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GENETIC DISORDERS – DEVELOPMENT

Familial juvenile hyperuricemic nephropathy: Detection of mutations in the uromodulin gene in five Japanese families

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Familial juvenile hyperuricemic nephropathy: Detection of mutations in the uromodulin gene in five Japanese families.

Background. Familial juvenile hyperuricemic nephropathy (FJHN) is an autosomal-dominant disease characterized by hyperuricemia of underexcretion type, gout, and chronic renal failure. We previously reported linkage on chromosome 16p12 in a large Japanese family designated as family 1 in the present study. Recent reports on the discovery of mutations of the uromodulin (*UMOD*) gene in families with FJHN encouraged us to screen UMOD mutations in Japanese families with FJHN, including family 1.

Methods. Six unrelated Japanese families with FJHN were examined for mutations of the *UMOD* gene by direct sequencing. To confirm the results of the mutation screening, parametric linkage analyses were performed using markers in 16p12 region and around other candidate genes of FJHN.

Results. Five separate heterozygous mutations (Cys52Trp, Cys135Ser, Cys195Phe, Trp202Ser, and Pro236Leu) were found in five families, including family 1. All mutations were cosegregated with the disease phenotype in all families, except for family 1, in which an individual in the youngest generation was found as a phenocopy by the genetic testing. Revised multipoint linkage analysis showed that the *UMOD* gene was located in the interval showing logarithm of odds (LOD) score above 6.0. One family carrying no mutation in the *UMOD* gene showed no linkage to the medullary cystic kidney disease type 1 (MCKD1) locus, the genes of hepatocyte nuclear factor-1β (HNF-1β), or urate transporters URAT1 and hUAT.

Conclusion. Our results gave an evidence for the mutation of the *UMOD* gene in the majority of Japanese families with FJHN. Genetic heterogeneity of FJHN was also confirmed. Genetic testing is necessary for definite diagnosis in some cases especially in the young generation.

Key words: familial juvenile hyperuricemic nephropathy, FJHN, uromodulin, Tamm-Horsfall protein, glycoprotein-2, linkage analysis.

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Familial juvenile hyperuricemic nephropathy (FJHN) (MIM 162000) is an autosomal-dominant disease characterized by hyperuricemia of underexcretion type, gout, and chronic renal failure. More than 50 families in various ethnic groups have been described since Duncan and Dixon first noted the disease in 1960 [1]. Affected family members show the impairment of urate excretion before puberty and usually develop hyperuricemia and gout after adolescence [2]. Renal function gradually deteriorates and results in end-stage renal failure within 10 to 20 years. Elucidation of the molecular defects accounting for this disease should help understand the pathogenesis, early diagnosis, and improvement of therapy. It may also help identify the mechanisms underlying reduced urinary excretion of urate [3]. We have previously performed parametric linkage analysis on a large Japanese family with FJHN and mapped the candidate gene locus on chromosome 16p12 [4, 5]. Several groups also reported linkage to chromosome 16p11-p13 for European families with FJHN [6–9].

Autosomal-dominant medullary cystic kidney disease (MCKD) (MIM 174000) is a renal disorder characterized by the presence of small medullary cysts, a reduction in urine concentrating ability, and a decrease in sodium conservation. MCKD also progresses toward end-stage renal failure during adulthood. Hyperuricemia and gout have been reported in MCKD [10–12]. Several groups reported linkage of MCKD on chromosome 1q21 [13–15]. Histopathologic findings at the late stage of both MCKD and FJHN are common and characterized by chronic tubulointerstitial nephropathy with focal areas of interstitial fibrosis and inflammatory cell infiltration, thickening of tubular basement membrane, and glomerulosclerosis. These findings, however, are not specific and shared with other familial renal diseases such as autosomal-recessive juvenile-onset nephronophthisis (NPH) (MIM 256100) [16, 17].

Scolari et al [18] mapped a responsible gene locus on chromosome 16p12 in an Italian family with MCKD, which was designated later as MCKD2 (MIM 603860). Hence, MCKD showing linkage to chromosome 1q21 was designated as MCKD1. The same group screened mutations in the uromodulin (*UMOD*) gene as a positional candidate for MCKD2, but they reported failure in finding consistent mutations [19]. Dahan et al [7] confirmed the linkage between FJHN and markers within the 16p12 locus in a Belgian family and proposed that FJHN and MCKD2 might be allelic disorders based on the similar location of the gene loci as well as the clinical and pathologic resemblance between the diseases. Recently, Hart et al [20] succeeded in positional cloning of a responsible gene for FJHN in the locus on 16p11-p13. They found four heterozygous mutations in the *UMOD* gene in three families with FJHN and in one family with MCKD2, proving the theory of allelism of FJHN and MCKD2. Turner et al [21] also reported five heterozygous missense mutations in the *UMOD* gene in five unrelated families with FJHN.

In the present study, we describe a novel mutation in the *UMOD* gene found in the large Japanese family in which we have localized a responsible gene for FJHN to 16p12 [4, 5]. We found a phenocopy case in the family and solved the inconsistency between the proposed disease candidate intervals of us and those of other groups [6–9, 20]. We also report four different mutations in the *UMOD* gene in another four Japanese families with FJHN. Besides, we confirmed the genetic heterogeneity by finding one FJHN family showing no mutation in the *UMOD* gene and no linkage to other known candidate loci or genes [6, 8, 9, 22]. We suppose that the remaining inconsistency in the proposed candidate intervals for FJHN among the research groups is probably derived from phenocopy and/or genotying errors. Genetic heterogeneity of FJHN might also complicate the linkage analyses. Possible mechanisms for the mutations of the *UMOD* gene in pathogenesis of FJHN are discussed.

METHODS

Pedigrees

Six unrelated Japanese pedigrees with FJHN were studied (Fig. 1). Of the largest pedigree designated as family 1 in the present study, the clinical and the biochemical findings were reported previously by Yokota et al [4] and a genome-wide linkage study by Kamatani et al [5]. Informed consent was obtained from all subjects. In family 1, DNA was extracted by the phenol extraction method from the lymphoblastoid cell lines established using Epstein-Barr virus. In families 2 to 6, DNA was extracted from mononuclear cells separated from heparinized peripheral blood. Criteria for diagnosis of affection with FJHN were described previously [4, 5]. Briefly, an individual was considered to be affected if he or she had either definitive severe renal failure or impaired urate excretion as indicated by a fractional clearance of uric acid (C_{UA}/creatinine clearane) of less than 5.5% for men or less than 7.8% for women.

Sequence analysis

For the sequence analysis of the *UMOD* gene, the Genbank data NM_003361 for cDNA, NP_003352 for protein, and AC106799 for genome were used. Intronic primers for polymerase chain reaction (PCR) amplification of all 12 exons of the *UMOD* gene were designed. PCR was performed in a 50 μL reaction mixture containing 20 ng template DNA, 1 unit KOD-plus DNA polymerase (Toyobo, Osaka, Japan), 0.3 μmol/L each of primers, 1 mmol/L $MgSO_4$, and 0.2 mmol/L each of desoxynucleoside triphosphate (dNTP) in 1 × PCR buffer (Toyobo). Amplified DNA was either treated with a mixture of endonuclease I and shrimp alkaline phosphatase (ExoSAP-IT) (USB Corporation, Cleveland, OH, USA) or purified with agarose gel electrophoresis and was sequenced using the BigDye Terminator Cycle Sequencing Kit, version 1, on an ABI 3700 DNA analyzer (Applied Biosystems, Foster City, CA, USA).

Restriction endonuclease analysis in family 1

PCR amplification of exon 4 of the *UMOD* gene was performed using genomic DNAs as templates and the primers 5′-GGGGATGGATGGCACTGTGAGTG-3′ and 5′-TTCCAGGCCTGGGATGAGGA-3′. The amplified DNAs were digested with *Fok* I (New England Biolabs, Beverly, MA, USA) and electrophoresed on an 8% polyacrylamide gel. The gel was stained with ethidium bromide and photographed under ultraviolet light.

Allele-specific (AS)-PCR assay

AS-PCR assays were performed using AS primers (details of primers are available on request). PCR amplifications were performed using a SYBR Green I assay mixture (SYBR Green PCR Master Mix) (Applied Biosystems) with an ABI 7900HT system (Applied Biosystems).

Parametric linkage analysis

Pairs of primers in the Linkage Mapping Set (Applied Biosystems) were used for known polymorphic microsatellite loci. For novel microsatellite markers, the fluorescent dye primers were synthesized by Applied Biosystems Japan (Tokyo, Japan). PCR and analyses of data were performed as reported previously [5], except for using an ABI 3700 DNA analyzer (Applied Biosystems) for data collection and a Genotyper software, version 3.5 (Applied Biosystems) for analyses. The MLINK program, version 5.1, and the LINKMAP program,

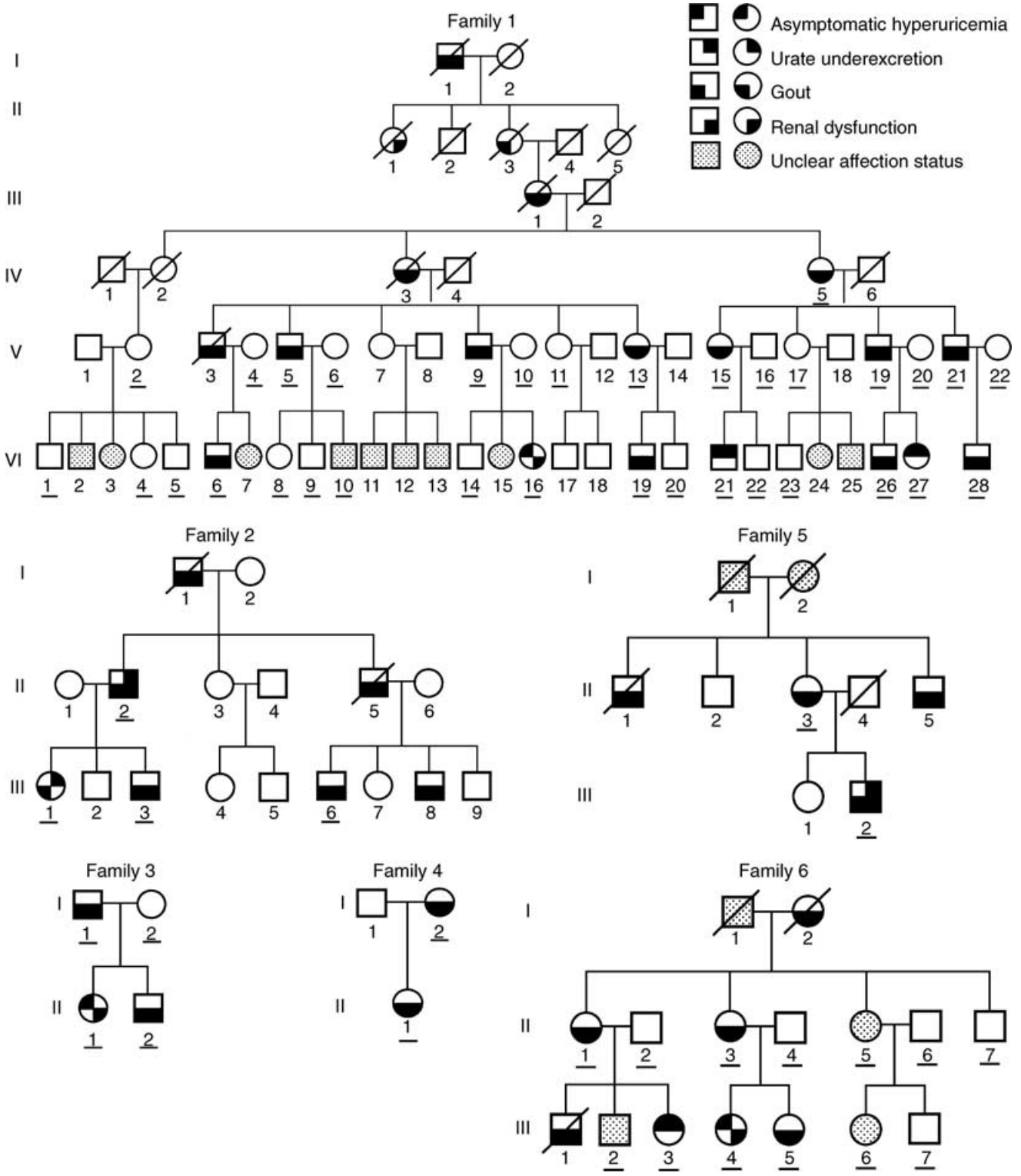

Fig. 1. Pedigrees of families with familial juvenile hyperuricemic nephropathy (FJHN). Hyperuricemia was defined as over 0.45 mmol/L (=7.56 mg/dL) of serum uric acid; urate underexcretion, a fractional urate clearance of less than 5.5% for men or less than 7.8% for women. The symbol of renal dysfunction indicates that an individual had undergone renal transplantation, was undergoing hemodialysis, or showed a serum creatinine concentration of over 133 mmol/L (=1.5 mg/dL). Underlined numbers represent individuals who supplied DNA samples.

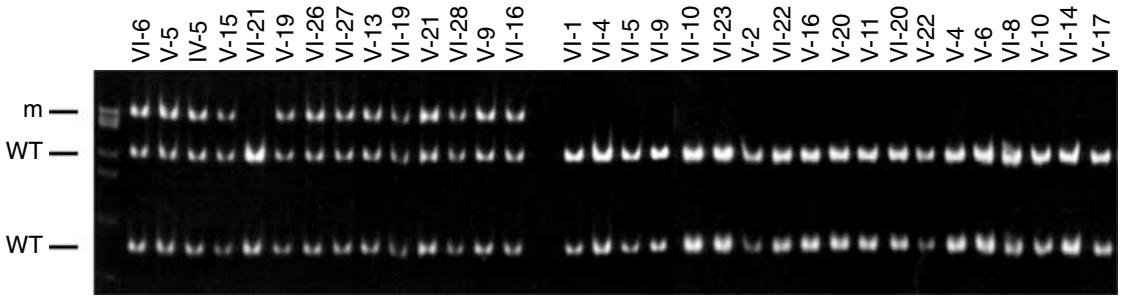

Fig. 2. Restriction endonuclease analyses of exon 4 of the uromodulin (*UMOD*) gene. Individuals in each lane are indicated by the combination of roman and arabic numerals. Roman numeral represents the number of the generation, and arabic numerals represent the number of individual in each generation as presented in Figure 1. The upper bands indicated by m are derived from the polymerase chain reaction (PCR) products containing the mutant allele, and the middle and lower bands indicated by WT are derived from the wild-type allele. Fourteen left-side lanes show the digested PCR products from the individuals who had been considered as affected, while the remaining 19 lanes are those from the individuals considered as unaffected. Note that the individual IV-21 carries only the wild-type allele of the *UMOD* gene.

version 5.1, supplied in the LINKAGE package [23] were used for two-point and multipoint analyses, respectively. Mapping information of known microsatellite markers was based on the Marshfield genetic map. Parameters were set as previously reported [5]. Briefly, the mode of inheritance was set as autosomal-dominant. The penetrances for a homozygote without the disease allele, a heterozygote, and a homozygote with the disease allele were set at 0, 1, and 1, respectively. The frequencies of marker alleles were set at even for every allele observed for each marker. The frequency of the disease gene was set at 0.001 in the general population.

RESULTS

Uromodulin sequence analysis

DNA sequence analysis of the 12 exons of the *UMOD* gene was undertaken in 33 members in family 1, including all 32 members who had previously been examined in the linkage analysis [5]. A heterozygous missense mutation of Pro236Leu was found in all affected individuals except VI-21 who belongs to the youngest generation (Fig. 2). Subsequently, we examined other five families with FJHN in the same way. In four of these five families, four different single nucleotide substitutions were found to cause heterozygous missense mutations (i.e., Cys135Ser in family 2, Cys195Phe in family 3, Cys52Trp in family 4, and Trp202Ser in family 5 (Table 1). No mutation was found in family 6. All mutations in families 1 to 5 altered an evolutionary conserved residue in the UMOD protein. In families 2 to 5, mutations were cosegregated with the disease phenotype. None of the five mutations was identified in any of 96 control genomic DNAs examined with sequencing and in other 180 control genomic DNAs tested by AS-PCR.

Clinical findings of the individual VI-21 in family 1

To dissolve the inconsistency between genotype and phenotype in VI-21 of family 1, we reexamined him clinically. He was considered to be affected before the first report of family 1 in 1991 [4] because of asymptomatic hyperuricemia. Besides, multiple renal cysts have been detected in the kidneys in repeated examinations with ultrasonography. He has hypertension with no symptoms of renal insufficiency at the age of 30 years. The hyperuricemia was at the level of 8.2 mg/dL. His fractional clearance of uric acid was 5.23%, which was in the range to be judged as affected in the criteria of the previous reports [4, 5]. Judging from the present genetic data and the recent reports [20, 21], the Pro236Leu mutation was the causative mutation in family 1. Thus, VI-21 was indicated to be a phenocopy case.

Table 1. Summary of the mutations in the uromodulin (UMOD) gene

Exon[a]	Mutation[b]	Amino acid position[b]	From	To	Phenotype	Reference
4	c·261T>G	52	Cys	Trp	FJHN	Present study (family 4)
4	c·335G>A	77	Cys	Tyr	FJHN	21
4	c·412G>T	103	Gly	Cys	MCKD	20
4	c·481T>C	126	Cys	Arg	FJHN	21
4	c·488A>G	128	Asn	Ser	FJHN	21
4	c·508T>A	135	Cys	Ser	FJHN	Present study (family 2)
4	c·548G>A	148	Cys	Tyr	FJHN	20
4	c·634_660del	177–185	—	—	FJHN	20
4	c·689G>T	195	Cys	Phe	FJHN	Present study (family 3)
4	c·710G>C	202	Trp	Ser	FJHN	Present study (family 5)
4	c·754T>C	217	Cys	Arg	FJHN	20
4	c·812C>T	236	Pro	Leu	FJHN	Present study (family 1)
4	c·869G>A	255	Cys	Tyr	FJHN	21
5	c·1003T>G	300	Cys	Gly	FJHN	21

[a] According to the designation of the exons by Hart et al [20].
[b] According to the sequence of the mRNA (GenBank NM_003361) and the amino acid (GenBank NP_003352) of human uromodulin.

Linkage analysis of family 1

We reexamined the parametric linkage analysis in family 1 under the setting that VI-21 was a phenocopy. A

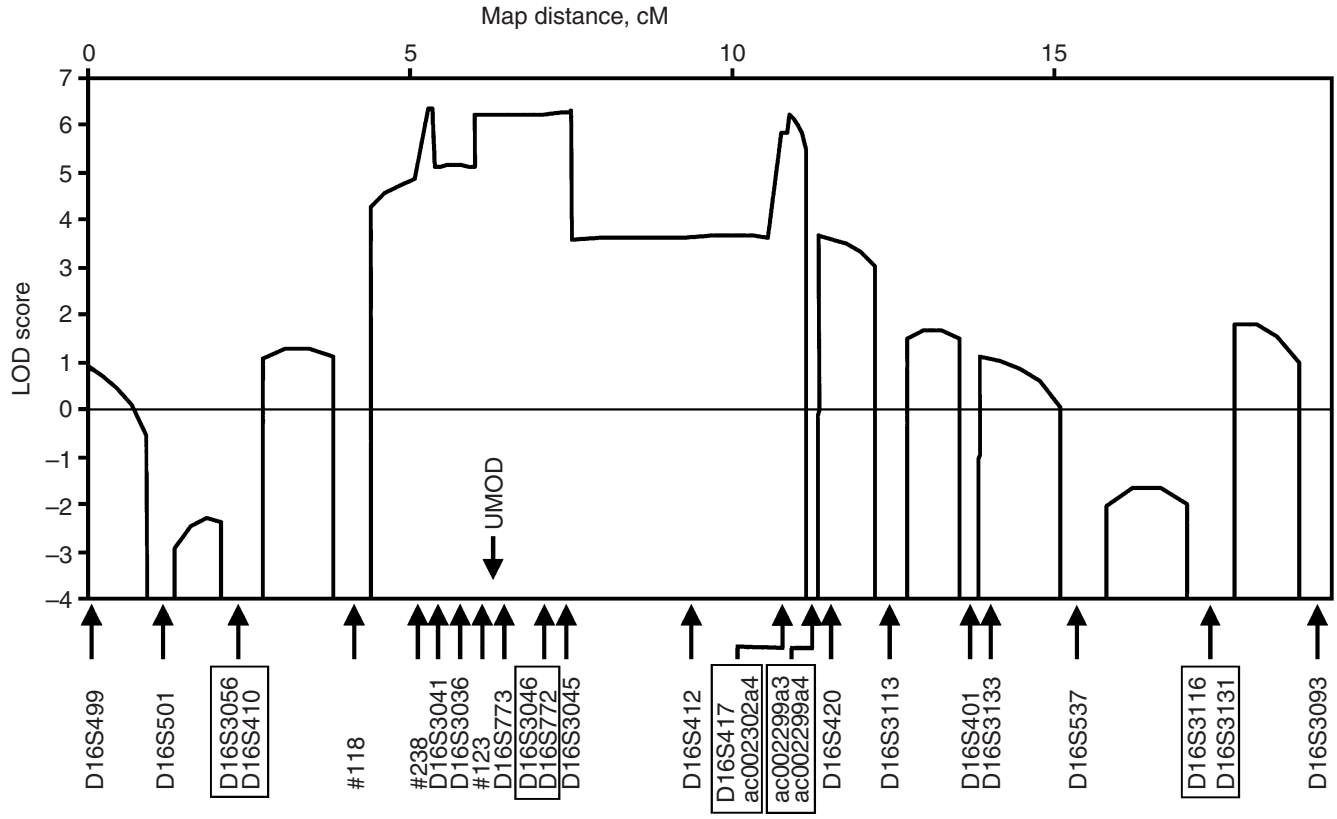

Fig. 3. Results of multipoint linkage analysis of the markers on chromosome 16p in family 1. The analysis was performed under the setting that individual IV-21 was a phenocopy. Primer sequences of novel microsatellite markers are 5′-GGAGGTCGAGACTGCAGTG-3′ and 5′-ATTTCAAAGTCAGTTGCTGATGT-3′ for #118, 5′-GTATCAGTATGGACTCAGGAG-3′ and 5′-TGAAGAATCTTGTAGTGCCAGA-3′ for #238, 5′-CAGCTCAAACCCAGGTCAG-3′ and 5′-TTCCCAAACGATTCTATGGAG-3′ for #123, 5′-TTATCACAAACCCTTGTAGCA-3′ and 5′-AAAAGTAAGAGAAGGAGCACG-3′ for ac002302a4, 5′-ACCTGCAGAGATTCTAATGGGA-3′ and 5′-GAGCTTGCAGT GAGTAGACAGA-3′ for ac002299a3, and 5′-TCCAATTCAATTCATCCTAAAGCC-3′ and 5′-AGGACCAAGATACGCCAGTC-3′ for ac002299a4.

two-point analysis by setting the penetrance at 1 showed that the logarithm of odds (LOD) score was the highest (5.76) for D16S772 at $\theta = 0$. Multipoint analysis yielded the maximum LOD score of 6.35 at a novel marker of #238 followed by 6.30 at D16S3046, 6.28 at D16S773, 6.25 at D16S772, 6.24 at #123, and 6.23 at ac002302a4 (Fig. 3). These results were compatible with that the disease in family 1 was caused by the missense mutation in the *UMOD* gene locating between #123 and D16S773.

Linkage analysis in family 6

In family 6, we performed additional linkage analysis to exclude the *UMOD* gene as the candidate gene and to examine other candidate loci and genes of FJHN reported or speculated so far. We examined known and novel microsatellite markers in the MCKD1 locus (1q21) [13–15] and those around the genes of *UMOD*, urate transporters of URAT1 [24] and hUAT [25, 26], and hepatocyte nuclear factor (HNF)-1β [27] (Table 2). The *UMOD* gene and the MCKD1 loci were clearly excluded for linkage. Moreover, none of the loci of URAT1, hUAT, or HNF-1β yielded the sufficient evidence for linkage.

DISCUSSION

We found five separate mutations in the *UMOD* gene in five of six families with FJHN (83%), indicating that most of Japanese families with FJHN are caused by mutations of the *UMOD* gene. This rate (83%) is comparable to a recent linkage study by Stacey et al [8] showing linkage to 16p11-p13 in five of seven European families with FJHN (71%). Another recent linkage study by Stiburkova et al [9] showing linkage to 16p11 in six of 15 European families with FJHN represented a much lower rate (40%). Both the difficulty of accurate diagnosis in some cases and the genetic heterogeneity of this disease probably complicated the linkage analysis. Recently, Bleyer et al [28] reported a clinical characterization of a family with FJHN caused by a deletion of in-frame 9 amino acids in the *UMOD* gene. They found in the members carrying the mutation that renal insufficiency was the most

Table 2. Two-point logarithm of odds (LOD) scores for family 6

Locus	Position[a] Genetic (cM)	Position[a] Physical (Kb)	θ 0.0	θ 0.1	θ 0.2	θ 0.3	θ 0.4
MCKD1 (1q21)							
D1S252	150.27	116705	−infinity	−0.80	−0.33	−0.12	−0.03
D1S498	155.89	148077	−infinity	−0.73	−0.35	−0.14	−0.03
D1S1153	161.05	152047	−infinity	−0.65	−0.27	−0.10	−0.02
D1S1595	161.05	152466	−infinity	−0.61	−0.28	−0.11	−0.03
D1S2635	165.62	155948	−infinity	−1.12	−0.47	−0.18	−0.04
D1S484	169.68	157545	−infinity	−1.13	−0.48	−0.18	−0.04
URAT1 (SLC22A12)							
D11S4191	60.09	60251	−infinity	−0.68	−0.32	−0.13	−0.03
URAT1		64620					
ac044790a2[b]		64634	−0.35	−0.14	−0.06	−0.02	−0.00
ac044790a1[c]		64665	−0.01	−0.04	−0.05	−0.03	−0.01
D11S987	67.48	68143	−infinity	−0.11	0.03	0.04	0.02
D11S4162	72.82	71198	0.29	0.24	0.18	0.10	0.03
UMOD							
D16S3036	39.04	19452	−infinity	−0.67	−0.32	−0.13	−0.03
#123		19631	−infinity	−0.78	−0.34	−0.14	−0.03
UMOD		20272					
D16S773		20608	−infinity	−0.31	−0.12	−0.05	−0.01
D16S3046	40.65	20814	−infinity	−0.30	−0.11	−0.04	−0.01
D16S772		20898	−0.10	−0.07	−0.04	−0.02	−0.00
hUAT (LGALS9)							
D17S1857	43.01	16358	−0.05	−0.07	−0.06	−0.03	−0.01
hUAT		25810					
D17S1824	49.67	26512	−infinity	−0.84	−0.38	−0.16	−0.04
D17S1878	50.74	25963	−infinity	−0.84	−0.38	−0.16	−0.04
D17S798	53.41	31139	−infinity	−0.71	−0.32	−0.13	−0.03
HNF-1β							
D17S927	58.25	34737	−infinity	−0.49	−0.22	−0.09	−0.02
HNF-1β		35777					
D17S1788	58.25	35817	0.51	0.37	0.23	0.11	0.03

Abbreviations are: MCKD1, medullary cystic kidney disease type 1; URAT1, urate transporter; UMOD, uromodulin; hUAT, human urate transporter; HNF-1β, hepatocyte nuclear factor-1β.

[a] According to the Marshfield genetic map and NCBI Map View build 33.

[b] Primer sequences are 5′-CATGCATACTGGTTCACACTCAC-3′ and 5′-GGAGCACACAGGCATACAG-3′.

[c] Primer sequences are 5′-TCCTCAGCATTGCTTGAATCA-3′ and 5′-CCTCCAGAAATCGACTGTCC-3′.

consistent finding after the age of 20 years and hyperuricemia was not universally present. Several unaffected and control individuals were found to have fractional clearance of uric acid of less than 5%. Our experience on the individual IV-21 in family 1 also indicates the difficulty in clinical diagnosis of FJHN, especially in the youngest generation and the importance of a genetic test. Results of the revised parametric linkage analyses of family 1 were consistent with the discovery of mutation in the *UMOD* gene and solved the inconsistency in the proposed disease candidate intervals between us and other groups [6–9, 20].

The exclusion of the involvement of *UMOD* gene in family 6 confirmed the genetic heterogeneity of FJHN [6, 8, 9, 22]. Moreover, other possible candidates such as MCKD1 locus [13–15], *URAT1* gene [24], *hUAT* gene [25, 26], and *HNF-1*β gene [27] gave no significant linkage. Genome-wide linkage study of this family should help finding another candidate locus responsible for FJHN.

In spite of extensive studies on physicochemical and biologic properties, in vivo functions of UMOD remain obscure [29]. UMOD is an 85 kD glycoprotein initially purified in 1985 from the urine of pregnant woman using lectin adherence columns as an in vitro immunosuppressive factor against T-cell and monocyte activity [30]. In 1987, UMOD was revealed to be identical to Tamm-Horsfall protein with the isolation of complementary DNA of human UMOD [31]. Tamm and Horsfall [32] isolated the protein in 1950 from urine using the salt precipitation method and characterized it as an inhibitor of viral hemagglutination. Tamm-Horsfall protein is the most abundant protein in normal urine and a major component of urinary casts [29]. UMOD is synthesized in kidney cells as a 640 amino acid precursor. Upon translocation into the endoplasmic reticulum, the 24 amino acid signal peptide and the hydrophobic portion of the C-terminus are removed, and then glycosylphosphatidylinositol (GPI) anchor is attached to the C-terminus. GPI-anchored UMOD is transported to

the cell surface by exocytotic vesicles. At the cell surface or in the endoplasmic reticulum lumen, UMOD is cleaved again near the C-terminus, resulting in urinary UMOD/Tamm-Horsfall protein [33–35]. UMOD contains 48 cysteine residues which potentially form 24 intramolecular disulfide bonds [31].

It is noteworthy that most of the mutations of the *UMOD* gene are missense mutations located in the exon 4 [20, 21] (Table 1). There are only two exceptions; one is an in-frame deletion of nine amino acids encoded in exon 4 [20], and the other is a missense mutation (Cys300Gly) encoded in exon 5 [21]. Furthermore, the frequent missense mutations of cysteine residue (9/13) apparently rule out the assumption that the mutations happened randomly in any residue [(48/640)^13]. These findings suggest that the pathogenesis of FJHN associated with the UMOD mutations is either the gain of function or the dominant negative effect of the mutant UMOD rather than the haplo insufficiency.

Aggregation of mutant proteins is one of the possibilities for the gain of function mutation. The extratubular UMOD/Tamm-Horsfall protein deposition as insoluble aggregates has been documented in MCKD [36, 37]. A treatment of HeLa cells expressing recombinant UMOD with an exogenous reducing agent such as 2-mercaptoethanol results in drastic delay in the conversion from a precursor to a mature UMOD [38]. The formation of a correct set of interchain disulfide bonds is required for UMOD to exit the endoplasmic reticulum [38, 39]. The UMOD molecules with aberrant folding due to the missense mutations may aggregate for the problems in posttranslational processing in the endoplasmic reticulum [40, 41]. Several experimental and clinicopathologic evidences demonstrated a proinflammatory potential of aggregated UMOD/Tamm-Horsfall protein such as activation of neutrophils [42–44], stimulation of monocytes to proliferate and release cytokines and gelatinases [45, 46], and induction of humoral and cellular immune responses [47]. Experimental inductions of autoimmune tubulointerstitial nephritis by immunization with Tamm-Horsfall protein were reported [48, 49]. These pro-inflammatory potentials of UMOD/Tamm-Hosfall protein may relate the tubulointerstitial nephritis in FJHN.

Studies of glycoprotein-2 (GP-2) suggest the dominant-negative effect of the mutant UMOD. GP-2 is a 78 kD membrane glycoprotein and the major component of zymogen granule membranes of the exocrine pancreas. GP-2 and UMOD define a new gene family based on the structural similarity and other common characteristics, including the GPI linkage, release from the apical membrane of cells, and large aggregate formation in solution after release from membrane [50–52]. The C-terminal regions of GP-2 (Asp54-Phe530) and UMOD (Asp175-His644) from rat show 53% identity, 86% similarity, and 26 conserved cysteine residues, including one epidermal growth factor motif [50, 51]. All of the mutations of the *UMOD* gene, which was located within the homologous region to GP-2 occurred on conserved amino acid of both proteins, namely, Cys195Phe, Trp202Ser, and Pro236Leu in the present study as well as Cys217Arg [20], Cys255Tyr [21], and Cys300Gly [21] in the previous reports. These six residues are also conserved in GP-2 of human, dog, mouse, and rat. As for the deletion mutation of nine amino acids (HRTLDEYWR) [20], two residues (L and R) are conserved in GP-2 of the four species. This evolutional conservation of these residues suggests their important roles for homologous functions of UMOD and GP-2. Both UMOD/Tamm-Horsfall protein and GP-2 showed pH- and ion-induced self-association mediated by hydrophobic interactions following pH-induced conformational changes [51, 53]. GPI anchors of both proteins may facilitate the self-association, because diffusion coefficients for GPI-anchored membrane proteins were about 10-fold higher than values for peptide anchored membrane proteins [51]. Based on these, Scheele, Fukuoka, and Freedman [52] claimed that the self-association of the GPI-linked forms of UMOD on the cisternal leaflet of trans-Golgi membranes enable the sorting of Na-K-2 Cl cotransporter to the luminal surface. The polymeric form of UMOD and GP-2 may function to maintain the patency of tubular lumen, and prevent its collapse by forming gel [54, 55]. The mutations of UMOD are likely to change these conformational properties and interfere self-association.

Renal urate transport is complex and not clearly understood [56]. Urate is freely filtered at glomeruli, and then nearly all urate is reabsorbed before the distal convoluted tubule, with the majority of urinary urate derived from secretion. Both secretion and postsecretory reabsorption are supposed to occur in the proximal tubule. Based on the parallel location of UMOD and Na-K-2 Cl cotransport system in epithelial cells of thick ascending loop of Henle (TALH) and the early distal convoluted tubule, UMOD may be playing a role for the extremely low water permeability that is necessary to maintain the countercurrent multiplier system [57–61]. The UMOD mutations may cause a defect in the impermeability of TALH, which will result in influx of water from tubular lumen to the medullary interstitium and lowering the urinary concentrating ability by reducing medullary tonicity. The mutations may diminish the number of Na-K-2 Cl cotransporter on the luminal epithelial membrane of TALH due to failure of the sorting mechanism. This condition is similar to that seen following the chronic administration of loop diuretics or osmotic diuretics [62], associated with hyperuricemia due to depletion of extracellular volume, a diminished glomerular filtration rate (GFR), and increased reabsorption of urate in the proximal tubule [62–64]. Similar mechanisms were supposed to be responsible for hyperuricemia in patients with MCKD2 or FJHN

[20]. The mutations of UMOD, however, may diminish GFR without depleting extracellular volume by activating tubuloglomerular feedback, because patients with FJHN are usually normotensive to hypertensive even in those without marked renal insufficiency [2, 4].

CONCLUSION

The present study confirmed the mutation of the *UMOD* gene in the majority of the examined Japanese families with FJHN. Genetic testing is necessary in some members of the families for definite diagnosis of affection status especially in the young generation. Discovery of the *UMOD* gene mutation as a cause of FJHN is the first genetic abnormality found as a cause for hyperuricemia of an underexcretion type. Studies of pathogenesis of FJHN associated with mutations of the *UMOD* gene should help understanding the mechanism of urate transport in the kidney and finding therapy for chronic progressive renal failure in FJHN.

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NOTE ADDED IN PROOF

During the proofreading process of this manuscript, Dahan et al [65] and Rampoldi et al [66] reported the clustering of mutations in exon 4 of the *UMOD* gene and the intracellular accumulation of uromodulin in tubular epithelia in patients with FJHN.

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