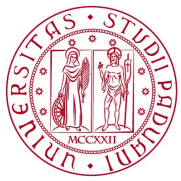

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SCUOLA DI DOTTORATO DI RICERCA IN SCIENZE CLINICHE E SPERIMENTALI
INDIRIZZO SCIENZE NEFROLOGICHE
CICLO XXII

ALTERATIONS OF UROMODULIN BIOLOGY AND FJHN/MCKD SYNDROME

Direttore della Scuola: Ch.mo Prof. Antonio Tiengo
Coordinatore d'indirizzo: Ch.mo Prof. Angela D'Angelo
Supervisore: Ch.mo Prof. Franca Anglani

Dottorando: Dalia Dali

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TAMM-HORSFALL PROTEIN (UROMODULIN)

Tamm-Horsfall glycoprotein (THP) is the most abundant protein in normal human urine. It was first described as "urinary mucoprotein" by Morner 1895 (43, 44). Then 1950 Igor Tamm and Frank Horsfall isolated a mucoprotein from the human urine by precipitation with sodium chloride, and showed that the protein was able to interact and inhibit viral haemagglutination (18,74,77, 79)

The 2 investigators sought to obtain evidence of the putative enzymatic activity associated with viruses and identify inhibitors preventing viruses from binding to susceptible cells.(74, 79).

This observation persuaded Gottschalk (28) and Odin (55) to analyze the carbohydrate moiety of urinary mucoprotein. Both studies established that carbohydrate content accounts for more than 20% to 25% of mucoprotein weight, and sialic acid is abundantly present. Urinary mucoprotein has since become known as Tamm-Horsfall glycoprotein or Tamm-Horsfall protein (THP)(18,74)

In 1985, Muchmore and Decker identified a 85kDa glycoprotein in the urine of pregnant women. The protein was named Uromodulin, due to its potent immunosuppressive activity reflecting its ability to inhibit antigen-induced T-cell proliferation and monocyte cytotoxicity in vitro . Besides the molecular mass and the abundance in urine, the characterization of Uromodulin revealed a number of resemblances with THP, including a 30% carbohydrate content, a tendency to form aggregates and a significant number of intrachain disulfide bridges (18,54) Based on sequence analysis, Pennica et al. later confirmed that Uromodulin was indeed THP. (59)

In consequence, the terms THP and UM or (THP/UM) was used interchangeably for this glycoprotein. However, it was reported that they differ functionally in that UM is significantly more immunosuppressive in vitro than THP. This was reflected particularly by higher binding of UM to cytokines, it was assumed that is the high mannose type of the carbohydrate chains.(73)

In 1998 Olczak.T et.al examined the carbohydrate parte of glycoproteins isolated from urines of two groups of female donors: healthy, non-pregnant (designed as THP) and pregnant women (UM)(56), these studies have shown that there are no significant differences in the carbohydrate content between THP and UM, and also in their reactivity with specific lectins, indicating similarity of their carbohydrate chains. These results together with previous results based on amino acid analysis(59) indicate that Tamm-Horsfall protein and Uromodulin are identical or very similar glycoproteins.

In 2001 Van Rooijen et.al studied Uromodulin which was isolated from monthly collected urine of three pregnant women. They fuond that that no significant changes in the negative charge distribution stemming from sialic acid and sulfate residues occur; it suggests that the branching patterns of the complex-type carbohydrate chains remain fairly constant. The composition of the oligomannose-type glycans does not undergo significant alterations during pregnancy.
However, comparison of uromodulin samples from pregnant and non-pregnant periods of each donor indicated small shifts in the molar ratio of the various oligomannose-type glycans.(82)

Uromodulin structure

Uromodulin is a large glycoprotein of about 105 kDa (70), that is exclusively expressed in the thick ascending limb (TAL) of Henle's loop and the early distal convoluted tubule (DCT) of the kidney. It is a glycosylphosphatidylinositol (GPI)-anchored protein (at the position 614) mainly localized at the apical plasma membrane of epithelial tubular cells(70) and basolateral poles of the cell membrane(51) from which it is released into the tubular lumen through a proteolytic cleavage by a yet to be identified protease.(70,86) It is excreted in urine at a rate of 50 - 100 mg/day (14), up to 200 mg daily under physiological conditions. Moreover, significant serum levels in healthy individuals have been described, ranging from 70 to 540 ng/ml (87)
Uromodulin is a 640 amino acid protein with a 24 amino acid signal peptide at the N-terminus, 48 cysteines that form 24 potential intra-chain disulfide bridges and are essential to establish the correct structure (5), and 8 potential N-linked glycosylation sites (as Y in the figure), of which 7 appear to be occupied .

Sequence homology indicates that Uromodulin is likely to contain three epidermal growth factor (EGF)-like domains, of which the second and third contain a calciumbinding (cb) motif ; a domain of eight cysteines (D8C) within a cysteine-rich region; and a zona pellucida (ZP) domain which is responsible for the polymerization of extracellular proteins into helical filaments(18,74,86)

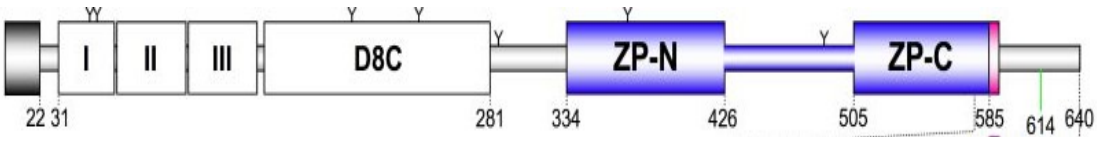

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Fig (1): Uromodulin protein structure.(53)

In its native form, THP exists in a polymeric form which can be dissociated into monomeric subunits of about 85 kD .The main part of the molecule is a polypeptide (about 70% of molecular weight) which is identical in different species . This glycoprotein contains a great amount of acidic amino acids which explains its low isoelectric point . About 30% of the THP/uromodulin molecule consists of carbohydrates, comprising at least five N-glycosidically bound sugar chains. Human THP contains large amounts of sialic (neuraminic) acid (about 5% of total molecular weight (18,43,47,74)

Electron microscopy (EM) studies on purified urinary uromodulin showed that it forms a three-dimensional matrixwith pores. This matrix is formed by long filaments having a width of about 100 Å and measuring 2,500 to 40,000 Å in length, with an average of 25,000 Å. High-resolution EM imaging shows fibrils with frequent occurrence of “zig-zag” configuration suggestive of a three-dimensional double helical structure.(70)

Tendency to Gelation/Aggregation

One of the most peculiar features of THP in solution is its tendency to gelation/aggregation when sodium chloride concentration is close to 100 mmol/L or calcium chloride is 1 mmol/ L.(6,81) Both conditions usually occur in normal urine, and a method based on this property has been set up to purify THP(74)Due to its ability to assemble into a meshwork of fibers forming a gel-like structure, uromodulin

has been hypothesized to have also a role in water/salt balance in the TAL and DCT(70)

Polymerization

Uromodulin belongs to the family of the ZP domain proteins. These proteins perform highly diverse functions but share the ability to extracellularly polymerize into filaments and matrices via their ZP domain (18,70,74)

Schaeffer et al. have shown the presence of two hydrophobic patches involved in the regulation of ZP domain-mediated polymerization:

An **IHP** is defined as a short stretch of seven amino acids, containing hydrophobic residues, adjacent to the end of the ZP-N subdomain , and an **EHP** which is a short stretch of seven amino acids localized in the C-terminal part between the cleavage site and the membrane-anchoring site(70)

It has been proposed that the functional relationship between EHP and IHP motifs could underlie a direct interaction of the two modules during the trafficking of the protein to the plasma membrane to keep the ZP domain in an inactive conformation that does not allow polymerization. Once the protein has reached the plasma membrane where it is cleaved, the interaction would be lost, leading to an active conformation allowing protein assembly.

In the case of a ZP domain protein mutated in either IHP or EHP motif, it's possibile to see premature polymerization of and lacking membrane anchoring (70).Therefore the presence of intracellular filaments rather than protein secretion should be considered as a more specific sign of polymerization dysfunction.(70)

A possible role for Uromodulin in renal development:

One study has been identifid a novel mutation of UMOD gene in a 13-year-old boy, which is clenicaly affected by glomerulocystic kidney disease (GCKD), the renal biopsy of this shown histological picture at the corticomedullary level of immature structures . PAX2- and vimentin-positive immunostaining confirmed the immature and developmental character of these elements. Vimentin is the universal marker of mesenchymal cells and PAX2 is a developmental renal protein expressed in nephrogenesis in both the ductal and mesenchymal component.

These findings suggest a possible role for UMOD in renal development.(5)

Uromodulin and kidney stones:

THP is a potent inhibitor of CaOx crystal aggregation and reduced urinary excretion of THP by stone formers has been reported (42) as the study of Glauser et al. Which fuond that Uric acid and calcium stone formers predict reduced THP excretion in comparison with healthy subjects, whereas female gender goes along with increased urinary THP excretion in healthy subjects. Possibly most relevant to kidney stone formation is the fact that THP excretion rises only in healthy subjects in response to increasing urinary calcium and oxalate concentrations, whereas this self-protective mechanism appears to be missing in stones formeres.(26),therefore THP can be used as a reliable biomarker for screening of kidney stone disease, irrespective of stone types .(47)

Another studies have been found that 14,3%, 16% of mice lacking THP spontaneously form interstitial deposits of calcium phosphate within the renal papillae (52,53). This percentage rised to 76% with calcium overload.

Research to date suggests that THP plays a dual role in modifying crystal aggregation both in vivo and in vitro. In solutions with high pH, low ionic strength and low concentrations of calcium and THP, this glycoprotein acts as a powerful inhibitor of CaOx crystal aggregation. Conversely, the combination of low pH, high ionic strength and high concentrations of calcium and THP causes the aggregation of the monomeric THP molecule, which lowers its inhibitory activity against CaOx crystal nucleation and aggregation. (37, 77)

THP under normal conditions may sequester calcium and thereby inhibit its interaction with oxalate. That can be explained by the presence of a normal content of sialic acid on the protein's outer coat, which gives a high negative charge to the protein, However, a positively charged calcium ion gains entry to the calcium-binding hydrophobic domains embedded in the protein's interior structure stabilized by the S–S bridges evidenced by the –SH content of the protein.(77)
A loss of several S–S bridges by oxidation, could cause alterations in the secondary structure of the protein. These alterations lead a reduction of inhibitory properties in a

crystallizing medium, and depletion of sialic acid. The normalization of the kinetic properties of THP following vitamin E supplementation (as antioxidant) supports these hypothesis.(35,77)

Although the reduced urinary excretion of THP by stone formers has been reported, conversely Jaggi et al. demonstrated that severely recurrent calcium stone formers with a positive family history excrete more THP than healthy controls, and their THP molecules are structurally different, contain more sialic acid SA, and they are weaker inhibitors of calcium oxalate te crystal aggregation than normal THPs(37) and they can promote crystal aggregation (42)

Uromodulin as antioxidant

Free radicals can produce cellular injury through lipid peroxidation of mitochondrial, lysosomal and plasma membranes, such injury could alter membrane function and structure. THP could bind the cell membrane of proximal tubules of the kidney through sialic acid. The binding to the cell membrane is important for the protection of epithelial cells against various potential damage. Therefore, enzymatic removal of sialic acid from THP could alter this protective effect and decrease the binding activity of THP to the cell membrane, that's lead to think that THP could be inhibitors of calcium oxalate stone formation due to their antioxidant ability (14)

Tamm-Horsfall protein protects the kidney from ischemic injury

THP is known to aggregate in highly tonic media, and is invariably found in casts formed during acute kidney injury (AKI). Therefore, it has been suggested that THP plays a crucial role in tubular cast formation and obstruction in AKI (20,29,75).

TLR4 is a member of the large Toll-like family of innate immune receptors, Indeed, these trans-membrane proteins are the primary mediators of the interaction between pathogens and cells of the innate immune system (21) TLR4 was first recognized as the endotoxin receptor that mediates the inflammatory response in gram-negative sepsis.

Its presence was documented in various segments of the tubular epithelium, recently it was demonstrated that a TLR4-dependent cyclooxygenase (Cox)-2 upregulation

was specific to THP-expressing thick ascending limbs (TAL) during sepsis . Furthermore, renal TLR4 was shown to play a detrimental role in renal ischemia-reperfusion injury (IRI) by promoting inflammation and apoptosis, therefore mice deficient in TLR4 had significantly less injury and inflammation after ischemia compared with a wild-type strain(91)

El-Achkar et al. studied the protective effects of THP in IRI and they found that its absence resulted in more severe inflammation, increased cast formation (that cast formation is an injury-driven event and does not depend on the presence of THP), and worse renal function. The absence of THP increased the expression of TLR4, and shifted the proximal tubular distribution of this receptor from the apical side to the cellular and basolateral compartments. (20)

The mechanisms by which THP exerts its protective function are likely very complex but potentially involve enhanced expression of TLR4 and its targeting to the apical membrane of S3 segments of the proximal tubular. This apical location of TLR4 could minimize injury by decreasing the interaction of TLR4 with proinflammatory interstitial ligands released after ischemia.(20)

Uromodulin and urinary tract infection

Tamm-Horsfall glycoprotein (THP), the most abundant protein in mammalian urine, has been implicated in defending the urinary tract against bacterial infections.(13, 57,61, 62, 68,74)

The studies of THP –/– mouse have been shown:

1- a greater bacterial load in the urine of the THP –/– mouse than in the urine of the THP +/+ mouse suggesting that the bladder colonization with uropathogenisis was more intense in the THP –/– mouse.

2- The bladders from THP –/– mice show an increased wall thickness with bacterial species.

3- The intensity of infl ammation was more prominent in the THP –/– mice.(61,62)

4- The kidneys of THP_/_ mice showed more frequent discoloration and abscess formation, and more intense pyelonephritis. (62)

.

THP acts by two principle nonmutually exclusive mechanisms:

- The capture of potentially dangerous microbes.
- The ability of this glycoprotein to induce robust protective immune responses against uropathogenic bacteria.(68)

The capture role of THP

This role was studied against common ueopathogenisis such as Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae and Staphylococcus saprophyticus.

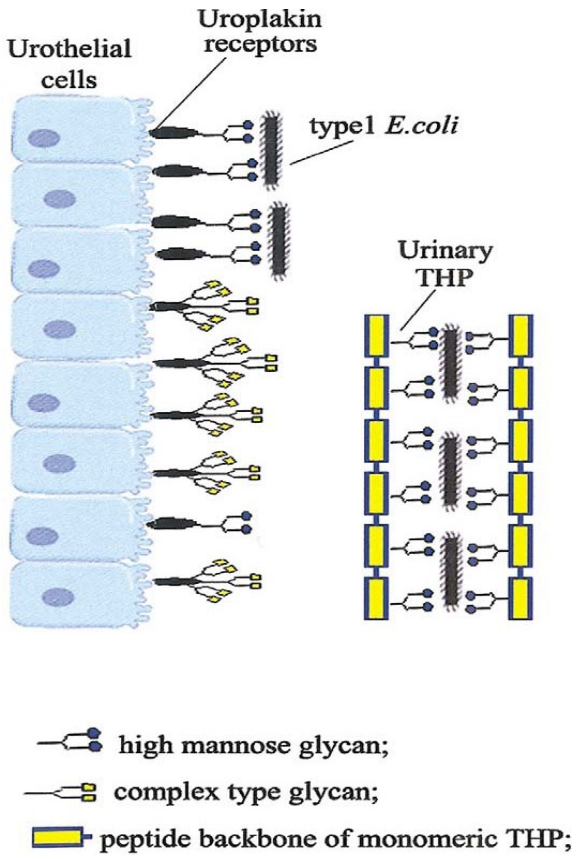


Fig (2) Binding of polymeric THP to type 1 Ecoli, mediated by high-mannose glycans. Uroplakins are the major integral-membrane glycoproteins exposed at the luminal face of the bladder and urothelial tract.(74)

The adhesion process between E.coli and their Uroplakin receptors on the surface of urothelial cells, frequently requires filamentous surface appendages of uropathogenic E. coli that are called fimbriae, or pili. Epidemiological studies have shown that .90% of all *E. coli* isolates from UTI patients elaborate type 1 fimbriae.(57)

Recent experimental evidence indicates that the defensive capability of THP relies on its single high mannose chain, which binds to E. coli FimH lectin and competes with mannosylated uroplakin receptors on the bladder surface.(13,57,68)

This mechanism can explain the capture role of THP against Proteus mirabilis, and Klebsiella pneumoniae which can adher to the urothelial cells is mediated by pili (fi mbriae) on the bacterial surface. Particolary type 1 pili which is located on the fimbrial shaft and is capable of binding to mannose residues, not only on the cell surface glycoproteins, but also in urinary glycoproteins, such as THP.(61,62)

Another Studies of carbohydrate structures on THP have demonstrated the presence of GalNac on the terminal end of some of the carbohydrate chains of THP. It is possible that the oligosaccharide GalNac in the THP molecule may play a role in inhibiting adherence of Staphylococcus saprophyticus to the bladder epithelium. Wheras mannose had no effect.(61)

Immune response mediated by THP against uropathogenic bacteria.

Presumably, healthy mammals do not raise antibodies against THP, as the exclusive localization of THP at the luminal surface of tubular epithelial cells shed the protein from the antibody-producing

machinery. This shedding might be abolished when the localization of THP is altered in cases of luminal/ basolateral ..

Healthy mammals do not raise Abs against THP because the exclusive localization of THP at the luminal surface of tubular epithelial cells keeps the protein from the Ab producing diseases by loss of cell integrity or of luminal/basolateral tubular come from its ability to immediately activate innate immune responses, also recruiting components of the adaptive arm of the immune system. THP could thereby provide a critical danger signal to prevent host invasion of potentially harmful bacteria in case of local injury or increased epithelial permeability.(18,69)

It was demonstrated that the abundant urinary glycoprotein THP is a strong activator of professional APCs, including DCs. Its immunostimulatory potential and its ability to induce an anti-THP Ab response depends critically on TLR4 and its subsequent signaling pathway.(27,69)

The contribution of THP towards adherence of Pseudomonas aeruginosa to uroepithelial cells and murine peritoneal macrophages was studied.(30).

In vitro it was observed decreased adherence of THP-coated P. aeruginosa to uroepithelial cells and phagocytes. In vivo,THP-coated P. aeruginosa showed increased renal bacterial load and tissue pathology in a mouse model of acute ascending pyelonephritis. This study showed that THP may not necessarily act as a host defense component; rather, it may help in renal colonization of P. aeruginosa in vivo(30)

Uromodulin and cast nephropathy :

Cast nephropathy is a form of progressive renal failure that occurs in the setting of multiple myeloma. Although it was well known that immunoglobulin light chains (Bence Jones proteins) participate integrally in the process, dissecting the pathogenesis of this complex problem proved difficult. More recently, evidence demonstrated an important role of THP in cast nephropathy. The resultant aggregation of these proteins produced intraluminal obstruction from cast formation (67,92). Using immunofluorescence microscopy, THP has been shown to be a component of the casts.

THP, a protein that is synthesized by cells of the thick ascending limb of the loop of Henle, In an experimental model of cast nephropathy, this area of the nephron was also the initial site of cast formation.(92)

By altering production of THP, intranephronal obstruction from light chain precipitation was prevented. Sanders PW et,al. demostrated that Tamm-Horsfall glycoprotein from colchicine-treated rats did not contain sialic acid and did not aggregate with Bence Jones proteins in vitro, and intranephronal obstruction was aggravated by decreasing extracellular fluid volume or adding furosemide. Therefore, by decreasing secretion and altering the carbohydrate moiety of Tamm-Horsfall glycoprotein, colchicine prevented intraluminal cast formation and obstruction of the rat nephron.(67)

Subsequent analysis of the THP interaction with light chains demonstrated a single binding site on THP, the identification of the CDR3 region as the single binding site of light chains for THP provides new insights into the pathogenesis of cast

nephropathy related to multiple myeloma. Differences in the CDR3 region accounts for the variable affinity of light chains for THP, when this process is examined under controlled conditions. (67)

Uromodulin in the blood

The function of uromodulin in the blood is unclear. It has been demonstrated in several animal studies that elevated serum uromodulin causes a cellular immune response that is directed against uromodulin, resulting in tubulointerstitial nephritis . It was determined the plasma uromodulin concentration in healthy volunteers to be (19.88 5,97) ng/ml.

Because uromodulin is targeted specifically to the apical membrane, its presence in the blood may seem puzzling. However, an alternative minor pathway for GPI-anchored proteins has been proposed, whereby the carboxy-terminal is proteolyzed intracellularly without addition of the GPI anchor . The intracellularly truncated form of the protein subsequently is secreted from the cell *via* exocytosis. Western blots of renal tissue homogenates exhibit a band with the same mobility as urinary uromodulin. This provides evidence that a fraction of uromodulin is present intracellularly with a cleaved C-terminus without the addition of GPI anchor. This non–GPIanchored fraction represents a secondary pathway for uromodulin secretion and may represent the major pathway for basolateral secretion into the interstitial space.(38)

These results indicate that the mutations studied do not impair glycosyl-phosphatidylinositol-mediated apical targeting of the protein but do affect apical secretion. Because the mutant proteins are secreted as efficiently as wild type to the basolateral compartment, the possibility arises that interactions with the immune system at the site of secretion are a contributing factor to the development of tubulointerstitial nephritis in FJHN.(38)

THP and polymorphonuclear leukocytes

THP can potentialy modulate essential functions of polymorphonuclear leukocyte (PMNL).

THP in its soluble form at concentrations similar to serum levels found in healthy subjects (350 ng/ml) and in patients with impaired renal function (70 ng/ml) increase

ithe directed polymorphonuclear leukocyte (PMNL) migration,but a significant inhibition of directed PMNL migration is seen at higher THP levels resembling urinary concentrations (1.75 and 8.75 mg/ml); that means that low concentrations of THP may enhance neutrophil migration towards the focus of inflammation, while higher doses cause the cells to remain at the site of inflammation.(87)

Higher levels of THP resembling urinary concentrations were observed to inhibit PMNL apoptosis and to stimulate PMNL phagocytosis. Inhibition of apoptotic cell death and the increase in phagocytosis suggest an activating effect of THP on PMNL at these concentrations. The stimulating THP effect observed on PMNL phagocytosis at higher concentrations appeared to be similar to a response caused by bacterial lipopolysaccaride (LPS) (87). On the other hand, THP at lower concentrations equalling physiological and pathological serum levels appeared to increase PMNL chemotaxis, suggesting an activating effect as well. However, the effects on apoptosis at low THP concentrations were controversial and PMNL showed a decrease in apoptotic DNA cleavage while the morphological characteristics displayed an increase in early apoptotic features. This might be explained by the fact that morphological features such as cell shrinking and chromatin condensation might be visible at an earlier stage of apoptosis before significant DNA cleavage occurs.
Finally, PMNL phagocytosis was not influenced by lower THP concentrations.(87)

THP and the complement

THP binds complement1q(C1q) as demonstrated using both ELISA and biosensor techniques. At least a portion of this interaction involves electrostatic forces between the positively charged C1q and the negatively charged THP molecules, because the binding affinity was greater in low-salt buffers than it was in physiological- strength solutions. While THP and C1q may bind in vivo under low ionic-strength situations, because urine frequently is hypotonic, even under physiological ionic conditions the THP/C1q binding affinity was still significant.(65)

The intact C1 complex, binding of THP to C1 could activate the classical complement pathway and potentiate an inflammatory reaction.(66)

THP and lymphocytes

Tamm-Horsfall protein (THP) purified from pregnancy urine was found to stimulate normal human mononuclear cell (MNC) proliferation at a concentration greater than 10 micrograms/ml. This stimulation was non-specific because the percentage of B and T cell subpopulations including CD20, CD3, CD4, CD8 and CD4/CD8 ratio was not changed by THP. THP not only bound to human mononuclear cells but depolarized the membrane potential, increased 22Na+ uptake and enhanced the expression of IL-2R and HLA-class II antigens on these cells. The concentrations of sIL-2R, sCD4 and sCD8 in the THG-stimulated MNC culture supernatants were significantly increased compared with control supernatants (93).

These results suggest that urinary THG activates monocytes to synthesize large amount of monokines through its membrane effect. The released monokines subsequently stimulate lymphocytes expressing IL-2R and HLA-class II antigens and finally lead to cell proliferation.(93)

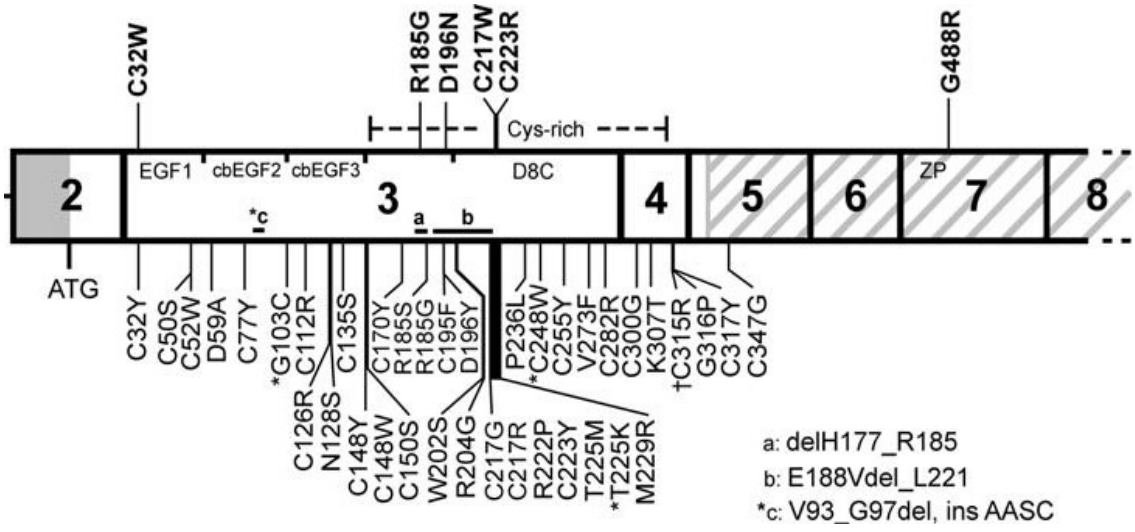
On the other hand THP stimulated human mononuclear cells to proliferate and secrete TNF-alpha.(76)

UMOD GENE

UMOD gene encodes Uromodulin, maps to 16p12(57), and comprises 12 exons(5)

UMOD gene mutations

Hart et al. (31) firs reported mutations of the UMOD gene (that encodes uromodulin) in FJHN as well as MCKD2 kindreds.



Human Molecular Genetics, 2009, Vol. 18, No. 16 (86)

Fig (3) Schematic representation of exons 2–8 of UMOD illustrating the locations of 46 mutations. The majority (.87%) of the mutations have been identified in FJHN patients, 10% in MCKD patients (*) and 2.5% in GCKD patients (†) Missense substitutions are indicated by single-letter amino acid codes, and three inframe deletions are indicated by horizontal lines a–c. Most of the mutations cluster in exons 3 and 4, which encode the three epidermal growth factor (EGF)-like domains, and the cysteine-rich region, which includes the domain of eight cysteines (D8C). The G488R mutation is the first to be described in exon 7, which encodes part of the zona pellucida (ZP) domain.

To date, more than fifty UMOD mutations have been reported in UAKD patients (6)

Most of the mutations cluster in exons 3 and 4 (86), that were subsequentely named exons 4 and 5 (11)

Mechanisms of injury of uromodulin alterations

The mutations in UMOD may critically affect the function and expression of uromodulin, resulting in abnormal accumulation of the mutated protein within tubular cells, resulting in increased apoptosis and tubular injury.

This conclusion was supported by Rampoldi et al. (63), who further analysed the trafficking defect of uromodulin in cultured cells. These experiments clearly established that UMOD mutations affecting cysteine residues cause a delayed export, and maturation (72) of mutant uromodulin to the plasma membrane, with a longer retention time in the ER probably reflecting an abnormal folding of the protein. (18,63,81).

Failure of correct folding triggers the so-called ER stress response, which involves 2 different pathways known as: unfolded protein response, and ER overload response, the latter involving nuclear factor-kB induction as mediator of an inflammatory response. Further investagation will be needed to asses whether cell stress by ER accumulation of unfolded mutant uromodulin may trigger the unfolded protein response and ER overload response and, eventually, programmed cell death. (72,81,83).

Vyle'al et al have studied Functional consequences of UMOD mutations, and they found that all the mutant proteins differed from the wild-type protein in their ability to reach the exoplasmic face of the plasma membrane. In these mutants, the associated pathogenic mechanism may thus be related to impaired intracellular trafficking, decreased ability of the protein to be properly internalized and exposed on the exoplasmic face of the plasma membrane, or defective assembly of UMOD filaments. In contrary, mutants lacking GPI cannot exit and remain retained within ER lumen (83).

In the same study; electron microscopy and immunohistochemical staining of available kidney tissues showed massive intracellular UMOD accumulation in the patients with UMOD mutation, and presence of UMOD in hyaline casts with low intracellular positivity, irregular pattern of UMOD staining or strongly reduced UMOD expression in patients with not yet established molecular defects. (34,83)

The absence of UMOD properly expressed on the plasma membrane might result from different molecular mechanisms such as reduced gene expression resulting from

a mutation of transcription factor as demonstrated in the case of the HNF1-b mutation or in hypothyroidism, from protein mistargeting, inability of the protein to be properly GPI-anchored, from gradual gene expression silencing resulting from aberrant developmental processes or from aberrant cellular differentiation or proliferation (83).

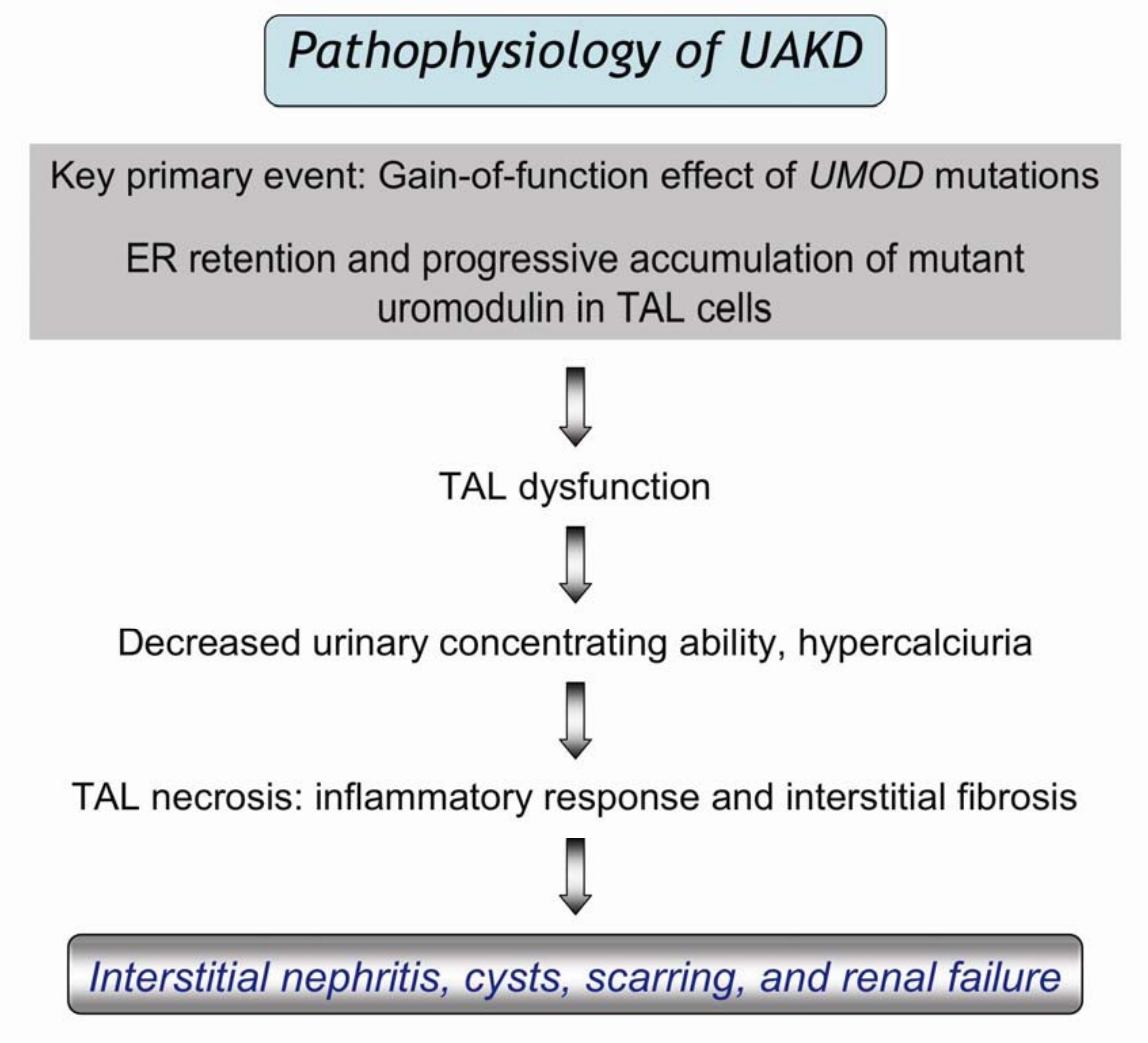
Takiue et al, produced transgenic (Tg) mice harboring the mutant human UMOD gene to demonstrate the mechanism of accumulation of uromodulin in the kidneys of FJHN/MCKD2 patients with UMOD gene mutations. They found that uromodulin accumulated in the kidneys of FJHN/MCKD2 patients with UMOD gene mutations is not the mutant type but the wild type because their studies have shown the progressive accumulation of uromodulin is not extrinsic human uromodulin but intrinsic mouse uromodulin in the kidneys of Tg mice that also harbors the mutant human UMOD gene. (78)

The reason may be associated with the unfolded protein response (UPR). The UPR is the cellular signaling pathway to ER stress that the disturbances of the correct protein folding by various conditions such as genetic mutation and oxidative stress lead to the accumulation of unfolded or misfolded proteins in the ER. The UPR includes the induction of molecular chaperones to promote protein folding, the translational attenuation to reduce new protein synthesis and the ER-associated degradation to facilitate misfolded protein degradation.(78)

Missense mutations of UMOD affecting cysteine residues are likely to alter disulfide bridges, cause misfolding of the global protein structure, and disrupt the correct folding of the protein.(63,72)

Similarly, mutations affecting the calcium binding affinity of the EGF domains will likely lead to structural destabilization or increased sensitivity to proteolytic degradation (18).

Figure (4) shows the proposed model of pathogenesis in uromodulin-associated kidney diseases (UAKD).



Fig(4): Proposed model of pathogenesis in uromodulin-associated kidney diseases.(6)

UMOD gene mutations are associated with 3 phenotypes:

1- ***Familial Juvenile Hyperuricemic Nephropathy (FJHN).***

2- Medullary Cystic Kidney Disease type 2 (MCKD2).

3- Glomerulocystic Kidney Disease (GCKD).

Because all three phenotypes can be caused by the same UMOD mutation, these three disorders (FJHN, MCKD2 and GCKD) have also been described as '**Uromodulin-associated kidney disease' (UAKD)**(88,94)

UROMODULIN-ASSOCIATED KIDNEY DISEASES (UAKD)

Scolari et al, have called these diseases as **Uromodulin Storage Diseases**, because their principal pathology is the storage of Uromodulin within the endoplasmatic reticulum (ER).(72).
Another recent study demonstrated that Uromodulin is expressed in renal primary cilia, and showed that the reduced ciliary UMOD staining is specific for UMOD mutations and cannot be found in kidney biopsies of patients with other tubulo-interstitial kidney disorders, therefore these diseases can belong to the group of diseases termed **ciliopathies**. (94).

These three disorders (FJHN, MCKD2 and GCKD) are autosomal dominant diseases.

***Manifestations*:**(72,88,94)
The cardinal feature of FJHN/MCKD2 diseases is chronic, progressive renal failure that is tubulointerstitial in origin. Hyperuricaemia was found in the vast majority, but not all family members identified with this disorder. Similarly, medullary cysts are frequently not seen in radiological examinations of these patients. Given that hyperuricaemia is not always present in FJHN and given that medullary cysts are not always present in MCKD2, and given that these two conditions result from mutations of the same gene,we believe it is appropriate to designate these two conditions as uromodulin associated kidney disease.(31,72).As the molecular pathogenesis is the same for FJHN and MCKD2, it seems questionable, whether a further differentiation between FJHN and MCKD2 is meaningful.(88).Tht some authors confremed that FJHN and MCKD2 are tow facets of the same disease.(17).

The table (1) shows the characteristics of (FJHN) and (MCKD2)The phenotype showed a variety of symptoms such as urinary concentration defect, vesicoureteral reflux, urinary tract infections, hyperuricemia, hypertension, proteinuria, and renal hypoplasia.(88)

Glomerulocystic Kidney Disease (GCKD)
It has been described as sporadic or familial (autosomal dominant). Clinical features include hyperuremia, impairment of urine-concentrating ability, and progression to end stage renal disease.(63)

Glomerular cysts are defined as Bowman space dilatation greater than 2 to 3 times normal size, the term glomerulocystic kidney is justified when at least 5% of glomeruli are cystic (48)

	FJHN	**MCKD2**
Age at presentation (years)	Childhood, adolescence, early adult life	Early to later adult life (about 32 years)
Age at ESRD (years)	20–40	
Symptoms	Hyperuricemia, sometimes gout, renal hypoexcretion of urate (FEurato12% in children), hypertension inconsistently, absent or minimal proteinuria	Polyuria, salt loss, hyperuricemia, sometimes gout, No data about FEurat in MCKD2, hypertension, no data about proteinuria in MCKD2
Ultrasound	Echogenic kidneys	Small, echogenic kidneys, often with small medullary cysts, but not necessary for diagnosis
Histopathology	Tubular atrophy, fibrosis, interstitial cell infiltration, segmentally sclerosed glomeruli, thickening of the basement membrane	Like in FJHN plus microcysts and tubular dilation

Table (1) Comparison of the characteristics of (FJHN) and (MCKD2)

Homozygous UMOD mutations

Rezende-Lima et al,(64) described a family with 3 individuals who carry a homozygous missense change in uromodulin (C255Y9).These data suggest:

1- C255Y homozygosity is associated with more severe phenotypes in terms of earlier onset age of hyperuricemia, starting age of renal impairment, and progression to ESRD.

2- one heterozygous patient compared with a normal control patient, both with normal renal function i s not a lethal condition, human embryos are viable, and three

affected individuals were able to live until an adult age. This situation is completely different than other cystic kidney diseases, such as autosomal dominant polycystic kidney disease type 1 and type 2.

3- Homozygotes did not have higher serum uric acid levels than heterozygotes suggesting that uromodulin does not act physiologically like a urate transporter.

4- The role of uromodulin in human kidney development is different from other products involved in the pathogenesis of cystic diseases, like polycystin 1 or polycystin 2.

5- The three homozygous patients had two mutated MCKD2 alleles in each renal tubular cell since their conception, and even so they did form just a small number of cysts, and only when they were in an advanced situation of renal failure.

6- Lower levels of urine THP in one heterozygous patient compared with a normal control patient, both with normal renal function.

Atypical familial juvenile hyperuricemic nephropathy associated with a hepatocyte nuclear factor-1 B gene mutation:

UMOD transcription is activated by the transcription factor hepatocyte nuclear factor-1B (HNF1B), it's gene locus is on chromosome 17cen–q21.3.

Mutations in HNF1B gene cause a phenotype similar to FJHN/ GCKD but also congenital anomalies of the kidney and the urinary tract (CAKUT), and early onset diabetes (7, 89)

The HNF-1 knockout mouse has evidence of renal proximal tubular dysfunction with a renal Fanconi syndrome with polyuria, aminoaciduria, phosphaturia, and glucosuria (7)

Bingham et al, studied 3 families which were clinicaly diagnosticated as FJHN hyperuricemia, reduced FEur, evidence of renal damage, and three members with young-onset gout. It was showed that one family of these 3 families had 7 members with HNF1B gene mutations.(7)

These finding suggest that HNF-1 mutations are a minor cause of FJHN.

Two subjects in this family had pelviureteric junction obstruction and required renal pyeloplasties. The pelviureteric junction region derives from the ureteric bud in the developing human embryo and this has been shown to be a site of HNF-1 expression.(7).

Of mouse and man: important notes:

1-The increased susceptibility to urinary tract infection (UTI) and renal stone formation observed in uromodulin KO mice is unlikely to be extrapolated to the patients harbouring UMOD mutations, because KO mice have no uromodulin excretion in the urine , whereas some wild-type protein is detected in the urine of patients with UMOD mutations. This small amount of bioactive, residual protein may be sufficient to prevent UTI or renal stone formation (18), although Wolf et al. have demostrated a case of female 24 years old, affected by FJHN, and has UMOD C744G mutation, which after a correct surgery for vesico-ureteral reflux, continued to suffer from recurrente urinary tract infections (88).

2-The uromodulin KO mice lack any structural abnormality in the kidney, whereas patients with UMOD mutations have an interstitial nephritis with focal tubular atrophy, occasional lymphocytic infiltration, thickening and splitting of tubular basement membranes, and cortico-medullary cysts The intracellular accumulation of mutated uromodulin may actually trigger an inflammatory response and a pro-fibrotic cascade leading to tubulo-interestitial fibrosis. The link with (non-constant) cystic changes is unclear: one hypothesis is that they could arise from tubule swelling due to luminal obstruction.(18)

3- Tg*Umod*C147W mice (Tg: transgenic) represent a clinical and pathological model of human UAKD and are a unique tool to further dissect the pathophysiology of renal damage associated to uromodulin mutations. (6)

4- In Tg*Umod*C147W mice we can found an absence or only moderate occurrence of hyperuricemia may be due to preserved uricase activity in mice that catalyses the conversion of uric acid to allantoin , which is absent in humans (41, 6)

Why hyperuricaemia?

Hyperuricaemia is the result of decreased urinary excretion of urate, theoretically, the decreased fractional excretion of urate could be due to 1) reduced filtration of urate, 2) enhanced reabsorption of urate, or 3) decreased secretion of urate. (33)

Hisatome et al, showed that tubular urate secretion was selectively reduced, while the presecretory reabsorption of urate remained normal but the postsecretory reabsorption of urate was slightly decreased. This reduction of the postsecretory reabsorption could be the result of decreases in the tubular secretion (33)

THP-Uric Acid binding:

Generally, it is believed that uric acid transport is complete by the end of the proximal tubule. However, there is some older evidence supporting the concept of distal uric acid transport . As mutations in THP produce a phenotype that frequently has a defect in uric acid excretion, it is tempting to hypothesize that that THP could bind to uric acid and that this may prevent distal urate transport.

Nevertheless, Gersch et al, used four independent methods to test the hypothesis that THP might bind to uric acid under physiological conditions. While each of the methods employed has some limitations, the combined results of these four experimental methods provide good evidence against the hypothesis that THP can bind to uric acid under physiological conditions (25).

Hyperuricaemia as a result of renal compensation for renal salt wasting:

The TAL plays an important role in the kidney's capacity to concentrate urine because Na reabsorbed in the TAL is critical for maintaining the countercurrent exchange mechanism by generating a hypertonic medullary interstitium; therefore, loss of Na-K-2Cl co-transport in the TAL abolishes the medullary concentration gradient, resulting in an inability to concentrate urine even in the presence of vasopressin., suggesting that these patients have lost their medullary concentration gradient secondary to failure of TAL Na transport (75) as uromodulin is produced in the thick ascending limb of the loop of Henle, and the relationships of THP with ion transporters involved in the function of the TAL, chiefly with NKCC2,(uromodulin is colocalized with (NKCC2) have been discussed in earlier studies (6,41,72), It is likely that uromodulin is important in maintaining the countercurrent gradient and the medullary loop impermeability, therefore, low urine osmolarity is an almost constant finding in patients with UMOD mutations (72).

The high rate of enuresis seen in the patients affected by UMOD mutations suggests that difficulties in urinary concentration are present. This decreased concentrating

ability probably results in increased urinary salt and water excretion, resulting in increased proximal tubular reabsorption of Na which is known to promote reabsorbtion of uric acid (The transport mechanisms of urate are localized in the PT) (18). Previous studies have documented increased proximal tubular uric acid uptake in patients taking loop diuretics and it is likely that a similar mechanism is responsible for hyperuricaemia in this group of patients.(3,6,18,31,41,80)

Interestingly, the presence of structural changes and kidney damage similar to the human condition in Tg*Umod*C147W mice suggest that hyperuricemia *per se* does not have a causal role in UAKD. (6).

Other ion changes

TAL of Henle loop cells express numerous ion transporters, such as NKCC2, ROMK, Na / H exchanger (NHE3), KCC4, and ClC-Kb.

Thus the ion transporter NKCC2 is essential for the passive paracellular reabsorption of Calcium and Magnesium . If NKCC2-mediated ion transport is impaired, this results in **hypercalciuria** and **hypermagnesiuria**, which was observed in homozygous *Umod*A227T mutant mice and might also be existent in humans with *UMOD* mutations (41). Therefore osteopenic phenotype of homozygous *Umod*A227T mutant mice is probably a long-term consequence of hypercalciuria. (41)

Another, there is an increased or fairly unchanged daily urea excretion and reduced fractional excretion of urea may be indicative of a urea-selective urinary concentrating defect in homozygous mutant animals. Renal urea transport is mediated by several urea transporters located at the thin descending limb or collecting duct of the nephron. As uromodulin is exclusively expressed in TALH cells, the *Umod* mutation may influence expression or activity of urea transporters indirectly as a consequence of TALH dysfunction. (41)

Why tubulointerstitial fibrosis?

Tubulointerstitial fibrosis is constituted mainly by deposition of type I and III interstitial collagens.

It should arise from a complex series of events culminating in renal infiltration by non-resident macrophages, cytokine production, and stimulation of collagen synthesis by tubular epithelial cells and fibroblasts.(72)
These events may be due to direct effect of uromodulin (27,69,72,74.87,93)

Another mechanism of renal fibrosis could be the consequence of inflammatory and immune responses mediated by upregulation of nuclear factor kB, which is a key step in the ER overload response pathway.(72)

Why Cystogenesis?

Cystogenesis in patients with MCKD2 and GCKD should follow different mechanisms. The small cysts described in patients with MCKD2 could arise from tubule dilatation, possibly caused by water income, which would be expected in the absence of uromodulin water barrier in the TAL.
In patients with GCKD it was hypothesized a mechanism of dilatation of Bowman's space linked to tubular obstruction that would determine urine reflux and in some way mimic an obstructive condition. (72, 63)

Diagnosis:

UMOD-related kidney disease is defined by:

1-The presence of a mutation in UMOD.

2-Increased Tamm-Horsfall protein (THP) immunostaining on renal biopsy.

3- Decreased uromodullin urinary excretion. (50)

Decreased uromodullin urinary excretion

Anthony et al, studied uromodullin urinary excretion in a family with UMOD mutation, they have found that all individuals with *UMOD* mutations had low THP excretion, irrespective of gender, glomerular filtration rate (GFR), or age.

The urinary excretion of THP was low, even at the earliest ages before developing chronic renal failure. Therefore tests of urinary THP excretion may provide a noninvasive way to clinically evaluate young children in families with known *UMOD* mutations (8.) Chemical analysis showed the presencs of only wild-type uromodulin in urine from patients with FJHN.(72)

Urinary THP excretion has been evaluated by either 24-hour excretion or in relation to creatinine excretion . Urinary THP decreases with declining glomerular filtration rate (GFR)**,** however Decreased THP excretion appears to be a general feature of chronic renal failure, but it was more decreased in patients with *UMOD* mutations when compared to THP of patients with other causes of renal failure.(8)

Other studies demonstrated undetectable urinary uromodulin levels, in a patient with UMOD homozygous mutation with renal insufficiency (64). Vyiet'al et al studied 19 families affected by FJHN/MCKD2 , they showed that UMOD excretion was absent or reduced in all families except one. Decreased UMOD excretion was present also in young patients with relatively preserved renal function . UMOD excretion normalized in a single patient with the UMOD mutation after kidney transplantation .(83)

Patients with FJHN who do not have UMOD mutations still show decreased THP excretion in urine, which suggests involvement of a protein involved in THP processing. (45)

Decreased fractional excretion of urinary uric acid

(usually <5%) is common in UMOD-related kidney disease .
Normal values: children 18.4+5.1%, men 8.1+3.2%, women 12.8+2.9%. (50)

The exact source of the very low FEur in FJHN is not yet clear, but superactivity or deficiency in one or other of the luminal anion exchangers and/or the luminal or basolateral voltage-dependent pathways of urate transport could result in a net decrease in FEur (23).
The reduction of urate excretion is an early event since it can be detected in affected children with preserved renal function . The fractional excretion of urinary uric acid (FEur) can be calculated as: (urine uric acid concentration x serum creatinine concentration) / (serum uric acid concentration x urine creatinine concentration).
Note: (1) The fractional excretion of urinary uric acid can be measured from a spot urine sample; however, a 24-hour urine collection is preferable. (2) Aspirin, diuretics, and nonsteroidal agents should be avoided during the collection. (3) Because the fractional excretion of uric acid rises above 5% as renal function worsens, when the creatinine clearance decreases below 80 ml/min, the fractional urate excretion

increases in any event (81). This test is not sensitive in individuals with UMOD-related kidney disease who have renal insufficiency (9).

Presymptomatic diagnosis

McBride et al, studied 34 apparently healthy children and 2 probands from kindred with familial juvenile hyperuricaemic nephropathy (FJHN). Hyperuricaemia associated with a grossly reduced FEur - the two biochemical hallmarks of FJHN – was found in approximately 50% of the children from these kindred, only 2 of whom were symptomatic. 42% of these hyperuricaemic children still had normal renal function.(50).Therefore measurement of FEur in children with either unexplained renal insufficiency or normal renal function, where there is a strong family history of renal disease, or gout, is also recommended.(50)

Urinary THP excretion may provide a noninvasive way to clinically evaluate young children in families with known *UMOD* mutations.(8)

Progression

The pathophysiologic changes leading to renal insufficiency do not take years to develop but are present in infancy. While the progression of kidney disease is slow, changes occur early in life.(71)

Schäffer et al, studied a family with UMOD mutation (father with his 3 children) The children in this family have a much faster rate of kidney disease progression than their affected father.

There is significant variation in the rate of progression of kidney disease both between families and within families with UMOD mutations and its cause is unclear (71)

It can be explained by:

1-Increased protein intake in succeeding generations could result in increased uric acid production , with subsequent deleterious effects on the kidney, in addition to the general negative effects of protein intake on kidney disease progression . However, this would seem unlikely to have a significant effect at very young ages, as seen in this family.

2- The presence of modifying genes that may slow the progression of kidney disease or decrease production of the abnormal uromodulin.(71)

Treatment:

Successful long-term prevention of renal damage in FJHN/MCKD requires therefore not only early diagnosis and treatment, but long-term patient compliance.(23)

Allopurinol

There is a significant debate as to whether allopurinol slows the progression of kidney disease.Schäffer et al demostrated that allopurinol was not found to prevent the progression of kidney disease (71)

Fairbanks et al. presented a long-term study in eight FJHN kindreds, now over 34 years,early allopurinol treatment when renal function is still normal, or only mildly reduced, is likely to result in continued stable renal function for a decade or more, or even improvement. Treatment started later, especially after renal damage has reduced function to half normal or less, is almost always accompanied by rapid progression to dialysis and transplantation or early death (23)

Uricosuric agents

The study of Fairbanks supported the suggestion that agents which increase FEur, such as **benzbromarone**, will help normalize the FEur by blunting urate reabsorption18 and may be of value in FJHN, together with allopurinol. (23)

Hisatome et al, showed that administration of allopurinol alone did not control the serum urate concentration. In contrast, combined treatment with **probenecid** with allopurinol decreased the serum urate concentration to a normal level, and thereafter the serum creatinine level decreased as well. However,at the stage of renal failure, combined treatment with uricosuric agents and allopurinol would be muchmore useful for controlling serum urate in FJHN compared with administration of allopurinol alone (33)

Colchicine

Let us take together the fact that UMOD mutations associated with MCKD-2 and FJHN result in the failure to process THP from the ER to the Golgi and secretion out

of the cell and the that THP retention in the ER seems to be associated with an increase in programmed cell death. Apoptosis of the TALH may account for the pathophysiology of progressive renal function seen in these diseases. Treatment of TALH cells that express mutant THP with either colchicine or 4-PBA reduced the accumulation of abnormal THP in the ER, facilitating its transport to the plasma membrane and out of the cell. This effect was associated with an increase in cell viability.

These findings suggest that treatment with colchicine or sodium 4-PBA may enhance the transportation of THP from the ER to the plasma membrane and secretion into the tubular lumen, reducing programmed cell death of the TALH. These chemical agents may provide therapeutic alternatives to prevent kidney damage in THP-associated kidney diseases.(15)

Renal transplantation (Tx)

Overall graft outcome does not differ from that currently recorded in other patients. The prevalence rate of reduced FEur and hyperuricaemia is similar to that commonly observed under anticalcineurin-based immunosuppressive regimens in patients transplanted for another nephropathy.(46)

The normalization of F E ur was not constant and not sustained in most patients is easily explained by several post-Tx factors lowering FEur, the two most common of which are the use of diuretics and that of anticalcineurins. Loop diuretics and thiazides are well known causes of reduced FEur and subsequent hyperuricaemia, through extracellular volume depletion and compensatory rise of proximal tubular reabsorption of urate, immunosuppressive regimens containing CSA also decreases the renal clearance of urate, with resulting hyperuricaemia, through a mechanism not completely understood.(46)

In other study it was found the disappearance of Tophi in a patient with Familial Juvenile Hyperuricemic Nephropathy after Kidney Transplantation, because the absence of the mutated gene in the transplanted organ probably led to normalization of the serum uric acid levels and the progressive disappearance of the tophi.

The risk of recurrence of interstitial nephritis in the transplanted kidney seems unlikely unless the transplant is from a living related donor, in which case genetic analysis of the donor should be performed.(51)

For the importance of hyperuricemia in the pathology of FJHN/MCKD , let's make a little summary about uric acid.

URIC ACID

Uric acid, a product of purine metabolism, is degraded in most mammals by the hepatic enzyme, urate oxidase (uricase), to allantoin, which is freely excreted in the urine. However, during the Miocene epoch (20 to 5 million years ago), 2 parallel but distinct mutations occurred in early hominoids that rendered the uricase gene non functional. As a consequence, humans and the great apes have higher uric acid levels (>2 mg/dL) compared with most mammals (<2 mg/dL).(12,39)

Sources of purine are either endogenous, from de novo synthesis and nucleic acid breakdown (approximately 600 mg/day), or exogenous, from dietary purine intake (approximately 100 mg/day). In the steady state, this daily production and ingestion of approximately 700 mg of uric acid is balanced by daily elimination of an equal amount of uric acid from the body. Approximately 30% of this daily loss is through the gut, with subsequent bacterial intestinal uricolysis. The other 70% (roughly 500 mg daily) needs to be excreted by the kidneys.(4,12,19)

Uric acid levels also vary significantly within humans as the result of factors that increase generation (such as high purine or protein diets, alcohol consumption, conditions with high cell turnover, or enzymatic defects in purine metabolism) or decrease excretion. Hyperuricemia also may result from increased net tubular absorption.(39)

Uric acid is higher in men and postmenopausal women because estrogen is uricosuric. In subjects with obesity, insulin resistance, and dyslipidemia (“the metabolic syndrome”), hyperuricemia frequently occurs because insulin stimulates sodium and urate reabsorption in the proximal tubule. Uric acid is increased in subjects with renal disease as the result of reduction in GFR and renal urate excretion. Diuretics, such as

thiazides, increase serum uric acid by stimulating both sodium and urate reabsorption in the proximal tubule. Alcohol intake results in elevated uric acid levels due to increased urate generation (from increased adenine nucleotide turnover) and decreased excretion (due to lactate blocking tubular transport of urate)(4,39,49).

Urate handling by the nephron:

The urate is freely filtrated at the glomerulus 100%. It is almost completely reasorbed along the early portion of the proximal tubule (PT) 99%, 50% of urate is secreted in the late convoluted segment of the PT, 40% is reabsorbed as post-secretory reabsorption along the straight portion of the PT. As a result up to12% execreted in the urine (12)figure (5).

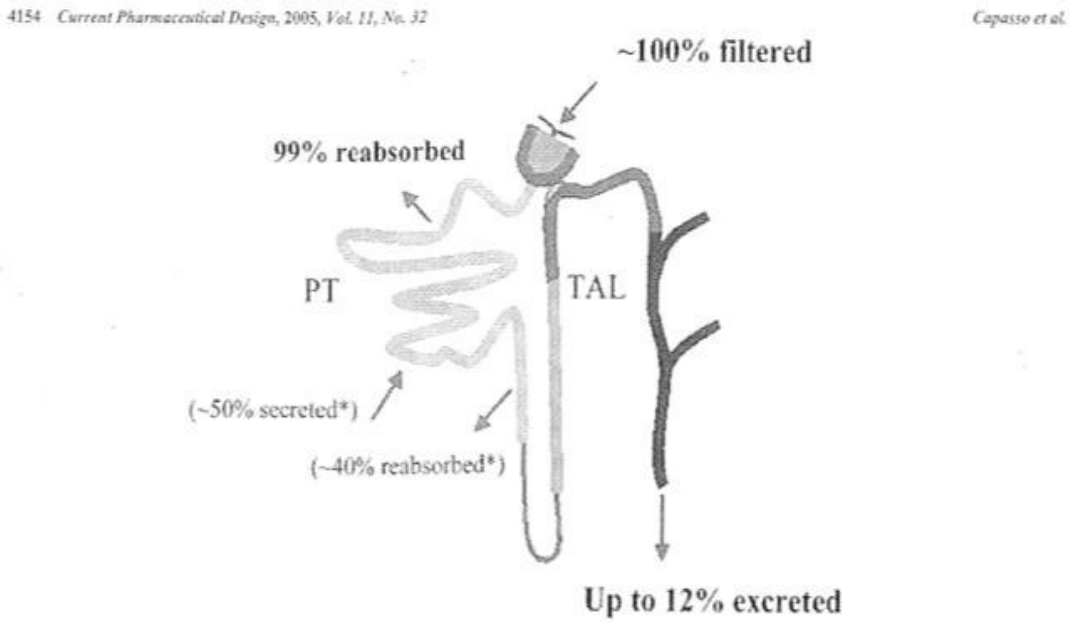


Fig.(5): urate handling by the nephron.

Molecular Mechanisms of Trans-Cellular Urate Transport

The serum urate level is regulated mainly by renal proximal tubular cells.

To date, 6 membrane proteins, namely, URAT1, OATv1, OAT1, OAT3, MRP4, and UAT, have been identified as urate transporters. (fig.6)

URAT1 (urate anion exchanger) is an apical urate/anion exchanger in the proximal tubular cells, responsible for reabsorption of urate from glomerular filtrate. OATv1(an

apical protein) is a voltage-driven organic anion transporter, which functions in urate excretion. OAT1 and OAT3 are basolateral organic anion transporter, play a role in uptake of urate from peritubular plasma. (12,22)

UAT is supposed to be an electrogenic urate channel, but its role in the apical membrane of proximal tubules remains to be elucidated (22), some studies suppose to be important in mediating metabolically derived urate efflux from cells (12).

MRP4 (an apical protein) is a membre of multidrug resistance-associated protein family, which mediates ATP-dependent urate transport (22). It is proposed to mediate secretion of urate and other organic anions such as cAMP, cGMP, and methotrexate across the apical membrane of human renal proximal tubular cells (32).

Uric Acid as an Antioxidant:

An important observation was that uric acid may function as an antioxidant (similar to vitamin C) (32), and possibly one of the most important antioxidants in plasma, this may account for the greater longevity of humans and the great apes compared with most other primates. Urate (the soluble form of uric acid in the blood) can scavenge superoxide, hydroxyl radical, and singlet oxygen in micro-vascular endothelium and in encephalic structures (49), and can chelate transition metals.

Peroxynitrite is a particularly toxic product formed by the reaction of superoxide anion with nitric oxide that can injure cells by nitrosylating the tyrosine residues (nitrotyrosine formation) of proteins, uric acid can also block this reaction.(39,49)

In addition Uric acid directly activates primary human T cells in the absence of antigen presentation. Furthermore, primary human T cells treated with uric acid overexpress the costimulatory molecule CD70, which plays an important role in T cell-B cell interaction and antibody production. This might play a mechanistic role in the inflammatory response observed in gouty arthritis and other immune-mediated diseases (84)

Also, urate can maintain blood pressure under lowsalt conditions via stimulation of the reninangiotensin system. Furthermore, recent studies suggest that urate may help arrest multiple sclerosis through scavenging the toxic compound peroxynitrite in the central nervous system.(32)

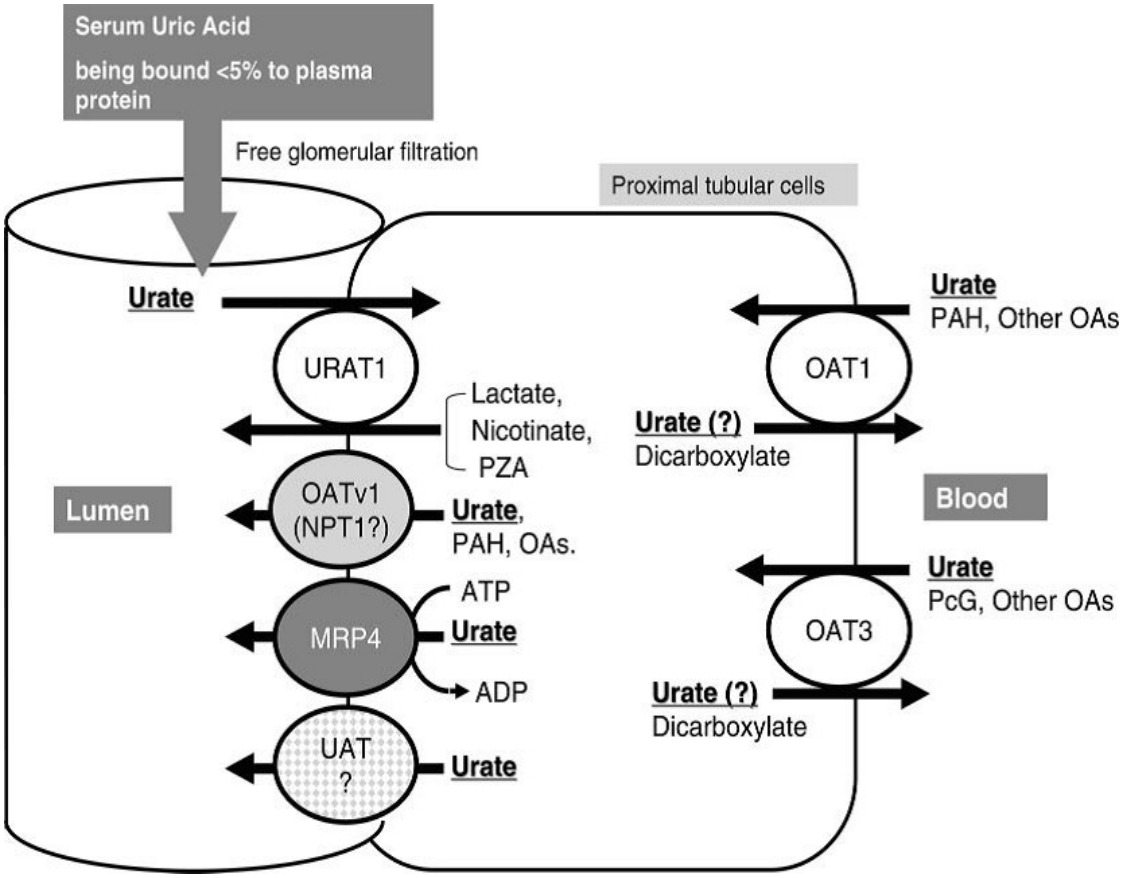


Fig. (6) Proposed model of transcellular urate transport in the proximal tubular cells

The pathogenic role of Uric Acid

The role of Uric Acid in the Progression of Renal Disease

Hyperuricemia has long been associated with renal disease. Approximately 20 to 60% of patients with gout have mild or moderate renal dysfunction ; before the availability of uric acid lowering agents, as many as 10 to 25% of patients with gout developed end-stage renal disease.(40)

Despite the association of gout with renal disease, controversy exists as to whether uric acid has an etiologic role . First, it has been difficult to ascribe the generalized renal injury in gout to the deposition of urate crystals, for they are often only focally present. Second, many patients with gout have hypertension or are elderly, and the renal lesions might simply reflect hypertensive or aging-associated renal damage . Third, results of the studies are mixed as to whether lowering uric acid will slow renal

progression in patients with gout . The inability to resolve this issue has emphasized the need for additional studies (40)

Weiner and others have been studied 13,338 participants with intact kidney function, for 8.5 +/- 0.9 yr of follow-up, and they have been found that elevated serum uric acid level is a modest, independent risk factor for incident kidney disease in the general population.(85)

Duk-hee Kang and others, have been studied the effects of hyperuricemia in rats, and they have been found: (40)

- Hyperuricemia Accelerates Renal Progression: causes hypertension, hypercreatiniemia, and proteinuria.

- Hyperuricemia Induces Severe Preglomerular Vascular Disease leads to glomerulosclerosis interstitial fibrosis, and vascular smooth muscle cells (SMC) proliferation, which is mediated by activation of Renin Angiotensin System (RAS), and cyclooxygenase 2

(COX-2) which is a major regulator of renin expression. (40) Others have been demonstrated that Uric acid can enter vascular smooth muscle cells and stimulate a number of factors, including platelet-derived growth factor and mitogen-activated protein kinase. These factors induce vascular smooth muscle proliferation and preglomerular arteriolopathy.(1,10)

Some studies had shown that preglomerular arteriopathy in hyperuricemic rats can be prevented by treatment with either ACE inhibitors or angiotensinII type 1 receptor (AT1) blockers.(40)

The relationship of serum uric acid and hypertension

It was found that there are strong association of uric acid with hypertension, particolary in new onset essential hypertension.(24)

Some mechanism of this relationship were explained above.

A recent autopsy study found that subjects with essential hypertension had nearly 50% fewer nephrons than control subjects. This fact can be explained by the presence of Endothelial dysfunction as a general mechanism linking low nephron number to hypertension, it was found high association between early essential hypertension, and an elevated uric acid level as well as elevated levels of sFLT-1(a major factor that is present in the circulation in preeclampsia) and ADMA (another circulating factor that causes endothelial dysfunction which blocks NO production) . Hence, a factor such as uric acid, which causes both endothelial dysfunction and vascular smooth muscle cell proliferation, would be expected to cause the renal lesion that would result in the development of hypertension.(24)

Childhood uric acid predicts adult blood pressure

Elevated childhood serum uric acid levels are associated with increased blood pressure beginning in childhood and higher blood pressure levels that persist into adulthood, in males and females, whites and blacks, suggesting that early elevations in serum uric acid levels may play a key role in the development of human hypertension (1).

The relationship of serum uric acid and a congenital reduction in nephron number

Elevated maternal serum uric acid levels cross the placenta into the developing fetus during nephron development, (uric acid is a small substance that is known to freely pass into the fetal circulation) (24).

Increases in fetal uric acid levels during the third trimester inhibit endothelial proliferation, a necessary step of nephron development, and reduce the final nephron number at birth, but why?

Studies have been demonstrated that: first, uric acid becomes a candidate to block nephron development because it potently inhibits human umbilical vein endothelial cell proliferation in vitro. Second, nitric oxide (NO) is also a trophic factor for endothelial cells, and uric acid is an inhibitor of NO production in vitro. Indeed, studies in humans also have found that uric acid levels correlate inversely with plasma NO and with acetylcholine-dependent vasodilation.(24)

As a result Uric Acid can be, also, a responsabile for intrauterine growth retardation (IUGR) and low birth weight, that which we can find in preeclampsia.(24)

The lower congenital nephron number may lead to hyperuricemia during adolescence as a consequence of increased proximal reabsorption.

AIM OF THE STUDY

In this study, we have investigated 3 families showing characteristics of the FJHN/MCKD phenotype. In the first two families the diagnosis of FJHN/MCKD was based on the familial occurrence of chronic renal disease associated with or preceded by early onset of hyperuricemia, however in the third family, which there wasn't familial story of chronic renal disease, the diagnosis was based on early onset of hyperuricemia, chronic renal insufficiency, and renal malformations.

The main aim of the study was to study the molecular bases of the disease and to analyse the functional significance of UMOD mutations in order to correlate them with the patient's clinical and family history.

MATERIALS AND METHODS

Patients

We have investigated 3 families that came to our attention, through patients who were refered to our renal division for investigation of chronic renal insufficiency.

DNA analysis

DNA analysis was conducted on peripheral blood genomic DNA, and all exons of the UMOD and HNF1B genes were screened by direct sequencing of PCR products.

Functional studies of UMOD mutations were conducted by immunofluorescence localization of wild type and mutant uromodulin isoforms, and by studying the trafficking of uromodulin to the plasma membrane. All these studies were performed at Laboratory of Pathophysiology of Uremia, Gaslini Hospital, Genova and at Dulbecco Telethon Institute, Molecular Genetics of Renal Disorders, San Raffaele Scientific Institute, Milano

RESULTS

The first family The first family came to attention through the proband III 16. The family pedigree is shown below, and the table (2) shows the clinical information on individuals of the first family.

This family is composed of three generations :

I1: affected from hyperuricaemia, gout, and CRI at age 30 years, he died at age 33 years for myocardia infarct. He had 3 sons and 4 daughters.

II1: died at age 10 years in car accident.

II2: 64years old, affected from gout at age 30 years, he was treated with Allopurinol and diet, the creatininemia was stable for 10 years about 1-2 mg/dl. After a myocardial infarction, renal function was further deteriorating, peritoneal dialysis was begun at the age of 57 years , three years later he underwent a renal transplantation.

III1: 39 years old, affected from hyperuricaemia (uricemia > 10 mg/dl), he was treated with allopurinol, his renal sonography showed renal cysts.

III2: 33 years old, affected from renal colic.

III3: 30 years old, healthy.

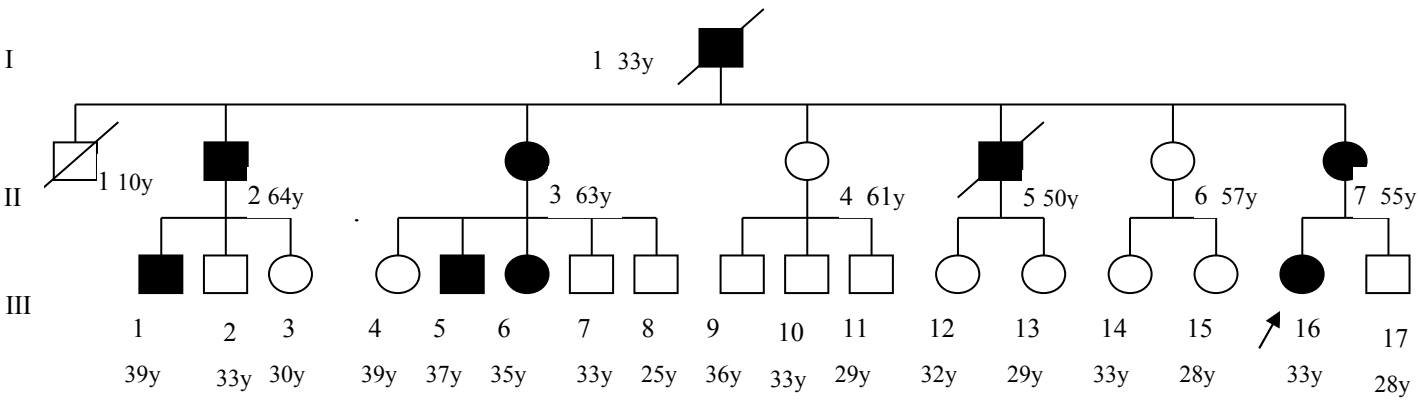


Fig (7): pedigree of the first family

II3: 63 years old, suffered from gout (uricemia 7,01 mg/dl), CRI (creatininemia 3,73 mg/dl), and hypercholesterolemia,At 59 years she underwent renal transplantation.

III4: 39 years old, healthy.

III5: 37 years old, affected from CRI, his sonography showed renal cysts.

III6: 35 years old, affected from CRI.

III7:33 years old, healthy.

III8: 25 years old, healthy.

	sex/age	Age of onset	gout	UA	CRI	Renal Tx	Renal cysts	Renal colic	notes
I 1	M	30Y	+	+	+	-	-	-	Died of myocardial infarction (33y)
II2	M/64	30Y	+	+	+	+	-	-	Myocardial infarction
III 1	M/39		-	+	-	-	+	-	
III 2	M/33		-	-	-	-	-	+	
II 3	F/63		+	+	+	+	-	-	
III 5	M/37					-	+	-	
III 6	F/35				+	-	-	-	
II 5	M	25Y	+	+	+	-	-	-	10Y dialysis, died of myocardial infarction (50 Y)
II 6	F/57		-	-	-	-	-	+	hypercolesterolemia
III14	F/33		-	-	-	-	-	+	Ovary cysts
III15	F/28	-	-	-	-	-	-	-	Hypercholesterolemia ovary cysts
II7	F/55	52 Y	-	+	+	-	+	-	
III16	F/33	28Y	+	+	+	-	-	-	Ovary cysts

Tx: transplantation.

Table (2): Clinical data on members of the first family

II4: 61 years old, she diden't have renal problems. She had 3 sons III9, III10, III11 (36, 33, 29 years old rispectly), they are healthy.

II5: affected from gout at age 25 years old, CRI for many years , but his renal function was further deteriorating after radiographic contrast medium. He died at 50 years old for myocardial infarction.

II6 :56 years old, affected from renal microlithiasis and hypercholesterolemia .

III14: 33 years old, affected from renal microlithisis, ovary cysts , with normal renal function.

III15: 28 years old, affected from hypercholesteroemia, ovary cyts, with normal renal function.

II7: (proband's mother) 55 years old: affected from moderate CRI, hypertension and hyperuricaemia at age 25 years, her renal sonography showed renal cysts. She was treated intermittently with Allopurinol and anti-hypertensive therapy. The table(3) shows laboratory parameters of mother's proband during 6 years of follow up.

	Sep-02 1°	Feb-03 2°	Jul-03 3°	Mar-04 4°	Feb-05 5°	Feb-06 6°	Dic-06 7°	Mar-07 8°	Jul-08 9°
Urea mg/dl	71	93	103	99	130	102	120	102	103
Creatiniemia mg/dl	1,61	2,28	2,09	2,11	2,46	2,33	3,05	2,41	2,74
Uricemia mg/dl	7		7,6	7,8	10,2	8,2	10		4,6
Creatinine clearance ml/min					28,6	35	33	32	
U-PS	1006	1005	1003	1006	1009	1017	1015	1010	

(1°: first control)

Table (3) Proband's mother laboratory parameters

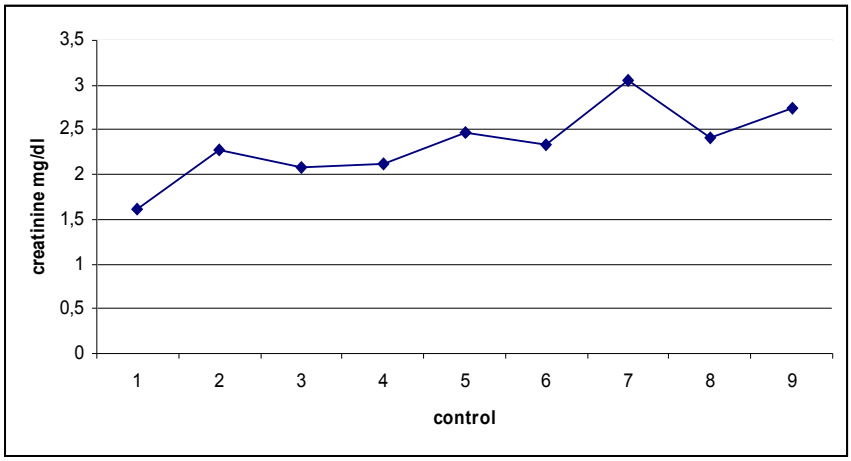


Fig (8):Creatininemia.of proband's mother

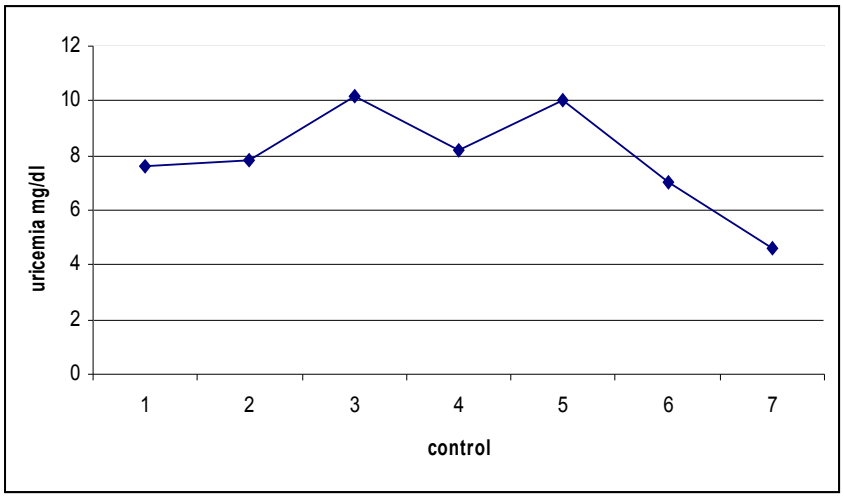


Fig (9): uricemia of proband's mother

The table shows an increase of creatinine level from 1,61 mg/dl, up to 3,05 mg/dl, with different values of uricaemia (maximum 10,2 mg/dl) mean 7,9 mg/dl, now uricaemia level is 4,6 mg/dl with Allopurinol therapy. Morever we observed reduction of creatinine clearance 32 ml/min, and a reduction of urine concentration.

Father's proband: 58 years old, affected from hypertension from about 10 years, in drug control. He has a familial story for hypertension: mother affected from hypertension at age <30 years, 2 brothers and 3 sisters affected from hypertension at age about 40 years.
His renal function is normal, and his renal sonography doesn't show renal cysts.

III17 (proband's brother): 28 years old. His renal function is normal: creatininemia(0,80- 0,92 mg/dl), uricemia (7- 5,9 mg/dl), creatinine clearance(137 ml/min), but he has reduction of FE
of uric acid (4,42).

III16 (the proband) :33 years old, affected from CRI at age 25 years, hyperuricaemia and gout at age 28 years. Microscopic evaluation of aspiration of synovial liquid, showed the presence of crystals of urate monosodium.
At age of 15 years old she underwent a surgical intervention for ovary cysts.

Renal sonography performed in august 2005 showed normal renal morfology, but renal sonografy 2 years later demostrated small kidneys with reduction of cortico-midullar differentiation, and microlithiasis.

She is affected from autoimmune hypothyroidism from 3 years, now TSH, FT3,FT4 are normal, with ANTI-TPO Anti-peroxidase : 359 IU/ml , she is treated by Eutirox. Now her therapy is Eutirox 50 mcg/day, Allopurinol 150 mg/day, and diet.

Her laboratory parameters during 12 years of follow up are shown in the table (4).

	NOV 94 1°	MAR 98 2°	JUL 2001 3°	JUN 2004 4°	AUG 2005 5°	SEP 2006 6°	DIC 2006 7°	APR 2007 8°	MAY 2007 9°	JUN 2007 10°	AUG 2007 11°	NOV 2007 12°	JAN 2008 13°	MAY 2010 14°
Urea mg/dl	63	61	71	86	73	89	60	63	65	74	84	64	68	56
Creatinin-emia mg/dl	-	1,34	1,50	1,53	1,6	1,7	1,5	1,4	1,5	1.17	1.39	1,42	1,4	1,5
Uricemia mg/dl	-	-	7,9	9,6	9,6	9,1	9,2	8,8	4,5	6,0	8.0	4,7	4,2	4,3
Creatinine clearance ml/min							46		56		51	42		43,8
U-PS				1009			1010	1008		1007		1010		
FE uric acid									3,42		3,08			
Uu.a/Ucrea									0,17		0,18			

Table (4): proband's laboratiry parameters.

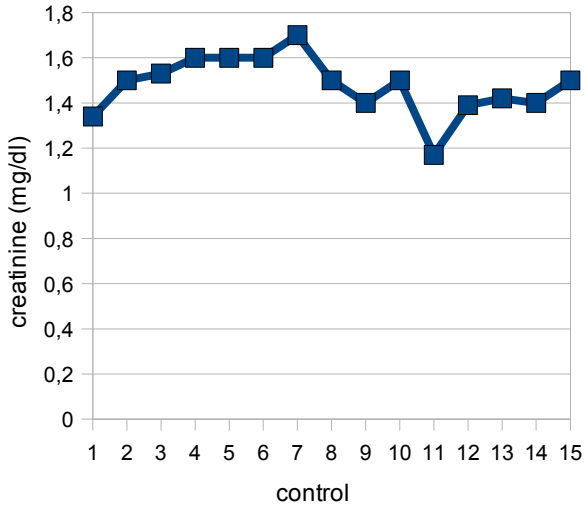
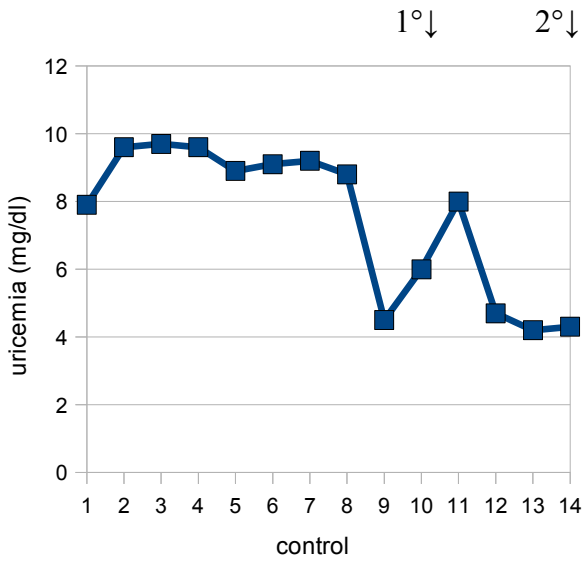


Fig (10): proband's creatiniemia.



(1°↓ Allopurinol start , 2°↓ allopurinol sospesion for 40 days)

Fig (11): proband's uricemia

The table and figures show the presence of CRI of third stage. Renal function was stable, with minor modification of creatinine level. Uric acid levels were high at the beginning of observation, and were normalized after treatment with allopurinol. Also these data show the reduction of:

-Urine concentration.

-Fractional excretion of uric acid.

-The relationship between urinary uric acid, and urinary creatinine (N.V 0,2 – 0,40).

DNA analysis

The presence in several family members of the maternal pedigree (4/7 sons and their father) of hyperuricemia, gout, and IRC, suggested an autosomal dominant pattern of inheritance of the disease. Proband's father and her brother were apparently healthy. DNA analysis demonstrated the presence of two novel nucleotide substitutions: c392G>A and c604 T>C in exon 4 relative to ATG translation start point (Fig. 12) . These substitutions cause the Trp202Arg and Gly131Asp non conservative missense mutations. Since the disease was inherited from the mother, the presence of a complex allele causing the disease was suspected. Mutation analysis was extended to proband's mother, father and brother and unexpectedly, the c604 T>C nucleotide substitution was detected in both father and brother DNA (Fig. 13). Therefore the proband was a compound heterozygous for two UMOD mutations. The mother with FJHN/MCKD, carried the c392G>A substitution, thus indicating that this substitution was a disease-causing mutation.

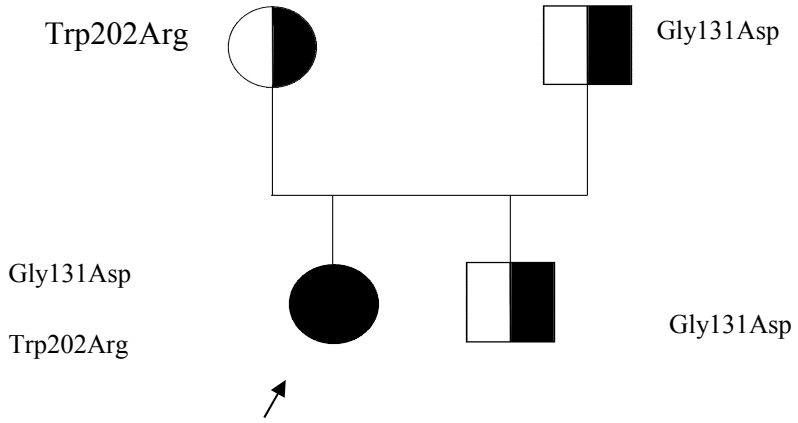


Fig (13): proband's family with the two mutations

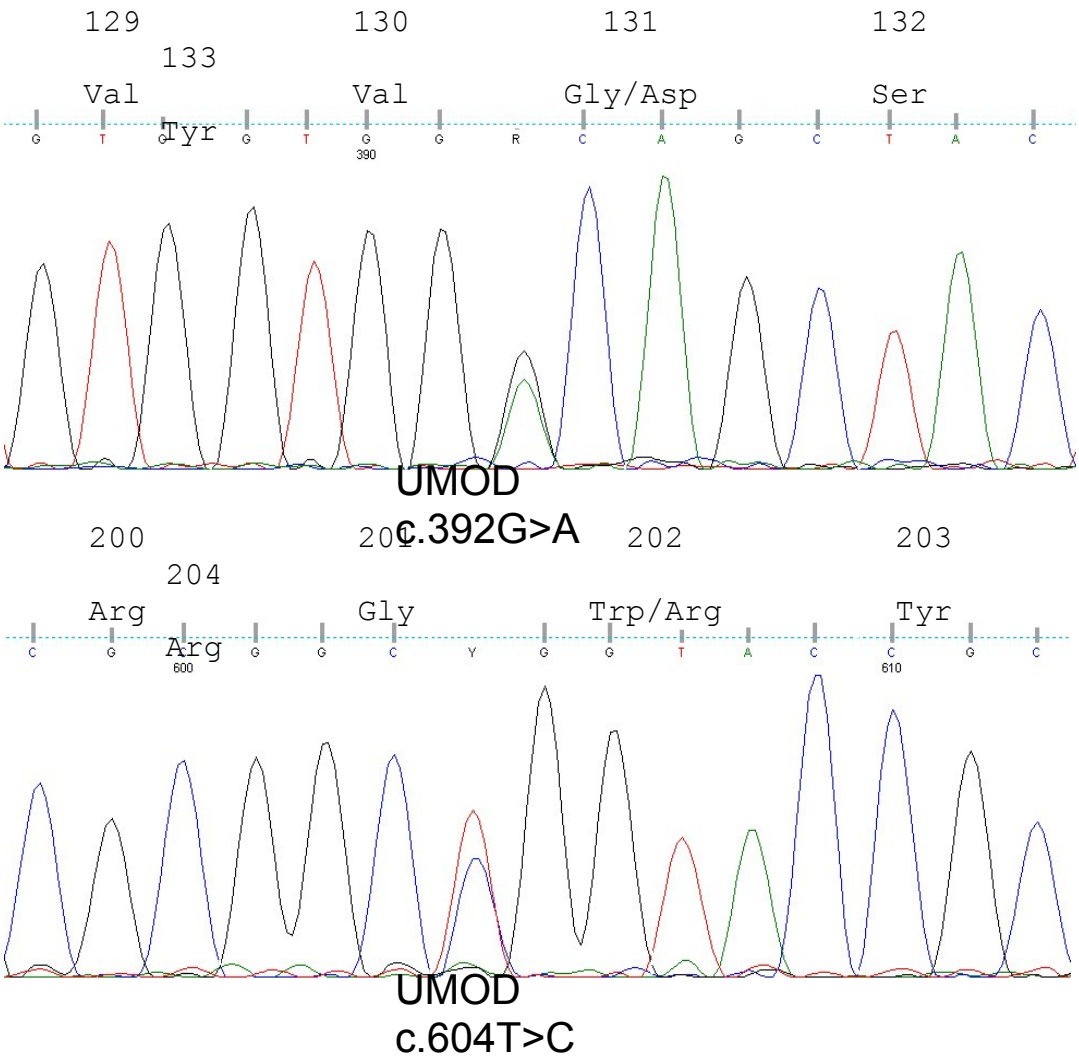


Fig (12) UMOD mutations site in DNA sequence.

The second family:

This family came to attention through the proband (IV 9) 46 years old with hyperuricemia with allopurinol therapy, proteinuria, microematuria, and CRI, she has a healthy son and 2 healthy daughters. Her therapy is: allopurinol 150 mg/day, zestrol 20 mg ½ cp/day. Crestor.

Her sister (IV6) has a congenital double ureter.

The mother (III3) 79 years old with CRI , is treated with dialysis from 17 years, while her father (III6) 83 years old affected from diabetes.

Her maternal uncle (III2) 72 years old suffers from CRI, he has a daughter (IV3) 39 years old suffers from CRI.

Her maternal grandmother (II1) died at age of 92 with CRI and reported nephritis?

Her paternal grandmother (II4) died at age 37 years old for renal problems?.

The pedigree of the family is shown in Figure (14)

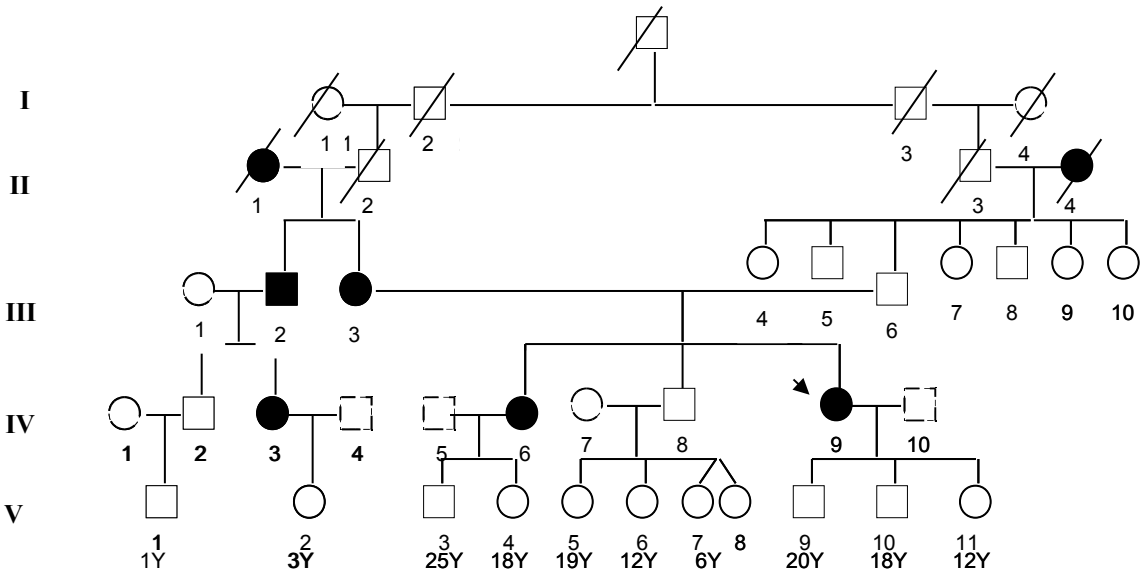


Fig (14): pedigree of the second family

	Jul-2007 1°	Oct-08 2°	Jan-09 3°	Sep-09 4°	Feb-10 5°
Creatininemia mg/dl	1,16	1,33	1,23	1,38	1,34
Uricemia Mg/dl		5,92	6,2	6,2	7
Creatinine clearance Ml/min		63,4			69,1
U-PS	1013	1012		1014	1016
Proteinuria g/24 ore	0,64		0,34	0,53	0,28

Table (5): proband's laboratoy parameters

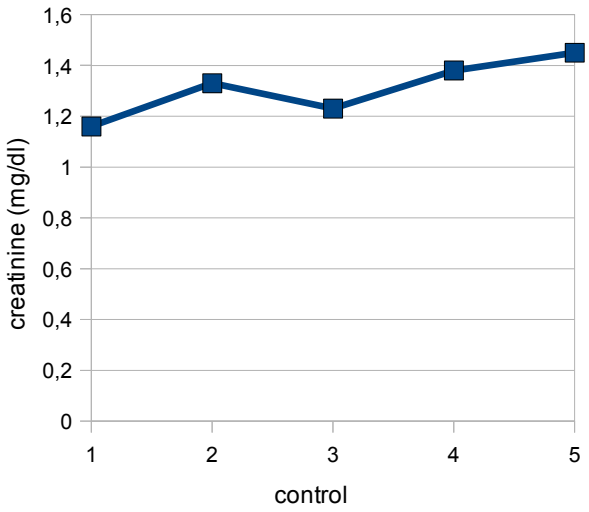


Fig (15): proband's creatiniemia

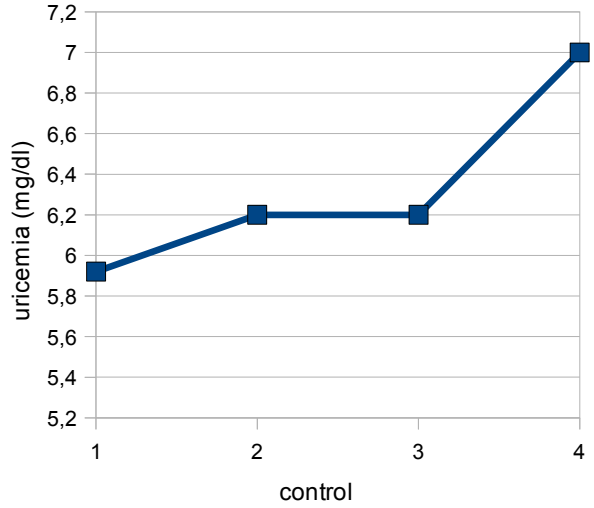


Fig (16): proband's uricemia

The table (5) shows the presence of second stage of CRI, with hyperuricemia, although the constant therapy with Allopurinol and proteinuria in the proband, also there isn't reduction of the ability of urine concentration, beside the presence of renal cysts. However, the pedigree of this family demonstrates that the member II1 transmitted the diseases to 2/2 sons, and the member III3 transmitted the diseases to 2/3 sons, thus suggesting an autosomal dominant pattern of inheritance of the disease. These facts together raised the suspicion of FJHN/MCKD syndrome.

DNA analysis:

DNA analysis was performed in the proband (IV 9), her mother (III3), and her cousin (IV 3).

UMOD gene was screened without identification of UMOD mutations.

Comparative Genomic Hybridization (CGH) was used to detect the presence of insertion/deletion in other genes but it gave negative results. the platform arrays of Agilent G3 400 was used for complete cover of genomic DNA.

Furthermore, it was examined HNF1B gene (to exclude atypical FJHN, for the presence of diabetes), and all of 9 exons were screened without identification of mutations.

The third family

This family came to attention through the proband, who was affected from CRI, malformative nephrouropathy, and hyperuricaemia. There is no family story for any renal pathology.

The proband is an only child, she is 26 years old, and she was followed from the birth for CRI and nephrourology malformations.

She was born at 39 GW, after a regular pregnancy, fetal sonography was normal.

Age Y	Creatininemia mg/dl	Uricemia mg/dl	Proteinuria g/24 h	U-PS	Ccr ml/min
1	1,2	7,1			
2	0,8	6,4			
3	0,7	6,8			
4	0,8	6,3			
5	0,78	5,2			74,9
6	0,9	6,85			51,3
7	0,99	7,52			63
8	0,97	7,1			
9	0,92	7,4	0,6		
10	1,24	5,7	<0,1		
11	0,94	6,9	-		

12	0,94	6,3	0,48		
13	1,19	8,4	1,5		
14	1,34	8,9	0,3		
15	1,3	7,9	0,34		
16	1,31	8,8	0,44	1005	
17	1,29	7,4			
18	1,52	8,4	0,46		
19	1,42			1015	
20	1,49		0,86	1005	
21	1,55				
22	1,75		0,83		
23	1,68				
24	1,69	8,9	0,45	1005	42,42
25	1,81	10,5		1004	50
26	1,96	6			

Table (6): proband laboratiry parameters

Normal values:

creatiniemia: mg/dl
newborn 0,3 – 1
infant 0,2 – 0,4
child 0,3 – 0,7
adolescent 0,5 – 1
adult F 0,6 – 1,2

uricemia: mg/dl
1 – 5 Y 1,7 – 5,8
6 – 11 Y 2,2 – 6,6
F 12 – 19 Y 2,7 – 5,7

For low weight at the birth (2,300g), she was subjected to investigations. Laboratory parameters demonstrated alteration of creatininemia (1 mg/dl NV 0,3-1 mg/dl) at age of 15 days. She was investigated with sonography, voiding cystourethrogram VCUG, renal scintigraphy, that demonstrated the presence of unique kidney at the right, with vesicoureteral reflux IV grade, and on the left side evidenced tow liquid formations.

At the age of 3 months, she underwent a corrective surgery for vesicoureteral reflux. Subsequently, she had one episode of urinary tract infection. VCUG after surgery showed moderate vesicoureteral reflux at the right.

Then she underwent periodic follow up, the renal function, however, is still stable with light CRI.

Hyperuricemia appeared at the age of 2 months, Allopurinol therapy was initiated from one year.

Proteinuria appeared from 13 years, and it is controlled by ACE- inhibitor therapy.

The growth was normal, as well as arterial pressure.

The last renal sonography showed: "Right kidney with diameter about 10,5 cm, showes regolar morphology, but with diffuse parenchimale hyperecogenecity, and reduction of cortico-medullary differentiation, the left kidney is non displayed, but there are some fluid formations the major has a diameter about 2,5 cm. No dilatation of urinary tract, bladder is completely empty.

Her therapy now is: Rocaltrol 0,25 ug/day, Unipril 2,5 mg/day.

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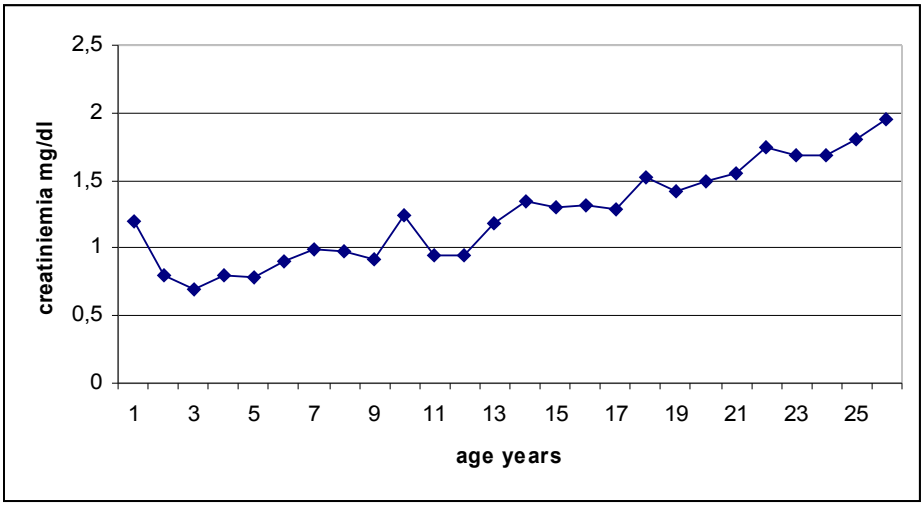
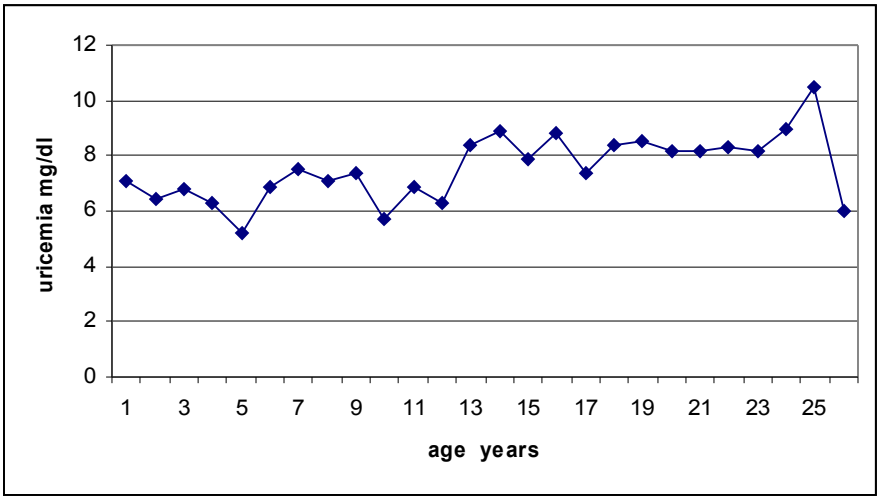


Fig (17): proband's creatininemia



Fig(18): proband's uricemia

The table and figures show the presence of CRI from the birth, with important hyperuricemia, which arrives at 6 mg/dl after the use of Allopurinol. Also we fuond the presence of proteinuria which is treated with ACE- inhibitor from age of 14 years old, and reduction of urine concentration. Although the proband suffers from CRI from 26 years, renal function is, however, stable during 26 years.

DNA analysis:

For the suspicion of FJHN, DNA analysis for UMOD mutations was conducted. Only exon 4 and exon 5 of UMOD gene were studied without identification of mutation. HNF1B gene was also analysed without identification of mutation, but 2 polymorphisms in intron 2 and intron 7 in heterozygosity were found, which excluded a gene deletion.

DISCUSSION

In the first family, there were 9 members with chronic renal disease associated with or preceded by early onset of hyperuricemia, and renal cysts, therefore they presented the typical phenotype of FJHN/MCKD syndrome. Genetic analysis confirmed the clinical diagnosis. However two nucleotide substitutions were found in the proband; these novel substitutions cause the Trp202Arg and Gly131Asp non conservative missense mutations therefore the proband was a compound heterozygous for two UMOD mutations.

The mother, who had a phenotype similar to the proband, had one of the proband mutations (Trp202Arg mutation), father and young brother who were apparently healthy had the other mutation (Gly131Asp).

The Trp202Arg mutation, which was found in the proband and her mother, did not change cysteine residues, but it was associated with FJHN/MCKD syndrome phenotype since, as well as the functional studies indicated, Trp202Arg mutant protein was retained in the endoplasimic reticulum (ER). Therefore we can say that Trp202Arg is a pathogenic mutation for FJHN/MCKD syndrome although it doesn't modify cysteine residues.

Since the presence of the two mutation did not seem to worsen the proband's clinical phenotype in respect to that of mother, and since proband's brother and father did not show any sign of FJHN/MCKD, we wondered if the Gly131Asp variant was indeed a polymorphisms.

In silico analysis performed by SIFT and Polyphen softwares predicted that both the two novel UMOD missense mutations were pathogenecs. Functional studies indicated, however, that the Gly131Asp isoform behaved as the wild type protein (Fig 19). This last result is in agreement with the clinical and familial data and suggests that Gly131Asp is not a FJHN/MCDK disease causing mutation. We decided to determine the frequency of the UMOD Gly131Asp substitution in the control populations. We searched among 220 children with urinary infections, 88 control DNA samples (from umbilical cords), 100 blood donors, but the variant was not found, thus suggesting that this variant is very rare. This finding prompted us to acquire more details on the clinical history of paternal pedigree, and to conduct

laboratory and instrumental investigation in proband's father and brother. Except for the low borderline value of fractional excretion of uric acid (FE_{ur}) in the brother and essential hypertension in the father since the age of 40, no clinical and laboratory signs of FJHN/MCDK were seen.

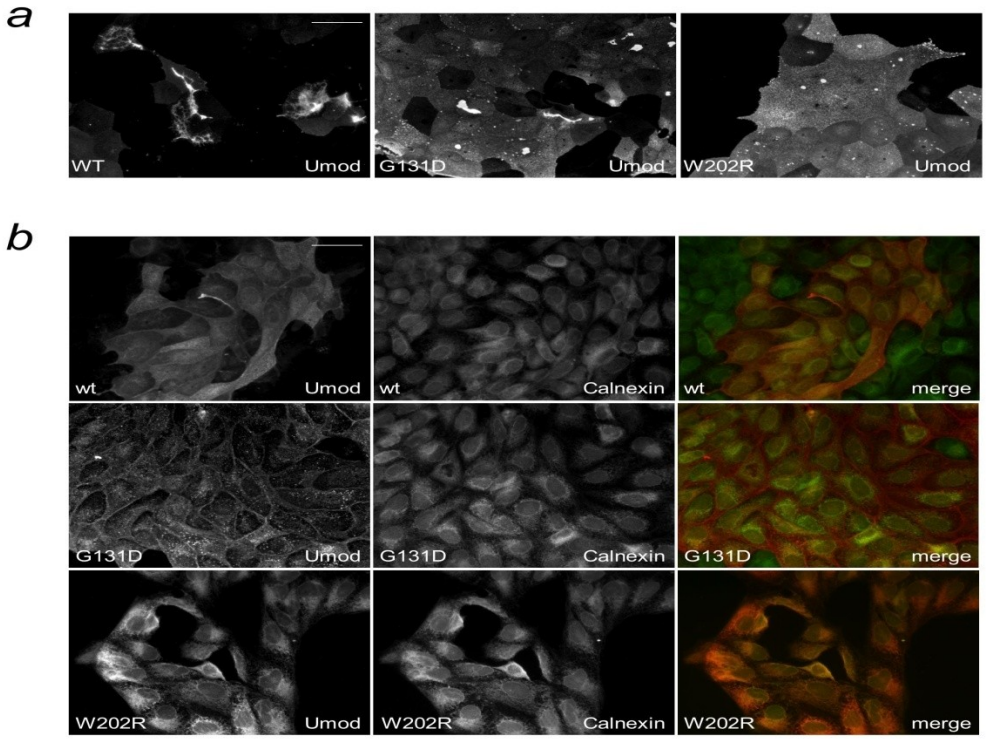


Fig (19): immunofluorescence analysis of G131D and W202R.

Immunofluorescence localization of wild type and mutant uromodulin isoforms. (*a*) Uromodulin signal on the cell surface of unpermeabilised cells. All isoforms are trafficked to the plasma membrane but mutant W202R is not able to assemble into filaments as opposed to wild type or G131D isoforms. (*b*) Uromodulin and calnexin (ER marker) signal in permeabilised cells. In the merged picture uromodulin signal is shown in red, calnexin one in green. Mutant W202R is ER retained as shown by strong colocalisation with calnexin. Wild type and G131D isoforms are only partially colocalised with calnexin and show a strong plasma membrane signal.

The family history in the paternal pedigree was negative for IRC, gout, or renal diseases, but positive for hypertension. In fact paternal grandmother, and 5 uncles suffered from hypertension (segregating as autosomal dominant trait) since about the age of 40. Hypertension was reported to be the unique symptom in a patient with uromodulin mutation (88), and hypertension has been found to be associated with polymorphisms in UMOD gene (36), we hypothesized that UMOD mutations could be responsible of essential hypertension in this family.

At the other hand the presence of low FE_{ur} in the proband brother is a very important sign, some authors, in fact, consider the reduction of FEur a hallmark of FJHN syndrome, and an important index for presymptomatic diagnosis**,** and they recommend measurement of FEur in children with either unexplained renal insufficiency or normal renal function, where there is a strong family history of renal disease, or gout.(50). A careful follow up was strongly suggested in proband 's brother.

In this family we show the association between CRI, hyperuricemia , and myocardial infarction, and this association was explained before in many studies (24,40)

In the second family, we have seen 6 members suffered from CRI, we lack a lot of informations about the major part of these members, but at least we know that the proband has phenotype compatible with MCKD, and the presence of double ureter can indicate FJHN/MCKD syndrome, although in the absence of UMOD and HNF1B genes mutations. In this family CGH array analyses were also conducted to search for other gene involvement without success however.

In the third family, we have seen the case of a proband, who although in absence of any positive family history had a clinical phenotype compatible with FJHN. In addition, she was affected from vesicoureteral reflux, and has unique kidney, however on the left side there were fluid formations probably indicating an immature structure of kidney or renal hypoplasia. In literature several studies confirmed the presence of UMOD mutation at early age, 9 months (71), in the absence of familial story (83), in association with vesicoureteral reflux, renal hypoplasia (88), and immature kidney (5), we can not exclude the diagnosis of FJHN, despite the fact that she hasn't UMOD and HNF1B mutations.

The question which raises now is:

Why there is FJHN/MCKD phenotype with no UMOD mutation?

UMOD mutations are not the only cause of the FJHN/MCKD phenotype. The other candidate loci for FJHN/MCKD were identified on chromosome 1q21, chromosome 1q41 (34,83,86), and a disease causing mutation in HNF1B gene was found in a single family with features of FJHN and diabetes (34,83,86). The mechanism linking the genetic heterogeneity to common disease symptom development in families with no UMOD mutations is not clear but it was suggested that UMOD dysfunction might be a common pathogenic mechanism. (34,83).

In these patients with FJHN with no UMOD mutation, a reduction of UMOD expression was indeed found (16,34,83). Reduced UMOD expression may be explained by the presence of mutations in a transcription factor or hormone involved in UMOD gene transcription activation as it is in atypical FJHN associated with diabetes which is caused by HNF1B mutations. Reduced UMOD expression may also be caused by a mutation affecting a protein involved in post-translational modification or cellular traffic of UMOD (34).

Also methodological differences in sequencing of UMOD gene may account for the different results. Usually, only exons 4 and 5 are screened since in these exons reside about 99% of UMOD mutations. This is the case of the third family where not all UMOD gene was analysed.

However, other possibilities must be considered: for example deletion of an entire exon could result in PCR amplification of only the wild type allele, masking the presence of a mutation (31).

Another explanation may be undetected mutations in UMOD promoter sequence or the existence of synonymous exonic mutations and/or intronic mutations affecting proper UMOD mRNA processing (83). Pirulli et al (60) studied a family with MCKD without UMOD mutations, but they did not analyse the non-coding exons 1 and 2, nor the 5' regulatory region of *UMOD*. It is possible that mutations in exon 1, exon 2, or in the regulatory region could result in loss of uromodulin production. Alternatively, genetic heterogeneity may exist with another kidney specific gene located in the candidate interval (31).

Williams et al (86) studied 20 FJHN probands, 70% of them did not have mutations in the UMOD gene, and this is consistent with previous reports (16,83,90), although

these studies have not searched for intronic mutations or whole gene UMOD deletions.

CONCLUSIONS

Limits of the study

We couldn't study all the members of the three families, because some of them live far from Padua,
and others refused to collaborate.
We couldn't complete all of biochemical exams to evaluate the nephropathy in probands.
We didn't analyse urinary uromodulin, and we didn't perform renal biopsy, and immunohistochemistry studies, which are perfect methods to understand UMOD biology
Evaluation of genotype–phenotype correlations in FJHN/MCKD2 has long been impossible due to
the limited number of patients and the individual character of each mutation for a kindred.

In spite of the limits, from the study of these 3 families we have get new insights in the molecular biology of UMOD gene:

1- Double mutant individuals occur in FJHN/MCKD. We identifided , for the first time, the presence of two UMOD mutation in one patient, as compound heterozygote for 2 UMOD mutations.

2- Amino acids substitution not involving Cysteine residues cause ER retention.

3- A non conservative amino acid substitution, that functional behaves as a normal allele, i.e. it is not able to induce ER retention, is not associated with FJHN/MCKD diseases, rather it might be associated with intermediate phenotypes (reduction of F E of uric acid, hypertension).

4- We confirmed that UMOD mutations are not the only cause of the FJHN/MCKD phenotype.

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