

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References

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