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Molecular basis of familial hyperuricemic nephropathies

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1 Summary

In 1960 Duncan and Dixon described family whth chronic tubulointerstitial kidney disease associated with juvenile onset of hyperuricemia and gout. Based on combination of these clinical symptoms they named the disease familial juvenile hyperuricemic nephropathy (FJHN) [1]. Disease with very similar clinical presentation but different age of onset and kidney histology was described as a medullary cystic kidney disease (MCKD) in 1977 [2]. Until recently the molecular basis and pathogenesis of this syndrome remained unknown.

The long term aim of our research group is to elucidate the genetic basis of the disease and to solve pathogenetic mechanisms leading to the individual clinical and biochemical symptoms (e.g. hyperuricemia) and kidney damage in general. We systematically identify patients with this disease and healthy family members and collect relevant clinical information and samples for classification (urine, blood, tissue biopsies) and subsequent clinical, biochemical, molecular biology and cell pathology correlations.

We [3, 4] and others [5-7] proved genetic heterogeneity of FJHN and defined four FJHN loci on chromosomes 1q21, 1q41, 16p11.2. and 17q21.3. Further research defined disease causing mutations in three genes - uromodulin (*UMOD)* [8], hepatonuclear factor 1-beta (*HNF-1β)* [9] and renin (*REN)* [10], which explain only about 40% of the FJHN cases.

We also found that most of the FJHN cases have altered expression and urinary excretion of UMOD which suggest central role of UMOD protein in development of hyperuricemia and FJHN pathogenesis [11]. Our surprising finding, has been corroborated recently by results of genome-wide association studies (GWAS) showing association of *UMOD* polymorphisms with development of chronic kidney disease [12, 13]. This is a reason of revived and currently growing interest in still mysterious UMOD biology and function and positioning FJHN as a very hot topic in current nephrology research.

2 Introduction

Until recently the molecular basis and pathogenesis of familial hyperuricemic nephropathies was unknown. In 2000 based on the linkage analysis UMOD was for the first time selected as one of the hot candiate gene to be associated with the disease condition [3].

The diagnosis of FJHN/MCKD was based on characteristic features of the disease, which include clinical evidence of gouty arthritis or renal failure, biochemical evidence of hyperuricemia, reduced fractional excretion of uric acid, and elevated creatinine serum levels.

2. 1 Familial hyperuricemic nephropathies (FHN)

Familial hyperuricemic nephropathies (FHN) are group of primary chronic forms of tubulointerstitial nephropathies with distinct clinical and histological features, various types of inheritance with either juvenile or adulthood onset. FHN with autosomal dominant inheritance, based on clinical and pathological finding, comprise following kidney diseases:

i) familial juvenile hyperuricemic nephropathy type 1 (FJHN1, OMIM 162000)

ii) familial juvenile hyperuricemic nephropathy type 2 (FJHN2, OMIM 613092)

iii) medullary cystic kidney disease type 1 (MCKD1, OMIM 174000)

iv) medullary cystic kidney disease type 2 (MCKD2, OMIM 603860), [Fig. 1].

2. 1. 1 Clinical signs and symptoms of FHN

The typical clinical features for FHN are alteration of urinary concentration ability, hyperuricemia and tubulointerstitial fibrosis [14]. Renal impairment usually reaches clinical significance between 15 and 40 years of age and inevitably leads to end-stage renal failure (ESRF) within 10 to 20 years [15-18].

FJHN1 as well as MCKD2 are characterized by juvenile onset of hyperuricemia, gouty arthritis, and progressive renal failure

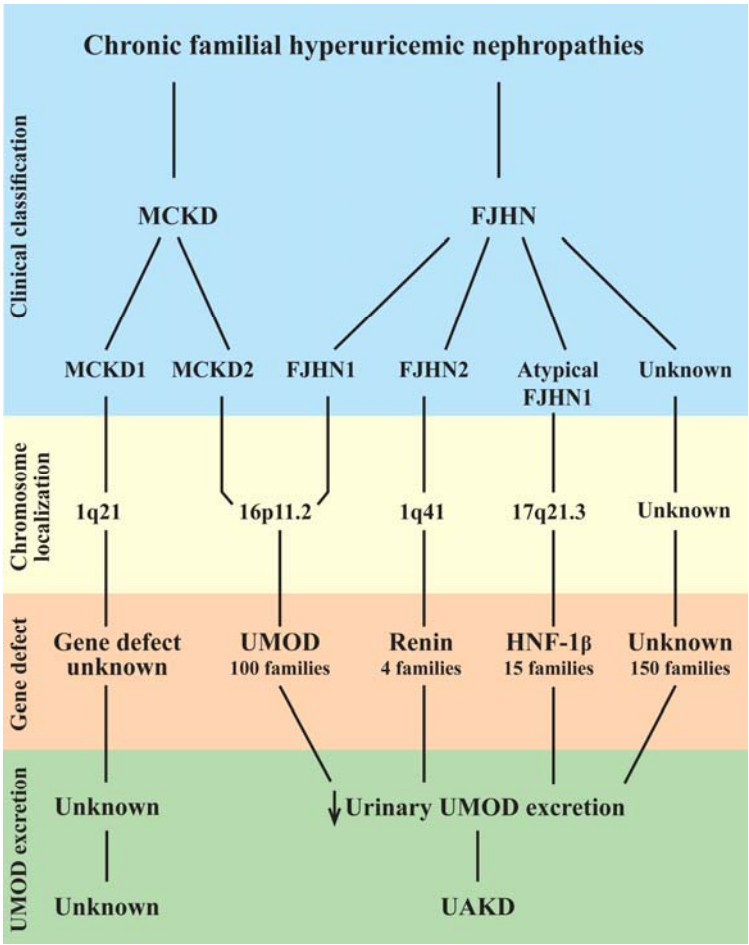

Figure 1: Chromosome localization of gene defect associated with chronic familial hyperuricemic nephropathies.

Abbreviations: MCKD - medullary cystic kidney disease; FJHN - familial juvenile hyperuricemic nephropathy; UMOD - uromodulin; HNF-1β - hepatocyte nuclear factor-1β; UAKD - uromodulin-associated kidney disease

at an early age. Several investigators suggest FJHN1 and MCKD2 to be the same disease.

FJHN2 phenotype is characterized by early anemia, hypouricosuric hyperuricemia and progressive kidney failure resulting in end-stage renal failure between ages 43 - 68 [4, 10].

MCKD1 is characterized by late onset of hyperuricemia, gouty arthritis, progressive renal failure and hypertension.

2. 1. 2 Genetic heterogeneity of FHN

FHN are genetically heterogeneous diseases [3, 5]. FJHN1 and MCKD2 segregate with overlapping regions on 16p11.2 and 16p12 respectively [3, 18-21]. FJHN2 is linked with chromosome 1q41 [4]. MCKD1 was further associated with region on chromosome 1q21 [6, 22] [Fig. 1].

2. 1. 3 Molecular basis of FHN

FJHN1 and MCKD2 were found to be associated with mutation in uromodulin *(UMOD)* which is localized in candidate region 16p11.2 [8, 11, 14, 23-26].

In families with features of FJHN1 complicated by early onset of diabetes was identified heterozygous mutation in *HNF-1β* gene [9]. However, *HNF-1β* region on 17q21.3 suggests that *HNF-1β* mutations are a minor cause of FJHN1.

Within the other locus, linked with FJHN2, on chromosome 1q41 mutation in signal peptide of renin gene *(REN)* was found recently [10] [Fig. 1].

2. l. 4 Uromodulin (UMOD)

UMOD is expressed in the thick ascending limb (TAL) of the Henle´s loop and the early distal convoluted tubule [11, 27]. The microscopic observations revealed that UMOD is targeted to the apical cell membrane of the TAL epithelial cells. From the apical pole of TAL epithelial cells is UMOD cleaved by unknown protease into the tubule lumen and excreted into the urine.

2. 1. 5 Renin

Renin is an aspartyl protease expressed from early stages of human kidney development by epitheloid juxtaglomerular cells which are located in the wall of renal afferent arterioles at the

entrance of the glomerular capillary network [28]. Juxtaglomerular apparatus (JGA) is the main source but not the only one of renin. Messenger RNA as well as protein of renin was observed in other segments of kidney as well. The renin was detected in cells of glomeruli and proximal and distal nephron [10, 29, 30]. Other tissues that were found to produce renin are adrenal glands, brain, lung and vasculature [31, 32].

3 Specific Aims

To elucidate the molecular basis of FJHN/MCKD we proposed the following specific aims:

1. Identified gene(s) defect of familial hyperuricemic nephropathies using linkage analysis and direct sequencing of candidate genes

2. Functionally characterize identified gene(s) defect using combination of molecular biological and biochemical methods

3. Correlate genotype-fenotype and suggest pathogenetic mechanism

4 Methods

4. 1 General Methods

Nucleic acid isolation from cells and tissues
Polymerase chain reaction
DNA electrophoresis in agarose gel
DNA cycle sequencing
Reverse transcription of total RNA (generation of cDNA)
Restriction analysis of DNA
Statistical analysis using the Mann-Whitney test, t-test

4. 2 UMOD related methods

Enzyme-linked immunosorbent assay (ELISA)

We developed sandwich enzyme-linked immunosorbent assay (ELISA) for quantitative UMOD analysis.

Thawed urine samples were diluted (1:250) in tetraethylammonium (TEA) buffer according toKobayashi and Fukuoka [33]. UMOD was quantified with antihuman Tamm-Horsfall protein mouse IgG2b monoclonal antibodies (Cedarlane, Hornby, Ontario, Canada) as capture antibodies and rabbit antihuman Tamm-Horsfall protein polyclonal antibodies (Biogenesis, Pool, England) and goat antirabbit IgG-horseradish peroxidase conjugate as detection antibodies.

SDS-PAGE and Western blot analysis

For qualitative analysis of urinary UMOD, 250 μL of urine was concentrated on Microcon YM-30 filters (Millipore, Bedford, MA, USA) and total protein was recovered. For analysis of UMOD in sediment, 35 μL of total urine was centrifuged at 5 000*g* for 10 minutes. About 10 μg of total urinary protein and entire sediment pellet were dissolved in sodium dodecyl sulfate (SDS) sample buffer. Proteins were separated by SDS polyacrylamide gel electrophoresis (PAGE) and either stained by SYPRO Ruby (Molecular Probes, Eugene, OR, USA), or blotted in a semidry system (Biotec-Fischer, Reiskirchen, Germany) on polyvinylidine difluoride (PVDF) membranes (Immobilon-P) (Millipore). Western blot analysis was performed with UMOD monoclonal mouse antibodies (Cedarlane) (primary antibodies) and antimouse Ig antibody conjugated to horseradish peroxidase (Pierce, Rockford, IL, USA) (secondary antibodies). Chemiluminescent signal was obtained using SuperSignal West Pico Chemiluminescent Substrate Kit (Pierce).

Cell culture and transfection experiments

AtT-20 pituitary cells were maintained in DMEM high glucose medium supplemented with 10% (vol/vol) fetal calf serum, 100 U/ml penicillin G, and 100 mg/ml streptomycin sulfate (PAA Laboratories GmbH, Pashing, Austria). Cells were used at 80% confluence. Transfection was carried out with 4 μg of DNA using Lipofectamine 2000TM (Invitrogen, Paisley, UK).

Flow cytometry

AtT-20 cells were seeded in 6-well tissue culture plates (BD Falcon, Palo Alto, CA, USA). Following 24-h incubation, the cells were transfected and harvested at the indicated time after the transfection. $2x10^5$ cells were washed, stained for 30 min with 1.5 μg of fluorescein isothiocyanate-labeled (Fluorescent Labelling Kit, Roche, Prague, Czech Republic) anti-UMOD – rabbit polyclonal IgG (Biogenesis, Pool, UK), washed and fixed in 2% paraformaldehyde. Fluorescence was measured using FACSCalibur flow cytometer and analyzed using the Cell Quest software version 3.3 (Becton Dickinson, San Jose, CA, USA). Cell surface expression of UMOD was quantified as the geomean of the fluorescence of gated fluorescein isothiocyanate-positive cells.

4. 3 Renin related methods

In silico analysis

Preprorenin signal sequences from the mammals were obtained from the UniProtKB/Swiss-Prot database (web address: http://www.uniprot.org/uniprot). Multiple alignment and evaluation of the amino acids conservation were performed by ClustalW2 software (EMBL-EBI database) (web address: http://www.ebi.ac.uk/Tools/clustalw2/index.html). Properties of the signal sequences were assessed with the SignalP 3.0 server9 (web address: http://www.cbs.dtu.dk/services/SignalP/) and the Kyte and Doolittle method [34].

Transient expression of REN

HEK293 cells were maintained in DMEM high-glucose medium supplemented with 10% (vol/vol) fetal calf serum (PAA), 100 U/ml penicillin G (Sigma, Prague, Czech Republic), and 100 mg/ml streptomycin sulfate (PAA Laboratories GmbH, Pashing, Austria). Transfections were carried out with Lipofectamine 2000 (Invitrogen, Paisley, UK) with either 1.5 μg or 4 μg DNA for 1.5 x 10^5 or 8 x 10^5 cells, respectively.

REN-expressing stable cell lines

HEK293 cells were maintained as described above and transfected at 85% confluence with Amaxa nucleofector system

(Amaxa, Koln, Germany). Three days after nucleofection, cells were trypsinized, diluted, and cultured in selective medium containing 0.8 mg/ml G418 (Invitrogen-GIBCO, Paisley, UK). REN-expressing clones were selected with PCR, sequencing, and western blot analyses.

Renin secretion measurement

Stably transfected HEK293 cells were cultured in 96-well plates in standard, serum-supplemented medium without phenol red. After 20 hr, the medium was replaced with medium containing renin substrate conjugated with 5-FAM and QXL520 (part of SensoLyte 520 Renin Assay Kit, AnaSpec, San Jose, CA). Fluorescent signal was monitored at 520 nm every 5 min for 8 hr at 37°C on Synergy 2 microplate reader (BioTek, Winooski, VT).

Immunofluorescence analysis

Transfected HEK293 cells were grown on glass chamber slides (BD Falcon - 4Chamber Polystyrene Vessel Tissue Culture Treated Glass Slide). After 48 hr, the cells were washed with PBS, fixed with 100% ice-cold methanol, blocked with 5% FBS, and incubated with either rabbit anti-preprorenin (288-317) of rabbit anti-preprorenin (21-64) antibody.As organelle specific primary antibody were used: anti-PDI – mouse monoclonal IgG1 (Stressgen, San Diego, CA, USA) for ER localization; anti-GS28 mouse monoclonal IgG (Stressgen, San Diego, CA, USA) for Golgi localization, and anti-pan Cadherin mouse monoclonal IgG (Abcam, Cambridge, UK) for plasma membrane localization. For fluorescence detection, secondary antibodies – Alexa 568 goat anti-rabbit IgG and Alexa 488 goat anti-mouse IgG (Molecular Probes, Invitrogen, Paisley, UK) were used. Prepared slides were mounted in fluorescence mounting medium Immu-Mount (Shandon Lipshaw, Pittsburgh, PA, USA) and analyzed by confocal microscopy.

Growth rate analysis

Stably transfected HEK293 cells were seeded in a 6-well plate at 4×10^5 cells per well and cultured in the selective, G418-containing medium. Cells were counted every 24 hr for 7 days via a standard Bu¨rker cell counting chamber. The medium was changed at the third, fifth, and sixth days.

Detection of induced ER stress (XBP1 analysis)

Total RNA was isolated from cells via TRIZOL (Invitrogen, Carlsbad, CA) and reverse transcribed with oligo-dT primer and SuperScript II (Invitrogen). XBP1 was PCR amplified from the corresponding cDNA with gene-specific primers.

Renin granulosity determination

Renin granulosity was assessed by the fluorescence activated cell sorter (FACS) assay, measuring the differences in the fluorescence intensity and the number of granular cells after the transient transfection of HEK293 cells with WT^{REN}, $C20R^{REN}$ and empty pCR3.1 expression vectors and labeling of low-pH secretory granules with fluorescent aminoacridine dye quinacrine. For this assay, 8 x 10^5 HEK293 cells were transfected with 4 µg of the corresponding vectors. Cells were mechanically harvested 24 hours post-transfection using 1.2 mL of supplemented Dulbecco's Modified Eagle's Medium (DMEM) without phenol red. Five minutes before the analysis, 400 µl of 8 µM quinacrine dihydrochloride (Sigma, Prague, Czech Republic) in DMEM without phenol red was added to 400 µl of the cell suspension containing aproximatively 500,000 cells. Fluorescence was measured using FACS Calibur flow cytometry, and data were analysed using Cell Quest software version 3.3. (Becton Dickinson, San Jose, Ca, USA). Granular cells were counted in gated side scatter high population. The number of low-pH granules was quantified as the mean fluorescence intensity (MFI) of gated quinacrine positive population of 100,000 cells.

5 Results and discussion

To elucidate the genetic basis of hyperuricemic nephropathies and to solve pathogenetic mechanisms leading to the clinical and biochemical signs and subsequent kidney failure we systematically collect samples (urine, blood, tissue biopsies) from patients and families and analyze clinical, biochemical, molecular and physiological results.

We use linkage analysis, candidate gene prioritizing, candidate gene sequencing and functional analysis of identified mutated genes. Finally, we correlate clinical, biochemical and

histological findings with our experimental data to identify pathophysiology of the disease.

5. 1 Identification and characterization of mutations in *UMOD* gene (locus 16p.11.2) in FJHN1/MCKD2 patients

In 19 families, we investigated relevant biochemical parameters, performed linkage analysis to known disease loci, sequenced uromodulin gene, expressed and characterized mutant uromodulin proteins, and performed immunohistochemical and electronoptical investigation in kidney tissues. We proved genetic heterogeneity of the disease. Uromodulin mutations were identified in six families. Expressed, mutant proteins showed distinct glycosylation patterns, impaired intracellular trafficking, and decreased ability to be exposed on the plasma membrane, which corresponded with the observations in the patient's kidney tissue. We found a reduction in urinary uromodulin excretion as a common feature shared by almost all of the families. This was associated with case-specific differences in the uromodulin immunohistochemical staining patterns in kidney.

Our results suggest that various genetic defects interfere with uromodulin biology, which could lead to the development of the common disease phenotype. 'Uromodulin-associated kidney diseases' may be thus a more appropriate term for this syndrome [11].

5. 2 Quantitative and qualitative changes in urinary UMOD excretion in Fabry disease patients

To gain knowledge of the spectrum of UMOD changes in various genetic diseases with renal involvement we examined urinary UMOD excretion and found significant quantitative and qualitative changes in 15 male patients at various clinical stages of Fabry disease. In untreated patients, the changes ranged from normal to a marked decrease, or even absence of urinary UMOD. This was accompanied frequently by the presence of aberrantly processed UMOD lacking the C-terminal part following the K432 residue. The abnormal patterns normalized in all patients on enzyme replacement therapy and in some patients on substrate reduction therapy.

Immunohistochemical analysis of the affected kidney revealed abnormal UMOD localization in the thick ascending limb of Henle´s loop and the distal convoluted tubule, with UMOD expression inversely proportional to the degree of storage.

Our observations warrant evaluation of tubular functions in Fabry disease and suggest UMOD as a potential biochemical marker of therapeutic response of the kidney to therapy [35].

5. 3 Identification of a new locus for uromodulin-associated kidney disease (UAKD) on chromosome 1q41

We identified a new candidate *UAKD* locus on chromosome 1q41 in family BE1.We analyzed and found no linkage to this region in eight additional families, who did not map to the previously established loci (16p11.2, 1q21). We noted that affected individuals showed, in addition to the characteristic urate hypoexcretion, significant reductions in urinary excretion of calcium and UMOD.

The immunohistochemical analysis of kidney biopsies showed that low UMOD excretion in family BE1 originated from significantly reduced UMOD expression. This observation is clearly different from the characteristic intracellular UMOD accumulation observed in the previously studied cases of FJHN with UMOD mutations and suggests that various defects affecting UMOD biology may play a central role in development of UAKD [4].

5. 4 Identification and characterization of dominant renin gene mutations associated with early onset anemia, hyperuricemia, and chronic kidney failure (FJHN2)

Through linkage analysis and candidate gene sequencing, we identified four unrelated families with the autosomal-dominant inheritance of early onset anemia, hypouricosuric hyperuricemia, progressive kidney failure, and mutations resulting in: **i)** the deletion (p.Leu16del); **ii)** the amino acid exchange (p.Leu16Arg) of a single leucine residue; **iii)** the amino acid exchange (p.Cys20Arg) of a single cysteine residue; in the signal sequence of renin.

All mutations decrease signal sequence hydrophobicity and are predicted by bioinformatic analyses to damage targeting and cotranslational translocation of preprorenin into the endoplasmic

reticulum (ER). Transfection and *in vitro* studies confirmed that mutations affect ER translocation and processing of nascent preprorenin, resulting either in reduced (p.Leu16del) or abolished (p.Leu16Arg and p.Cys20Arg) prorenin and renin biosynthesis and secretion.

Expression of renin and other components of the renin-angiotensin system was decreased accordingly in kidney biopsy specimens from affected individuals. Cells stably expressing the p.Leu16del protein showed activated ER stress, unfolded protein response, and reduced growth rate.

It is likely that expression of the mutant proteins has a dominant toxic effect gradually reducing the viability of renin-expressing cells. This alters the intrarenal renin-angiotensin system and the juxtaglomerular apparatus functionality and leads to nephron dropout and progressive kidney failure.

Our findings provide insight into the functionality of renin-angiotensin system and stress the importance of renin analysis in families and individuals with early onset hyperuricemia, anemia, and progressive kidney failure [10].

5. 5 Future research

In recent years the pathological and clinical approach to the diagnosis of FJHN and MCKD has been shown to be insufficient to distinguished different molecular mechanisms responsible for clinical signs and symptoms. Using positional cloning approach we identified and described mutations in two genes with very different kidney localization and biological function. It implies that future research must be aimed at further dissection of genetic heterogeneity of FJHN by genetic mapping approach. This will be greatly facilitated within next couple of years by availability and application of novel sequencing technologies which will enable massive resequencing of candidate loci identified in previous linkage studies and also by whole-exome and later also whole-genome sequencing studies in affected individuals. This approach will inevitably lead to definition of all of FJHN causing genes and together with subsequent targeted molecular studies it will suggest key mechanisms and pathways involved in pathogenesis of clinical and biochemical symptoms. This may open an avenue to early presymptomatic

diagnosis in affected individuals and families, development of targeted treatments and early intervention reducing and/or preventing development of kidney disease.

6 Conclusions

Within the frame of long-term aim of our research group my work resulted in and contributed to the following points:

- **We confirmed extensive genetic heterogeneity of familial hyperuricemic nephropathies**
  - We identified new locus on chromosome 1q41 segregates with the disease.
  - Only 40% of cases link to known loci associated with the disease (16p11.2, 1q21, 17q21.3 and 1q41).
- **We identified and functionally characterized several mutations in *UMOD* gene (locus 16p.11.2) in FJHN1/MCKD2 patients**
  - Mutated UMOD showed aberrant glycosylation patterns, impaired cellular trafficking, and decreased ability to be exposed on the plasma membrane of transfected cells compared to wild type UMOD. These changes in UMOD protein processing led to various degree of UMOD accumulation in endoplasmic reticulum which could induce stress of endoplasmic reticulum.
  - The observed accumulation of mutated UMOD in the endoplasmic reticulum corresponded with the histological finding in patient´s kidney.
- **We suggested UMOD as a key molecule in pathogenesis of development of hyperuricemic nephropathies, FJHN1/MCKD2 and FJHN2**
  - We found that FHJN1/MCKD2 and FJHN2 diseases of different genetic background are commonly associated with reduction of UMOD urinary excretion.
- **We defined and characterized molecular and pathogenetic mechanisms of new disease now named familial juvenile hyperuricemic nephropathy type 2 (FJHN2)**

- We described patients with early onset hyperuricemia, anemia and chronic kidney failure and identified mutations in renin signal sequence (locus 1q41) segregated with this phenotype in 4 unrelated families.
- This phenotype was enrolled into database of human genes and genetic disorders Online Mendelian Inheritance in Man (OMIM) administrated by National Center for Biotechnology Information (NCBI) as new disease under evidence number 613092 and named familial juvenile hyperuricemic nephropathy type 2 (FJHN2).
- All identified mutations significantly impaired prorenin and renin biosynthesis, secretion and enzymatic activity.
- We proposed that the identified mutations in renin signal sequence likely expose juxtaglomerular cells to chronic ER stress and lead to site-specific attenuation of renin biosynthesis and RAS dysregulation. Reduced viability of juxtaglomerular cells and limited renin availability then affect renal development, intrarenal RAS homeostasis, and kidney blood flow autoregulation resulting in anemia, reduced glomerular filtration rate, and hyperuricemia.
- Important therapeutic implications are based on our finding and are currently tested on selected patients

➢ **We established ongoing co-operation with nephrology clinicians and researchers from Czech Republic, Europe and USA.**

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8 List of author´s publications, awards, presentations and grants

8. 1 List of author´s publications

Hodanová K, Majewski J, **Kublová M**, Vyletal P, Kalbácová M, Stibůrková B, Hůlková H, Chagnon YC, Lanouette CM, Marinaki A, Fryns JP, Venkat-Raman G, Kmoch S.**:** *Mapping of a new candidate locus for uromodulin-associated kidney disease (UAKD) to chromosome 1q41.*
Kidney Int. 2005 Oct; 68(4):1472-82., **IF 4, 927**

Vylet'al P*, **Kublová M***, Kalbácová M, Hodanová K, Baresová V, Stibůrková B, Sikora J, Hůlková H, Zivný J, Majewski J, Simmonds A, Fryns JP, Venkat-Raman G, Elleder M, Kmoch S.: *Alterations of uromodulin biology: a common denominator of the genetically heterogeneous FJHN/MCKD syndrome.*
Kidney Int. 2006 Sep; 70(6):1155-69. Epub 2006 Aug 2, **IF 4, 773** *** these authors contibuted equally**

Vylet'al P, Hůlková H, **Zivná M**, Berná L, Novák P, Elleder M, Kmoch S.: *Abnormal expression and processing of uromodulin in Fabry disease reflects tubular cell storage alteration and is reversible by enzyme replacement therapy.*
J Inherit Metab Dis. 2008 Aug;31(4):508-17. Epub 2008 Jul 27, **IF 2,691**

Zivná M, Hůlková H, Matignon M, Hodanová K, Vylet'al P, Kalbácová M, Baresová V, Sikora J, Blazková H, Zivný J, Iványek R, Stránecký V, Sovová J, Claes K, Lerut E, Fryns JP, Hart PS, Hart TC, Adams JN, Pawtowski A, Clemessy M, Gasc JM, Gübler MC, Antignac C, Elleder M, Kapp K, Grimbert P, Bleyer AJ, Kmoch S.: *Dominant renin gene mutations associated with early-onset hyperuricemia, anemia, and chronic kidney failure.*Am J Hum Genet. 2009 Aug;85(2):204-13. Epub 2009 Aug 6, **IF 10,153**

Anthony J. Bleyer, **Martina Živná**, Helena Hůlková, Kateřina Hodaňová, Petr Vyleťal, Jakub Sikora, Jan Živný, Jana Sovová, Thomas C. Hart, Jeremy N. Adams, Milan Elleder, Katja Kapp, Robert Haws, Sharon M. Moe, Lynn D. Cornell, Stanislav Kmoch, and P. Suzanne Hart: *Clinical and Molecular Characterization of a New Disorder Resulting From Dominant Renin Gene Mutations and Response to Treatment with Fludrocortisone* (submitted manuscript)

8. 2 List of author´s awards

2003 **3rd place in poster session on conference 18th Metabolic Days**, Slusovice, CR
Title of poster: Experimental annotation of a transcription map of familial juvenile hyperuricemic nephropathy (FJHN) critical region on 16p13.12

2005 **Among three best posters on conference 20th Metabolic Days**, Lednice, CR
Title of poster: Familial juvenile hyperuricemic nephropathy (FJHN), molecular analysis of 23 families, identification and functional consequences of 6 uromodulin mutations

2008 **Best poster award on Gordon Research Conference** Proprotein Processing, Trafficking & Secretion, Colby-Sawyer College, New London, NH, USA
Title of poster: Preprorenin signal peptide mutation in a family with uromodulin-associated kidney disease

2009 **Bolzano´s award** – the academic award intended for students who published innovative publication on the medical, natural and humanities field of science
Title of project: Study of molecular basis of familial hyperuricemic nephropathies

8. 3 List of author´s grants (PI)

2005 – 2006 Grant awarded by Grant Agency of Charles University, registration number of grant 1/2005 (evidence number 203 200)
Title of project: Pathogenetic mechanisms of mutations in uromodulin and in other genes causing familial juvenile hyperuricemic nephropathy (FJHN)

2007 Grant awarded by Grant Agency of Charles University, registration number of grant 67207
Title of project: Familial juvenile hyperuricaemic nephropathy (FJHN): Identification and characterization of disease causing gene in chromosome 1q41 candidate region

2009 Grant awarded by Grant Agency of Charles University, registration number of grant 44309
Title of project: Analysis of new patients and families with familial juvenile hyperuricemic nephropathy and functional characterization of novel uromodulin (Tamm-Horsfall) mutations

8. 4 List of author´s presentations

Kublová M., Kmoch S.: Experimental annotation of a transcription map of FJHN critical region on 16p13.12, Poster presentation. 18th workshop Inherited Metabolic Disorders, Slušovice, Czech Republic, 2003

Kublová M., Vyleťal P., Hodaňová K., Kalbáčová M., Blažková H., Majewski J., Simmonds A., Matthijs G. and Kmoch S.: Uromodulin urinary excretion in patients with familial juvenile hyperuricaemic nephropathy (FJHN). Poster presentation. 19th workshop Inherited Metabolic Disorders, Podbanské, Slovakia, 26. - 28. 5. 2004.

Kublová M., Vyleťal P., Hodaňová K., Kalbáčová M., Hůlková H., Majewski J., Simmonds A., Matthijs G. and Kmoch S.: Uromodulin urinary excretion in patients with familial juvenile hyperuricaemic nephropathy (FJHN). Poster presentation. 45th Annual Short Course

in Medical and Experimental Mammalian Genetics, The Jackson Laboratory, Bar Harbor, Maine, USA, 18. - 30. 7. 2004.

Kublová M.: Familial Juvenile Hyperuricemic Nephropathy (FJHN): Pathogenetic mechanisms of mutations in *UMOD*. Oral presentation. 6th student´s scientific conference 1st Faculty of Medicine, Charles Univerzity, Prague, 23. 5. 2005

Kublová M., Vyleťal P.**,** Hodaňová K., Barešová V., Kalbáčová M., Sikora J., Živný J., Sovová J., Majewski J., Marinaki A., Simmonds A., Fryns J.-P., Venkat-Raman G. and Kmoch S.: Familial Juvenile Hyperuricaemic Nephropathy (FJHN), molecular analysis of 23 families, identification and functional consequences of 6 uromodulin mutations. Poster presentation. 37th European Human Genetics Conference, Prague, Czech Republic, 7. - 10. 5. 2005.

Kublová M., Vyleťal P., Hodaňová K., Barešová V., Kalbáčová M., Sikora J., Živný J., Sovová J., Majewski J., Marinaki A., Simmonds A., Fryns J.-P., Venkat-Raman G. and Kmoch S.: Familial Juvenile Hyperuricaemic nephropathy (FJHN), molecular analysis of 23 families, identification and functional consequences of 6 uromodulin mutations. Poster presentation. 20th workshop Inherited Metabolic Disorders, Lednice, Czech Republic, 18. - 20. 5. 2005.

Kublová M., Hodaňová K., Majewski J., Vyleťal P., Kalbáčová M., Stibůrková B., Hůlková H., Chagnon Y. C., Lanouette Ch. M., Marinaki A., Fryns J.-P., Venkat-Raman G. and Kmoch S.: Mapping of a new candidate locus for uromodulin-associated kidney disease (UAKD) to chromosome 1q41. Poster presentation. Functional Genomics and Disease, 2nd ESF Functional Genomics Conference, Oslo, Norway, 6. - 10. 9. 2005.

Živná M., Vyleťal P., Kalbáčová M., Hodaňová K., Barešová V., Stibůrková B., Sikora J., Hůlková H., Živný J., Majewski J., Simmonds A., Fryns J.-P., Venkat-Raman G., Elleder M. and Kmoch S.: Alterations of Uromodulin Biology - a Common Denominator of the Genetically Heterogeneous FJHN/MCKD Syndrome. Poster presentation. The American Society for Cell Biology - 46th Annual Meeting, San Diego, USA, 9. - 13. 12. 2006.

Vyleťal P., Hůlková H., **Živná M.**, Berná L., Novák P., Elleder M. and Kmoch S.: Abnormal processing of uromodulin (UMOD) in Fabry disease patients reflects kidney tubular cell storage alteration and is reversible by enzyme replacement therapy. Poster presentation. The American Society for Cell Biology - 47th Annual Meeting, Washington, DC, USA, 1. - 5. 12. 2007.

Zivna M, Hodanova K, Vyletal P, Sikora J, Baresova V, Hulkova H, Kalbacova M, Zivny J, Ivanek R, Kapp K, Elleder M and Kmoch S: Preprorenin signal peptide mutation in a family with uromodulin-associated kidney disease. Poster presentation. Proprotein Processing, Trafficking and Secretion**,** Gordon Research Conference, New Hampshire, USA, 13. – 18. 7. 2008

K. Hodanova, **M. Zivna**, P. Vyletal, M. Votruba, G. Venkat-Raman, T. Ring, S. Kmoch: Novel uromodulin mutations identified in 8 out of the 21 probands with uromodulin associated kidney disease (UAKD). Poster presentation. The American Society of Human Genetics – 59th Annual Meeting, Honolulu, Hawaii, USA, 20. – 24. 10. 2009

J. N. Adams, A. J. Bleyer, T. C. Hart, **M. Zivna**, S. Kmoch, P. S. Hart: Identification of the second North American family with *REN*-associated medullary cystic kidney disease. Poster presentation. The American Society of Human Genetics – 59th Annual Meeting, Honolulu, Hawaii, USA, 20. – 24. 10. 2009