Mutations of the *Uromodulin* gene in MCKD type 2 patients cluster in exon 4, which encodes three EGF-like domains

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Mutations of the *Uromodulin* gene in MCKD type 2 patients cluster in exon 4, which encodes three EGF-like domains.

**Background.** Autosomal-dominant medullary cystic kidney disease type 2 (MCKD2) is a tubulointerstitial nephropathy that causes renal salt wasting, hyperuricemia, gout, and end-stage renal failure in the fifth decade of life. The chromosomal locus for MCKD2 was localized on chromosome 16p12. Within this chromosomal region, *Uromodulin (UMOD)* was located as a candidate gene. *UMOD* encodes the Tamm-Horsfall protein. By sequence analysis, one group formerly excluded *UMOD* as the disease-causing gene. In contrast, recently, another group described mutations in the *UMOD* gene as responsible for MCKD2 and familial juvenile hyperuricemic nephropathy (FJHN).

**Methods.** Haplotype analysis for linkage to MCKD2 was performed in 25 MCKD families. In the kindreds showing linkage to the MCKD2 locus on chromosome 16p12, mutational analysis of the *UMOD* gene was performed by exon polymerase chain reaction (PCR) and direct sequencing.

**Results.** In 19 families, haplotype analysis was compatible with linkage to the MCKD2 locus. All these kindreds were examined for mutations in the *UMOD* gene. In three different families, three novel heterozygous mutations in the *UMOD* gene were found and segregated with the phenotype in affected individuals. Mutations were found only in exon 4.

**Conclusion.** We confirm the *UMOD* gene as the disease-causing gene for MCKD2. All three novel mutations were found in the fourth exon of *UMOD*, in which all mutations except one (this is located in the neighboring exon 5) published so far are located. These data point to a specific role of exon 4 encoded sequence of *UMOD* in the generation of the MCKD2 renal phenotype.

**Key words:** MCKD2, uromodulin, Tamm-Horsfall protein.
two different FJHN loci, which are overlapped by the MCKD2 locus [8]. Two years after publication of the chromosomal candidate region of MCKD2, Uromodulin (UMOD) was published as excluded from representing the disease-causing gene [9]. In contrast however, Hart et al [10] later detected four different mutations in the UMOD gene, indicating that this is the gene responsible for FJHN and MCKD2.

UMOD encodes the Tamm-Horsfall protein (THP), which is expressed primarily at the luminal side of renal epithelial cells of the thick ascending loop of Henle and of early distal convoluted tubules. THP is the most abundant protein in the urine of humans [11]. Functional roles of UMOD have been described in urinary tract infections, in binding to complement factors, in myeloma kidney, and nephrolithiasis [12–15]. The UMOD protein contains (1) a zona pellucida domain (ZP), which is necessary for polymerization into the supramolecular structure of a filament, (2) an elastase-sensitive fragment containing three calcium-binding epidermal growth factor (EGF)-like domains and a signal peptide, and (3) a potential glycosyl-phosphatidylinositol (GPI)-anchor cleavage site. UMOD is a transmembrane-bound protein, which can be secreted into the urine by cleavage of the GPI-anchor [16].

We here report three novel mutations in UMOD and confirm UMOD as the disease-causing gene. So far, eight mutations published are located in exon 4 and one mutation was found at the beginning of the neighboring exon 5 of the UMOD gene that encodes three EGF-like domains [10, 17].

METHODS

Patients

We ascertained 25 MCKD families (224 individuals, of which 84 were affected). Fourteen families were from Germany, three from the United States, two each from the United Kingdom, Hungary, and Turkey, respectively, one each from Belgium and China, respectively. The age at diagnosis, the age at onset of end-stage renal disease (ESRD), hyperuricemia, imaging data, and biopsy results were reviewed if available. Clinical criteria necessary for inclusion were (1) compatibility of pedigree with autosomal-dominant inheritance, (2) chronic renal failure, (3) defective urine concentration (<800 mOsm/L after overnight dehydration) with polyuria (>3 L/day), and (4) at least one pedigree member with chronic renal failure in whom renal biopsy showed tubulointerstitial fibrosis with infiltrates, tubular atrophy, and thickening of the tubular basement membrane. Optional clinical criteria were normal or small-sized kidneys with occasional small cortical cutaneous cysts. Hyperuricemia was defined as serum uric acid concentration >1 SD greater than the normal values for age and gender (both genders 5 to 10 years, 4.1 ± 1 mg/dL; female, 12 years, 4.5 ± 0.9 mg/dL; 15 years, 4.5 ± 0.9 mg/dL; ≥18 years, 4.0 ± 0.7 mg/dL; male, 12 years, 4.4 ± 1.1 mg/dL; 15 years, 5.5 ± 1.1 mg/dL; ≥18 years, 6.2 ± 0.8) [18].

The study was approved by the Ethics Committee of the Albert-Ludwigs-University Freiburg and all participating family members provided informed consent.

Haplotype analysis

Genomic DNA was isolated by standard methods directly from blood samples using the QIAamp blood kit (Qiagen, Valencia, CA, USA) or from blood lymphocytes after Epstein-Barr virus (EBV) transformation. Haplotype analysis was performed in 224 individuals (including 84 affected individuals) and inferred in 18 additional individuals (nine additional affected individuals), using 13 consecutive polymorphic microsatellite markers that span the critical MCKD2 region in the following order: cen-D16S3116-D16S401-D16S3113-D16S420-D16S417-D16S412-D16S3036-D16S3041-D16S3056-D16S501-D16S499-D16S3060-D16S764-tel. Fluorescently labeled polymerase chain reaction (PCR) products were detected by a Genetic Analyzer 3100 (Applied Biosystems, Foster City, CA, USA) and were analyzed by the GENOTYPER software.

Mutational analysis of the UMOD gene

Mutational analysis was performed by exon PCR of the Uromodulin gene. Primer sequences were determined using the UCSC sequence (November 2002 freeze) encompassing the coding sequence of UMOD [19]. The following primers and conditions were employed: exon 3, 5'-CACATCAACAGCAGTCCCTCCAG-3' and 5'-GACAGGTGTCATATTGCTTCC-3'; exon 4 was divided into two different overlapping parts: exon 4a, 5'-CCTGGAAGATGAGGAGGAGG-3' and 5'-CTG GACGAGTACTGCGC-3'; exon 4b, 5'-GACGACT CCTTGTGCGTA-3' and 5'-CTCTGCACTGCTTACC-3'; exon 5, 5'-GTCTCCCAAGCAGTCTCTC ATC-3' and 5'-GGCAGTGACAGGTGGTCTCAAC-3'; exon 6, 5'-GGCCCCCAAGCTAGACAC-3' and 5'-CCATGAATTGTCTTTTATTTG-3'; exon 7, 5'-TCATGGCCCTTTCTCTCATC-3' and 5'-GCTCATGTTAGAGG-3'; exon 8, 5'-TGCTCAGG ATGCAATCTCAAG-3' and 5'-CTTTACCTGCTCCT-3'; exon 9, 5'-TCAGCTCGTCCATAG-3' and 5'-TTGCCCAATGTTTCTTGC-3'; exon 10, 5'-AACCCACATTTGGCTTAC-3' and 5'-AGGTCCAGGCTGGTCTTAC-3'; exon 11, 5'-GTCAGATGTTTGGCAGG-3' and 5'-CAGTCTCAGG-3' using a “touch down” program with a starting annealing temperature of 72°C (exons 4a, 5, and 10), decreasing every step by 0.7°C for 24
times and a next round of amplification with an annealing temperature of 55°C for 20 times. An annealing temperature of 60°C was used for the exons 3, 4b, 8, and 9, 59°C for exons 7, 11, and 12, and 57°C for exon 6. PCR products were purified using the Marligen Rapid PCR Purification System (Ijamsville, MD, USA). Purified PCR products were sequenced, using a Genetic Analyzer 3700 (Applied Biosystems) and resulting sequences were evaluated with the Sequencher Software (Gene Codes, Ann Arbor, MI, USA).

RESULTS

Clinical data

The three families presenting with UMOD mutations included 12 living individuals affected with MCKD (three in F524, three in F739 and six in F762) (Fig. 1). Six of 12 also suffered from hyperuricemia (Table 1). The patients presented with hyperuricemia between 17 and 59 years of age (Table 1). ESRD developed between 29 years and 60 years. Imaging by magnetic resonance imaging (MRI) or ultrasound revealed in all families suspicious results with small kidneys, decreased parenchyma, or cysts. Occasional small cysts were only visible in three individuals by ultrasound (Table 1). Renal histology in all cases was compatible with MCKD showing microcysts in four out of 12 cases and in the others dilated or atrophied tubules, global sclerosis, extensive tubulointerstitial atrophy with fibrosis, and signs of chronic diffuse inflammation (Table 1).

Linkage analysis

In 25 families with 224 individuals, haplotype analysis for linkage to MCKD1 and MCKD2 was performed. In one kindred (F762), we found significant linkage to MCKD2. In six kindreds, significant linkage to chromosome 1 [logarithm of odds (LOD) score >3.0] or exclusion of linkage to chromosome 16 was found. Linkage for family F762 was previously published with a maximum multipoint LOD score of 3.75 in the interval between D16S3017 and D16S417 [20]. In 19 families too small for linkage analysis, haplotype analysis was compatible with linkage to MCKD2.

Mutation analysis

We performed mutational analysis in affected individuals from 19 families compatible with linkage to MCKD2 examining all 10 coding exons of the UMOD gene by exon PCR and direct sequencing of the forward strands of exon PCR products.

We used an “affecteds only” approach for definition of the affected status of MCKD. Specifically, if diagnostic data supported MCKD, the affected status was assumed. If there was no data demonstrating the presence of MCKD, the disease status was determined as unknown, since there is age-related penetrance in MCKD. On this basis, there was full-cosegregation of all found heterozygous UMOD mutations and the affected status for MCKD.

In three of these families, novel mutations were detected (Fig. 1). All three mutations were located in exon 4. In family F524, a nucleotide exchange (C779A) was found, resulting in the amino acid exchange Thr225Lys. The mutation was identified in all three affected brothers. Because I-1 in F524 is deceased, we cannot prove that he had the same mutation. His treatment of 15 years of dialysis makes his affected status very likely.

In family F739, a C849G mutation was detected, causing the amino acid exchange Cys248Trp. The mutation in F762 is an inframe deletion/insertion at position 383 (383del12/ins9) that deletes five amino acids and inserts four new amino acids.

Moreover, none of the mutations were found in 100 healthy controls. Therefore, our detected mutations are unlikely to be common polymorphisms.

In exon 6, an additional substitution (G1477T, Val458Leu) was found in two families that were compatible with linkage to 16p12. Nevertheless, this substitution was realized to be a new single nucleotide polymorphism (SNP) because we detected it in four healthy controls out of 100.

DISCUSSION

We detected three novel heterozygous mutations in the UMOD gene, thereby confirming that UMOD is the responsible gene for MCKD2. All detected mutations are found in exon 4 of UMOD, thus underlining the specific role of exon 4-encoded sequence in the generation of the MCKD2 renal phenotype.

In previous studies, abnormal localization of UMOD protein in the interstitium in MCKD patients was shown, suggesting UMOD as a candidate gene for MCKD [21]. By identifying three novel mutations, we confirmed the UMOD gene as the responsible gene for MCKD2. All mutations described here affect amino acids conserved in the cow, mouse, and rat (Fig. 2). The mutation of F762 (383del12/ins9) is positioned in a domain of known function, the second calcium binding (cb) EGF-like domain. In F762, the cbEGF-like domain encoded by UMOD exon 4 is affected by a loss of five amino acids and their replacement by four new amino acids. Two of the five amino acids involved by the mutation of F762 are highly conserved in evolution, including the lower vertebrate zebrafish and the nematode Caenorhabditis elegans, in genes encoding EGF binding domains (for example, fibulin 1, an EGF containing extracellular matrix protein). In contrast to human diseases, including Marfan syndrome, cerebral autodomal-dominant arteriopathy with subcortical infarcts and leukencephalopathy (CADASIL), Alagille syndrome, protein S defi-
Fig. 1. Mutations in the Uromodulin (UMOD) gene. The upper sequence shows the wild-type (WT) sequence and the lower sequence reveals the mutation (MUT) in each family. The resulting amino acids are indicated below sequences. All mutations are located in exon 4. (A) In affected individuals of family F524, a heterozygous C779A substitution was detected resulting in a Thr225Lys amino acid exchange. (B) Affected individuals in family F739 showed a heterozygous C849G substitution, causing a Cys248Trp change. (C) Affected subjects in F762 carried heterozygously a 12 bp deletion together with an insertion of nine nucleotides (383del12/ins9). The deleted nucleotides are indicated in underlined and bold. The inserted nucleotides are shown in red. Because of this mutation five amino acids are replaced by four different ones. The pedigree was recently published [20]. Arrows denote individuals in whom DNA was available for haplotype analysis.
Table 1. Synopsis of clinical data, imaging results, renal histology and Uromodulin (UMOD) mutation of the patients investigated by our study

<table>
<thead>
<tr>
<th>Family number</th>
<th>Gender</th>
<th>Age at presentation</th>
<th>Age at ESRD/death</th>
<th>Clinical symptoms</th>
<th>Imaging</th>
<th>Histology (kidney biopsy)</th>
<th>Heterozygous mutation (protein changes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F524</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II-1</td>
<td>M</td>
<td>27</td>
<td>29</td>
<td>CRI, hyperuricemia</td>
<td>NA</td>
<td>Tubulointerstitial atrophy (50%) and fibrosis with interstitial cell infiltration, microcysts</td>
<td>C779A (Thr225Lys)</td>
</tr>
<tr>
<td>II-3</td>
<td>M</td>
<td>18</td>
<td>34</td>
<td>CRI, gout, hyperuricemia</td>
<td>US (reduced parenchyma, small kidney, small cysts)</td>
<td>Thickened basement membrane, sclerosed glomeruli (20%), tubular atrophy</td>
<td>C779A (Thr225Lys)</td>
</tr>
<tr>
<td>II-4</td>
<td>M</td>
<td>29</td>
<td>NA</td>
<td>CRI, hyperuricemia</td>
<td>MRI (small right kidney, no cysts)</td>
<td>Tubular atrophy (70%), tubulointerstitial fibrosis, occasional microcysts, thickened basement membrane</td>
<td>C779A (Thr225Lys)</td>
</tr>
<tr>
<td>F739</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-1</td>
<td>M</td>
<td>39</td>
<td>45</td>
<td>CRI, hyperuricemia</td>
<td>US (small kidneys)</td>
<td>NA (reduced parenchyma, 1 cyst)</td>
<td>C849G (Cys248Trp)</td>
</tr>
<tr>
<td>II-1</td>
<td>M</td>
<td>18</td>
<td>NA</td>
<td>CRI, hyperuricemia</td>
<td>US (small kidneys, reduced parenchyma)</td>
<td>Interstitial fibrosis, atrophic tubuli, sclerosed glomeruli, thickened basement membrane</td>
<td>C849G (Cys248Trp)</td>
</tr>
<tr>
<td>II-2</td>
<td>F</td>
<td>17</td>
<td>NA</td>
<td>CRI, hyperuricemia</td>
<td>NA</td>
<td>NA</td>
<td>C849G (Cys248Trp)</td>
</tr>
<tr>
<td>III-5</td>
<td>F</td>
<td>59</td>
<td>60</td>
<td>CRI, hypertension</td>
<td>NA</td>
<td>Interstitial atrophy, occasional microcysts, thickened basement membrane</td>
<td>383del12/ins9 (ValCysProGluGly93-97AlaAlaSerCys)</td>
</tr>
<tr>
<td>III-10</td>
<td>F</td>
<td>36</td>
<td>37</td>
<td>CRI, hypertension</td>
<td>NA</td>
<td>NA</td>
<td>383del12/ins9 (ValCysProGluGly93-97AlaAlaSerCys)</td>
</tr>
<tr>
<td>III-11</td>
<td>M</td>
<td>NA</td>
<td>56</td>
<td>CRI, hypertension</td>
<td>US (small kidneys, multiple small cysts bilaterally)</td>
<td>NA</td>
<td>383del12/ins9 (ValCysProGluGly93-97AlaAlaSerCys)</td>
</tr>
<tr>
<td>IV-1</td>
<td>F</td>
<td>25</td>
<td>51</td>
<td>CRI, hypertension</td>
<td>NA</td>
<td>NA</td>
<td>383del12/ins9 (ValCysProGluGly93-97AlaAlaSerCys)</td>
</tr>
<tr>
<td>IV-2</td>
<td>M</td>
<td>49</td>
<td>52</td>
<td>CRI, hypertension</td>
<td>NA</td>
<td>Tubulointerstitial atrophy and fibrosis, occasional microcysts, thickened basement membrane</td>
<td>383del12/ins9 (ValCysProGluGly93-97AlaAlaSerCys)</td>
</tr>
<tr>
<td>IV-3</td>
<td>M</td>
<td>42</td>
<td></td>
<td>CRI, hypertension</td>
<td>NA</td>
<td>50% of glomeruli sclerosed, bands of tubulointerstitial atrophy, no microcysts, thickened basement membrane</td>
<td>383del12/ins9 (ValCysProGluGly93-97AlaAlaSerCys)</td>
</tr>
</tbody>
</table>

CRI, chronic renal insufficiency; NA, not available; US, ultrasound; MRI, magnetic resonance imaging. Age at presentation and age at end-stage renal disease (ESRD) is given in years.
Fig. 2. Comparison of the amino acid conservation in exon 3 and 4 (in which all mutations up to now were found) through evolution, using the CLUSTAL W (1.81) multiple sequence alignment software [27]. Shown is the amino acid sequence of the Uromodulin protein equivalent from Homo sapiens, Bos taurus, Mus musculus, and Rattus norvegicus. To underline the high degree of conservation with the zymogen granule membrane protein (GP2), the GP2 amino acid of Canis familiaris is indicated in the bottom row. The three calcium-binding endothelial growth factor (EGF)-like domains are highlighted in light gray, dark gray, and black. These domains were found using the consensus sequence of calcium-binding EGF-like domains in the NCBI Conserved Domain Database [28]. The amino acids affected by the mutations identified in patients with MCKD2 are indicated by arrows. Amino acid numbering is shown on the right margin for Homo sapiens UMOD.

Mutation in the UMOD gene in MCKD type 2 patients

---MGQPSLTLWMLMVVAVSWFITTAADTDSEARWCECHSNATCTEDEAVTCTCQEGFTG
Homo sapiens 58

MKCLFSPNWMMAAAVTVSTYIIAPADTDSSAKSCHECHSNATCTVVDAATTACAOEGFTG
Bos taurus

---MGIPWTLLVVMVTSEAFSEARWCECHNNATCTVVDTGGTSCQQTGFTG
Mus musculus

MGQLLSLLWMLVVMTVTAFVAGANDSPEREARWCECHNNATCTVVLDGTVTCQOAGFTG
Rattus norvegicus

---MGIPWTLLVVMVTSEAFSEARWCECHNNATCTVVDTGGTSCQQTGFTG
Canis familiaris

Clcn gene

---PGTICLVALECPGPAHGGCSNASSSSVNPFGSGCGVPEG---DALVCADDPCA
Homo sapiens 118

DGLCTVCYLDCEACPGHGGCSNASSSSVNPFGSGCGVPEG---DALVCADDPCA
Bos taurus

---PGTICLVALECPGPAHGGCSNASSSSVNPFGSGCGVPEG---DALVCADDPCA
Mus musculus

---PGTICLVALECPGPAHGGCSNASSSSVNPFGSGCGVPEG---DALVCADDPCA
Rattus norvegicus

---PGTICLVALECPGPAHGGCSNASSSSVNPFGSGCGVPEG---DALVCADDPCA
Canis familiaris

Clcn gene

---PGTECDVPGQPGPQEGCSCGDPGCQDPPAPRGGWHECSPGSCGPGDCLVPEG---DALVCADDPCA
Homo sapiens 176

DGLCTVCYLDCEACPGHGGCSNASSSSVNPFGSGCGVPEG---DALVCADDPCA
Bos taurus

---PGTECDVPGQPGPQEGCSCGDPGCQDPPAPRGGWHECSPGSCGPGDCLVPEG---DALVCADDPCA
Mus musculus

---PGTECDVPGQPGPQEGCSCGDPGCQDPPAPRGGWHECSPGSCGPGDCLVPEG---DALVCADDPCA
Rattus norvegicus

---PGTECDVPGQPGPQEGCSCGDPGCQDPPAPRGGWHECSPGSCGPGDCLVPEG---DALVCADDPCA
Canis familiaris

Clcn gene

---PGTECDVPGQPGPQEGCSCGDPGCQDPPAPRGGWHECSPGSCGPGDCLVPEG---DALVCADDPCA
Homo sapiens 236

HRTLDEYWRSTEGYAYACDTRLGRWFRFGQGGAECCTVPLCRCNAPAAMWNLGTHP
Bos taurus

HRTLDEYWRSTEGYAYACDTRLGRWFRFGQGGAECCTVPLCRCNAPAAMWNLGTHP
Mus musculus

HEETFYWRSTEGYVSDAGLHGRWTGQGVRMAETCPVPLRCNAPAAMWNLGNSH
Rattus norvegicus

HEETFYWRSTEGYVSDAGLHGRWTGQGVRMAETCPVPLRCNAPAAMWNLGNSH
Canis familiaris

Clcn gene

SSDEIGSVKARCAHWSGHilCWDA5SVQVKAAGGYVYLTAPEECNLYA
Homo sapiens 288

SSDEIGSVKARCAHWSGHilCWDA5SVQVKAAGGYVYLTAPEECNLYA
Bos taurus

SSDEIVSNRCAHWSGDCCLWDA5PVQKACAGGYVYNLTAPAECNLYA
Mus musculus

SSDEIVSNRCAHWSGDCCLWDA5PVQKACAGGYVYNLTAPAECNLYA
Rattus norvegicus

SSDEIVSNRCAHWSGDCCLWDA5PVQKACAGGYVYNLTAPAECNLYA
Canis familiaris

Clcn gene

ciency, hemophilia B, and familial hypercholesterolaemia missense mutations within cbEGF domains were found [22]. Mutations in the Fibrillin-1 (FBN1) cause the Marfan syndrome. FBN1 reveals an equivalent domain forming a part of a calcium coordinating segment that stabilizes the tertiary structure [23]. Tandem FBN1 cbEGF domain pairs, when saturated with calcium, exhibit a rod-like conformation [22]. In Marfan syndrome, missense mutations in FBN1 have been described affecting the same cbEGF domain as the mutation of F762 (383del12/ins9) [24, 25]. Smallridge et al [22] distinguished mutations within cbEGF domains as (1) mutations affecting cysteine residues and thereby disrupting disulphide bonds and (2) mutations affecting residues in the calcium-binding consensus sequence, which are likely to reduce calcium-binding affinity. As a consequence of the 383del12/ins9 mutation a cysteine is also affected, but the reason for the resulting impaired function remains theoretical because the importance of the four other replaced amino acids (ValProGlyGlu) is unclear.

The Thr225Lys mutation of F524 and the Cys248Trp mutation of F739 are located in a region of unknown function that is also encoded by exon 4. Hart et al [10] and Turner et al [17], respectively, also found two mutations in this region of exon 4, outside the known cbEGF-like domain. Interestingly, this region shows an identity of 56% with GP2, another ZP domain-containing protein. All three mutations found are not only conserved in the above-mentioned animals but also in this conserved part of GP2 of Canis familiaris. Two of these mutations (Cys248Trp in F739, 383del12/ins9 in F762) involve cysteine residues, which are highly conserved in the UMOD protein throughout evolution including C. elegans (Fig. 2). UMOD contains 48 cysteine residues per monomer form-
ing 24 intramolecular disulfide bonds. The amino acid changes in F739 and F762 are therefore expected to change the molecular conformation through impaired intra- or intermolecular disulfide bond formation. The high cysteine content of THP and correct formation of the disulfide bonds were suspected to be the rate limiting step for the export of the premature THP out the endoplasmatic reticulum and that this regulates the efficiency of THP maturation [11].

Impaired disulfide binding is well known as a reason for other disorders, for example, diabetes insipidus centralis with a less stable vasopressin precursor, which accumulates in the endoplasmic reticulum [26]. The mutations we found are very likely to cause MCKD2 in these patients, even if none of them is a frameshift or a nonsense mutation. Also Hart et al [10] and Turner et al [17] did not detect any frameshift or nonsense mutation, which would cause a truncated protein. In addition, and surprisingly, all up to now identified mutations with the exception of one mutation at the beginning of the neighboring exon 5 are located in exon 4. All mutations take place in front of the ZP domain. The N-terminal half of exon 4 encodes the three known calcium-binding EGF-like domains [16]. The C-terminal part of exon 4 includes a nine amino acid long immunoglobulin light chain–binding domain [14], which has not been affected by any mutation up to now. However, it seems that exon 4 plays an important role in the function of UMOD and that heterozygous mutations in this exon lead to the specific phenotype of MCKD2.

The clinical presentation of the phenotype has to be evaluated very carefully since renal cysts in imaging (only three of 12 affected individuals) and hyperuricemia (six of 12 affected individuals) do not occur in all of the patients. In all affected individuals, renal insufficiency (creatinine clearance <60 mL/min/1.73 m²) was diagnosed. The age of onset is varied between 17 and 59 years of age. Hart et al [10] also described a kindred, in which the vast majority but not all affected individuals have hyperuricemia. This underlines the heterogeneity of the phenotype and might show that neither hyperuricemia nor cysts are an obligatory feature of the clinical presentation. At the moment, a significant genotype/phenotype correlation is not possible due to the limited number of different mutations detected so far.

Finally, it was surprising to us to find only three mutations in 19 families, which were compatible with linkage to 16p12. This may be explained by the small number of affected persons in most families, possibly resulting in false positive linkage result in an autosomal-dominant disease. Alternatively, a second gene for FJHN and MCKD2 might exist in the more centromeric region of UMOD in the region described by Kamatani et al [7] and which excludes the UMOD gene. Reports about additional mutations in MCKD2 and studies concerning the function of the UMOD protein will help to clarify the role of exon 4 for the pathogenesis of MCKD.

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