PROJECT FINAL REPORT

Project acronym:	**EURenOmics**
Project title:	**European Consortium for High-Throughput Research in Rare Kidney Diseases**
Period covered:	**from 01.10.2012 to 30.09.2017**

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Table of Contents

Final Publishable Summary Report

1.1 Executive Summary

Rare kidney diseases impact markedly on life expectancy and quality of life. At the outset of this project, disease understanding and clinical management were compromised by a limited physiopathological knowledge base, low rates of diagnostic ascertainment, the absence of biomarkers predicting the risk and rate of disease progression, and a lack of effective therapies and disease models suitable for therapeutic research.

EURenOmics focused on steroid-resistant nephrotic syndrome (SRNS), membranous nephropathy (MN), hereditary tubulopathies, complement disorders, and malformations of the kidney and urinary tract (CAKUT). The consortium had access to >15,000 well phenotyped patients with >10,000 DNA, >3,000 serum, 2,000 urine, 500 amniotic fluid and 3,000 kidney biopsy specimens, and applied a wide range of high-throughput technologies, systems biology approaches and a plethora of *in vitro*, *ex vivo* and *in vivo* models to study disease mechanisms and explore novel therapeutic approaches.

A 'renal phenome' database integrating clinical information from various registries and a kidney-focused bioinformatic analysis pipeline were established. Exome sequencing performed in 315 families led to the discovery of 37 new disease genes and genomic rearrangements. Furthermore, NGS panels capturing all known genes per disease group were developed and applied in more than 4,200 patients, establishing a genetic diagnosis in 65% of tubulopathy, 40-50% of aHUS, 20-25% of SRNS, and 6-10% of CAKUT patients. Integration of clinical databases and genetic findings allowed deep phenotyping and genotype-phenotype association studies in more than a dozen large patient cohorts with defined genetic diagnoses.

The availability of large biorepositories fostered unbiased searches for diagnostic and prognostic biomarkers. Integrative bioinformatic analysis based on tissue transcriptome information unsurfaced urinary EGF as a novel biomarker of renal failure progression, which was confirmed in adult and pediatric cohorts. Peptidomic, metabolomic and microRNA profiling of body fluids yielded promising molecular signatures discriminating disease entities in SRNS and complement diseases, as well as poor from favourable prognosis in MN and prenatal CAKUT.

Fundamental progress in the understanding of genetic disease penetrance was achieved. For the first time, combinatorial mutation-dependent pathogenicity was described in compound heterozygous NPHS2 nephropathy. Common gene variants associated with disease risk were identified in MN, aHUS, and hypomagnesemia as novel mechanisms explaining variable phenotype expression.

Major progress was also made concerning molecular mechanisms of auto-immune disease. The target epitope of autoantibodies in MN and the HLA class-II peptidome were identified, the latter paving the way for unbiased identification of immune response targets in autoimmune diseases. In aHUS CFH autoantibody formation was found to emerge from deficient B-cell regulation by CFHR3 deficiency, demonstrating a mechanistic link between genetic disease and autoimmunity.

Remarkably, our research in rare nephropathies unsurfaced clues to general risk factors for common kidney diseases; e.g., common variants in the ATKD gene UMOD predispose to chronic kidney disease and hypertension, and abnormalities in the complement gene CFHR1 affect the risk and progression of IgA nephropathy.

In the search for new therapies, drug re-purposing allowed rapid progress in several areas such as rituximab for MN, eculizumab for secondary aHUS, and CoQ10 for hereditary mitochondrial podocytopathies. Promising new molecular therapies are tested experimentally, including monoclonal complement antibodies and flavaglines in hypomagnesemia. High-throughput compound screening in novel cell and animal models has identified repurposable drugs reversing the pathogenic effects of mutations in podocytopathies and ciliopathies, and models suitable for compound screening have been established for several hereditary tubulopathies, metabolic nephropathies and complement disorders.

In conclusion, the research results of EURenOmics will impact substantially on diagnostic management, risk assignment and therapeutic approaches in patients with rare kidney diseases, with potential implications also for patients with common kidney and other diseases and the society at large.

1.2 Summary description of project context and objectives

Project Context

More than 200 rare kidney diseases have been described, with an overall prevalence of about 60-80 cases per 100,000 total population. In contrast to many other rare diseases, patients with inherited or acquired kidney disorders rarely die when their disease progresses but - thanks to progress in organ replacement therapy - may **remain alive** for many years. However, this apparent advantage is frequently bought at the expense of **severely compromised health with poor quality of life**, and causes a tremendous **cumulative cost burden** to health care systems.

At the outset of the EURenOmics project, the **diagnostic and therapeutic management** of rare kidney diseases was **highly unsatisfactory.** Key unsolved issues included the inability fo explain the underlying inherited or acquired abnormality responsible for the disease phenotype, predict the individual risk and rate of disease progression, or quantitate the risk of relatives to develop the same disorder. Despite progress in gene discovery, the genetic and molecular basis of disease was still unknown in the majority of patients. Even in patients with known genetic disease causes, individual risk prediction was limited by considerable phenotypic variability putatively related to complex genetic disease transmission and/or gene-environment interaction. On the other hand, there was preliminary evidence suggesting that different initial events may converge to common pathways of disease progression. Neither the **clinical heterogeneity** within individual disorders nor the **commonalities between clinically disparate kidney diseases** were well understood, and clinically applicable technologies for the molecular characterisation of diseases and their risk of progression were lacking. Specific therapies effectively targeting disease activity and progression were, with a few notable exceptions, unavailable or limited to poorly defined disease subgroups; in the latter cases, biomarkers predicting individual susceptibility to pharmacotherapy were lacking. Finally, the **lack of suitable disease models** for many kidney disorders was conceived as a major barrier to progress in therapeutic development.

Hence, it was felt that to consistently improve the medical management and health outcomes of patients with rare kidney diseases, major progress would be needed in **four areas of research**:

- Identification of the genetic and environmental initiators and modifiers of disease and their molecular pathways;
- Re-definition of diseases according to molecular signatures and mechanistic principles beyond phenotypic or morphological description;
- Development of rapid and reliable diagnostic tests and biomarkers to allow better identification of progression risks and monitoring of disease activity;
- Development and application of disease models suitable to screen for novel molecular therapies.

Progress in -omics technologies had opened a window of opportunity for rapid substantial progress in each of these areas, and diseases of the **kidney appeared particularly suited to -omics approaches** due to the opportunity to examine molecular events in the end organ that is manifesting the disease: *kidney biopsy*, a standard diagnostic procedure, provides a unique opportunity to study intrarenal biological processes *ex vivo* using transcriptomic, proteomic and morphological approaches. *Urine* is a readily available non-invasive bio resource to study molecular readouts directly derived from the organ of interest, the kidney. *Amniotic fluid*, the fetal urine, allows prenatal epigenetic, proteomic and metabolomic profiling in the context of renal maldevelopment. Recent technological progress in exosome isolation from urine and amniotic fluid even has created the opportunity to study non-invasively cellular biomaterials originating from the diseased tissues. The availability of such samples greatly facilitates the development of biomarkers.

EURenOmics chose **five groups** of rare kidney diseases with an **urgent need and significant potential** for diagnostic and therapeutic progress: Steroid-resistant nephrotic syndrome (SRNS, WP2), membranous nephropathy (MN, WP3), tubulopathies (WP4), complement disorders (WP5) and congenital anomalies of the kidney and urinary tract (CAKUT, WP6). These disease entities were felt paradigmatic for the clinical challenges described above.

Objectives and Work Strategies

WP2: Steroid Resistant Nephrotic Syndrome (SRNS)

Objectives

- Identification and functional characterization of new disease-causing and modifier genes in SRNS
- Development and validation of a new disease ontology for SRNS by integration of phenotypic and genetic information with molecular signatures derived from tissue and urine analysis
- Development and implementation of rapid diagnostic tools and biomarkers
- Develop novel molecular therapies for genetic SRNS

Work strategy

Task 1: Identify novel disease causing and modifying genes underlying SRNS

a) Exome sequencing of 100 familial SRNS cases without mutations in known genes

b) Whole-genome sequencing of selected sporadic cases to catalogue all rare variants in coding and regulatory sequences of all podocyte genes

Task 2: Characterization of podocyte transcriptional and epigenetic network

Discovery of genomic key regulatory elements in podocytes by ChIP-Seq analysis via identification of genome-wide distribution of active epigenetic marks and transcription factor binding sites

Task 3: Functional characterization of novel disease associated genes

Studies of expression pattern and subcellular localization of normal and mutated gene products, functional studies in podocyte cell culture and animal models

Task 4: Deep phenotyping towards new disease ontology for SRNS

Definition of common data elements describing phenotypes in all available SRNS cohorts

Genotyping of all sporadic cases for all known SRNS genes

Comparative urine peptidome, metabolome, miRNAome profiling in different SRNS subtypes

Task 5: Development of diagnostic tools and prognostic biomarkers

a) Development of a gene diagnostic tool using a combination of multiplex PCR and high throughput sequencing Update of kit by the new disease genes identified in the course of the project

b) Biomarkers of disease progression and post-transplant recurrence Comparative analysis of urinary peptidome, miRNAome and tissue mRNA expression patterns

Task 6: High-throughput compound screening in cell models of genetic SRNS

In vitro bio-assays (cell lines stably expressing wild-type and mutant, untagged or GFP-tagged podocin and nephrin); high-throughput library screening for active compounds restoring intracellular trafficking of ER-retained mutated podocyte membrane proteins (nephrin, podocin)

Task 7: Testing of candidate substances in a knock-in mouse model

in vivo testing of candidate compounds inducible knock-in podocin nephropathy mouse model

Task 8: High-throughput compound screening in Zebrafish models

Development of R138Q-podocin-GFP transgenic line, high-throughput compound screening for molecules rescuing phenotype or retargeting mutant podocin to plasma membrane.

WP3: Membranous Nephropathy

Objectives

Our project objectives were i) to establish a large database of deeply phenotyped patients and related bioresources, ii) to identify pathogenic B-cell epitopes, novel antigens and gene variants responsible for disease initiation, progression, and recurrence in the graft, iii) to characterize T-cell epitopes and T-cell regulation, iv) to develop proprietary assays for the identified biomarkers and assess their diagnostic and predictive values in large cohorts, and v) to develop new therapeutic strategies and more personalized care and monitoring of patients with MN.

Work strategy

Task 1: Establish and harmonize a large database of deeply phenotyped patients and related bioresources

Task 2: Identify pathogenic epitopes responsible for disease initiation, progression and recurrence in the graft

Task 3: Identification of pathogenic gene variants

Task 4: Testing the pathogenic effects of epitopes and gene variants

Task 5: Analysis of antigen presentation and T-cell response

Task 6: Revisiting current therapies and testing new therapeutic approaches

Task 7: Development of proprietary assays for the identified biomarkers and evaluation of their diagnostic and predictive values: towards a new ontology

WP4: Tubulopathies

Objectives and Work strategy

- Identification of novel genes involved in causing or modifying tubular disorders
  - Whole-exome sequencing of families with unexplained tubulopathies
- Development of a novel diagnostic tool for rapid complete screening of mutations and variants in known renal tubular disease genes
  - Multiplex PCR/NGS based tubulopathy gene panel kit
- Reference -omics profiling and deep phenotyping to refine ontology and predict progression
  - Urinary metabolomics, proteomics, and miRNA profiling of different tubulopathy entities
  - Peptide microarray to identify novel antigens in autoimmune form of Sjogren syndrome
  - GWAS of tubular phenotypes in large cohort (>6,000 subjects)
- Functional characterization of mutated renal gene products (Functiomics)
  - in vitro protein function test, cell culture assays, in vivo models (zebrafish, KO/KI mice)
- Deep phenotyping of the SLC12A3 (Gitelman syndrome) carrier state
- Development of preclinical disease models and large-scale in vivo drug discovery screens
  - Xenopus models

WP5: Complement Disorders

Objectives

- To identify genetic and acquired initiators and modifiers of aHUS, MPGNI, DDD, and C3GN
- To develop technologies for diagnosis and screening of patients
- To find out disease-specific deep transcriptomic and proteomic profiles
- To discover biomarkers predicting response to therapies and post-transplant disease recurrence
- To develop in vitro, ex vivo and in vivo models to test preventive and therapeutic interventions

Work strategy

- Whole exome screening (WES), targeted NGS, copy number variation analysis and family and association studies
- in vitro and ex vivo assays of complement activation on recombinant and purified mutant proteins to characterize functional consequences of mutations and gene rearrangements.
- Geno-/immunotype-phenotype analysis in >3000 aHUS and 400 IC-MPGN/C3G patients, including analysis of response to therapies and outcome of kidney transplant.
- High resolution sequencing of polymorphic regions of HLA genes to search for genetic determinants of anti-FH autoantibodies in aHUS.
- Analysis of antibody interaction with FH, complement proteins, AP-C3 and C5 convertase in IC-MPGN and C3G to identify new autoimmune disease causes
- Design of NGS complement gene panels for rapid diagnostic screening
- Urine/plasma proteome, plasma exosome miRNA profiling in aHUS, IC-MPGN and C3G
- Development of new ex vivo and in vitro assays to predict response to eculizumab and to test new complement inhibitors developed during project period
- Mouse models of aHUS and C3G mutations obtained by transgenesis or knock-in strategies
- Development of Xenopus knockdown models of complement-related kidney diseases.

WP6: Congenital Anomalies of the Kidneys and the Urinary Tract

Objectives

- To identify novel candidate genes and elucidate the complex genetics of CAKUT.
- To establish the transcriptional networks driving renal development.
- To functionally characterize CAKUT genes
- To define prenatal (advanced) imaging and prognostic molecular biomarkers for CAKUT.
- To develop and implement a novel DNA diagnostic tool for CAKUT.

Work strategy

Task 1: Identification of CAKUT genes by next generation sequencing

- Exome sequencing of familial, syndromic and severe bilateral cases

Task 2: Establishment of the transcriptional networks driving renal development analysis

- In vivo ChIP-Seq analysis for key transcription factors involved in mouse kidney development

Task 3: Functional characterization of CAKUT genes

- In vivo-morpholino knockdown, ex vivo organ cultures, gene-targeting in animal models and iPSC

Task 4: Use of amniotic fluid -omics and advanced imaging to predict postnatal renal function

- Peptidomic, miRNomic and metabolomic profiling of fetuses with favourable vs. poor postnatal outcome
- Deep phenotyping by 3D ultrasound with automated volume count and virtual organ computer-aided analysis

Task 5: Development and implementation of a NGS-based diagnostic tool for CAKUT

- Newly identified CAKUT genes will be included to design CAKUTome NGS gene panel

WP7: Integrate Bioinformatics

Objectives

- Development of integrative biomedical knowledge generation architecture of rare renal diseases

- Multilevel and hierarchical deep data analysis along the genotype-phenotype continuum
- Human disease and model data integration beyond common ontologies
- Interaction and data integration with RD-Connect

Work strategy

Task 1: Implement central data and analysis domain providing computational processing, data and information transformation, and analysis and visualization of multiscale biomedical information from multiple sources. Semi-automatic conversion and analysis of high-throughput data to standardize -omics data including genomic, epigenetic and transcriptomic profiles by array and sequencing techniques, proteomic and metabolomic analyses and comprehensive clinical data

Task 2: Provide direct knowledge-based network analyses, co-citation analyses and relevance filtering of individual data with network-based stratification of entire patient cohorts. Screen disease-specific data sets for pathway perturbations and putative causal events using knowledge integration and extraction. Identify common networks correlating with a given disease, disease stage, or phenotype.

Task 3: Screen clinical and molecular datasets procured in task1 and 2 for common elements during renal disease.

Task 4: Coordinate activities with corresponding RD-Connect WPs to take full advantage of the integrative approach.

1.3 Description of the main S&T results/foregrounds

WP1: Project Management

Development of the Project website

A Consortium website (www.eurenomics.eu) was developed which informs the public about the project, its objectives and partners as well as of its dissemination activities. The website was constantly updated to disseminate news, inform about upcoming events and keep track of all publications emerging from the project. Meeting registrations were handled via the website as well.

Co-ordination activities

The Coordinator's Office guided the overall scientific and administrative implementation of the project. Regular face-to-face meetings and teleconferences of the steering committee, as well as meetings among project partners, were organized throughout the project's lifetime to adjust the strategies and measures required for the successful execution of the project. The Coordinator was also instrumental in establishing and strengthening collaborative links between partners within the consortium and in associating interested external research groups to the project. Over the years, strong collaborative ties were established with numerous European and international scientists, who were granted the status of Asscociated Partners.

Collaboration with RD-CONNECT

The Coordination team has worked closely with RD-Connect at multiple levels. This included issues such as central Omics data storage (EBI-EGA, Metabolights data archives) and data sharing, establishing an inventory of biobanks, harmonization of phenotyping, and the development of generic ethics documents. Towards the end of the project, EURenOmics partners started using the RD-Connect exome analysis pipeline and have uploaded sequencing data to the central platform. Furthermore, several EURenOmics partners participated in a Multi-Omics Task Force organized by RD-Connect. The members of the Ethics Advisory Board of EURenOmics contributed to the Patient and Ethics Council (PEC) of RD-Connect. In 2013, 2014 and 2017 joint meetings of RD-Connect, EurenOmics and Neuromics were organized.

To gauge the satisfaction of the EURenOmics beneficiaries with the overall execution of the project, we initiated a '**project end survey'** via Survey Monkey. Seventy-nine responses were received, and the results indicate a very positive outcome of this project. The mean scores are given in Table 1 below.

Table 1: Results of EURenOmics project end survey

	Mean Score (1-5; 5 being the best), n=79
How would you rate the coordination of EURenOmics (Scientific and Administrative Project Management)?	4.78
How would you rate the support through the Project Management Office?	4.65
How would you rate the execution of the scientific work in EURenOmics?	4.65
How would you rate the exchange of data in EURenOmics?	3.92
How would you rate the dissemination activities in EURenOmics?	4.39

Also, the majority of the participants indicated that they would not have done anything differently in terms of Science and Coordination (73%) or felt that any scientific aspect was left out (71%). Finally, most would also join another European funded project (82%) or have already done so (12.5%).

WP2: Steroid Resistant Nephrotic Syndrome

Tasks 1a and 3: Identification and characterization of new SRNS disease-causing genes

The main strategy to identify new causes of monogenic forms of SRNS was to perform exome sequencing in multiplex families. This was achieved in 103 families and causative mutations were found in 40.7% families (Partners 1, 2a, 4 and 7).

This allowed the identification of mutations in 11 new genes and 2 strong candidates genes. Among them, 7 were mutated in a specific subset of patients with Galloway-Mowat syndrome (GAMOS) associating SRNS, microcephaly and brain developmental disorders. In addition, Partner 7 contributed to the demonstration that mutations in the nuclear pore genes *NUP93, NUP205* and *XPO5* also cause SRNS (Braun D et al. Nat Genet 2016).

Functional studies using cell and animal models were performed in most cases and allow the identification of new pathophysiologic mechanisms underlying SRNS. The main results are summarized below:

Enzymes and biosynthesis/degradation pathways (Asraf S et al., J Clin Invest, 2013, Korkmaz et al., J Am Soc Nephrol 2016, Lovric et al., J Clin Invest 2017). Partner 1, 2a and 4 identified mutations in ADCK4 and SGPL1, two enzymes involved in the biosynthesis pathway of COQ10 and degradation of S1P, respectively, leading to the discovery of new pathophysiological pathways in AR SRNS pathogenesis that allow therapeutic approaches for individuals with this type of SRNS (COQ10 complementation, S1P receptor antagonist, respectively).

Regulation of microtubule dynamics and organization (Huynh Cong E et al. J Am Soc Nephrol. 2014; Colin E et al.; Am J Hum Genet. 2014). Partner 2a identified a homozygous missense mutation in the ciliary gene TTC21B which unexpectedly causes a dual phenotype of both primary tubulointerstitial and glomerular lesions, describing a new class of primary tubulo-glomerular disorders that might be due to mutations in ciliary genes. The product of the TTC21B gene, IFT139, mainly localizes at the base of the primary cilium in developing podocytes, whereas it relocalises along the extended microtubule (MT) network in nonciliated adult podocytes, suggesting a role in the regulation of MT organization and dynamics in mature podocytes. Such a tubulo-glomerular disorder was also described by Partner 7 who demonstrated the presence of glomerular lesions in patients with mutations in ANKS6, also encoding a ciliary protein (Taskiran EZ et al., JASN 2014).

A role on MT network regulation has also been attributed to WDR73, encoded by the first gene ***(WDR73)*** found mutated in patients with GAMOS (partner 2a). Partner 2a experiments and those of other teams, in various cell models and in zebrafish, clearly suggest a role for WDR73 in cell survival, MT regulation and neuronal progenitor proliferation and differentiation. Indeed, WDR73 is recruited at the spindle poles and MT aster during mitosis, a behavior similar to proteins mutated in primary microcephaly.

New pathophysiological pathways in GAMOS (**Braun et al. *Nat Genet*. 2017**). Very recently, Partner 2a identified, in collaboration with the group of F. Hildebrandt (Boston, USA), four genes (***LAGE3, OSGEP, TP53RK, TPRKB***) mutated in 37 individuals of 33 families with GAMOS. These genes encode the four subunits of the KEOPS complex, a very ancient and essential multi-protein complex that catalyses a universal chemical modification on tRNA, the formation of a N-6-threonylcarbamoyladenosine (t6A) at position 37 in ANN decoding tRNAs, necessary for translation initiation and translational efficiency. Functional *in vitro* and *in vivo* studies of these mutations led to the identification of alterations in multiple cellular processes, such as proliferation, apoptosis, migration and protein translation. In addition, an induction of endoplasmic reticulum (ER) stress with subsequent activation of the unfolded protein response has been observed in podocyte cell lines inactivated for the OSGEP, TP53RK and TPRKB, likely contributing to the observed proliferation defects. Some of these altered cellular processes are known to contribute to the pathogenesis of SRNS or microcephaly. Partner 2a also identified two other genes, ***C14orf142*** and ***YRDC (in collaboration with Partner***, mutated in five and two families with GAMOS, respectively, which encode proteins, involved in the KEOPS pathway. Indeed, *YRDC* encodes the second enzyme necessary for t6A formation (in addition to OSGEP in the KEOPS complex) and we

demonstrated that *C14orf142* encodes the fifth subunit of the KEOPS complex and our experimental data suggest that it stabilizes the KEOPS complex.

Potential digenic inheritance (Partner 2a in collaboration with M. Simons, Imagine Institute, Paris) **(Goncalves S et al., *submitted*).** Partner 2a also identified two homozygous missense variants in two candidate genes, ***ADD3*** and ***KAT2B***, both expressed in podocytes, in a consanguinous family with a complex phenotype associating SRNS, cardiomyopathy and neurological impairment. *ADD3* and *KAT2B* encodes adducing gamma, an important regulator of the actin cytoskeleton, and the lysine acetyl transferase2B involved in histone acetylation, respectively. The studies in podocyte cell lines and in the Drosophila model pointed to a major effect of *ADD3* and *KAT2B* in podocyte biology and showed that *ADD3* and *KAT2B* mutations are pathogenic. However, the results suggest that *ADD3* mutations are responsible for the neurological manifestations, cataracts and skeletal defects whereas *KAT2*B mutations are responsible for the heart and kidney phenotype, either alone or on a susceptible genetic background (*ADD3* mutation). These results validate the use of Drosophila for the *in vivo* study of candidate genes for SRNS.

Characterization of two new candidate genes in zebrafish: successful data-sharing strategy. Partner 2a identified potentially pathogenic mutations, one in *TRIM3* and one in *TBC1D8B* (encoding proteins involved in cytoskeleton regulation), in families with AD and X-linked SRNS respectively. Functional studies in podocyte cell lines and zebrafish allowed to confirm the pathogenic role of these mutations in the podocyte. Unfortunately, only one family was found mutated for each of these candidate genes by Partner 2a. However, during the course of EURenOmics, a list of all rare potentially pathogenic variants identified by exome sequencing in the families without identified disease-causing gene mutations has been established and made available to the partners**.** Through this strategy of data-sharing, partners 4 and 8 identified a new mutation in *TRIM3* and TBC1D8B, respectively, confirming the previous results of Partner 2a.

Deciphering the functional role of podocin (Tory K et al., *Nat Genet*, 2014; Serrano-Perez MC et al., **article in revision in *J Biol Chem***). Podocyte cell models have also been instrumental to identify a novel mechanism of mutation pathogenicity. Podocin, encoded by the *NPHS2* gene, is a membrane-associated component of the slit diaphragm in podocytes. Partner 2a demonstrated that the pathogenicity of the *NPHS2* variant, p.R229Q, is dependent on the trans-associated mutations: specific-C-terminal missense mutations exert a dominant negative effect on podocin R229Q resulting in its abnormal oligodimerization and mislocalization explaining the pathogenicity of this variant when it is associated only with certain specific mutations. More recently, this work was completed by the demonstration that some distal C-terminal truncating mutations are endocytosed and that this internalization can be prevented by the coexpression of membranous podocin variants suggesting the possibility of interallelic complementation in compound heterozygous patients (Straner P, submitted – This work has been performed mainly by K. Tory who started this work in Partner 2a's laboratory and continued it back to Hungary as associate partner to Eurenomics).

In parallel, Partner 2a studied podocin biogenesis, trafficking and degradation, in particular for the mutant p.R138Q which accounts for up to 32% of all *NPHS2 mutant* alleles, and demonstrated that this ER-retained mutant is glycosylated, adopting a transmembanous conformation at the ER and that the non-glycosylated form of p.R138Q is massively degraded by the proteasome, whereas the wild-type podocin is degraded by both the proteasome and the lysosome. These results are important for the development of therapeutic strategies for SRNS due to *NPHS2* mutations.

Task 2: Mapping the epigenetic landscape of podocytes in vivo

By combining ChIP-Seq analysis with genomic profiling in model systems (e.g. mouse model for Frasier syndrome), Partner 2d identified new important genes involved in podocyte formation and function. We demonstrated that WT1 is a major regulator of glomerular differentiation regulating >50% of podocyte-specific genes. Moreover, we showed that one of its targets - *Magi2* - is essential for podocyte foot-process development. This gene has therefore been included as a novel candidate involved in screening

protocol for SNRS. Indeed, mutations in *MAGI2* have recently been identified in patients suffering from nephrotic syndrome.

Task 4a: Deep phenotyping towards new disease ontology for SRNS

A consortium-wide **Renal Phenome Database** was established, which incorporates relevant clinical (family history, disease features at first manifestation, treatment response features, disease progression to renal failure), histopathological and genetic information. All phenotypic data elements were annotated to the terms of the Human Phenotype Ontology (HPO). The database contains information from more than 3000 SRNS patients from 72 specialized units in 21 countries, including those of partners 2a, 4 and 7. The results of NGS gene panel screening (see Task 4b) were included in the Renal Phenome Database to allow efficient genotype-phenotype analysis.

Another collaborative effort towards deep phenotyping of the SRNS patients comprised the development of an infrastructure for standardized histopathological phenotyping of renal biopsy specimens, the ”**Digital Kidney Biopsy Project**”. Slides from almost 200 digitalized biopsies of SRNS patients were stored on the central server.

The collected **comprehensive phenotype** information resulted in two landmark publications (**Trautmann et al. cJASN 2015**) and 2017 (**Trautmann et al., JASN 2017**). The first publication described the age distribution, clinical and histopathological features, responsiveness to immunosuppressive therapy and post-transplant recurrence risk, stratified by the genetic diagnosis. The second publication informed about the long-term outcomes of SRNS. Ten-year renal survival rates was 27% in children with a genetic diagnosis as compared to 94%, 72% and 43%, in children achieving complete, partial remission or no remission upon intensified immunosuppressive therapy. Immunosuppression responsiveness, presence of a genetic diagnosis, histopathological diagnosis, as well as age, serum albumin concentration, and CKD stage at disease onset all independently affected the risk to develop end-stage renal disease. Notably, patients with familial SRNS in whom no genetic diagnosis could be established showed an intermediate long-term course and some of them were immunosuppression-sensitive.

In addition to these global analyses of the entire cohort, several cohorts with **specific genetic diagnoses** were characterized in the course of the project. The publications described the largest patient cohorts by far ever compiled for these rare diseases.

Disease prevalence, phenotype spectrum, and genotype-phenotype correlations of 61 patients with **WT1 nephropathy** were reported relative to 700 WT1-negative SRNS patients (**Lipska et al. Kidney Int 2014).** Focal-segmental glomerulosclerosis was equally prevalent in both cohorts, but diffuse mesangial sclerosis was largely specific for WT1 disease and was present in 34% of cases. Sex reversal and/or urogenital abnormalities (52%), Wilms tumor (38%), and gonadoblastoma (5%) were almost exclusive to WT1 disease. Missense substitutions affecting DNA-binding residues were associated with diffuse mesangial sclerosis (74%), early steroid-resistant nephrotic syndrome onset, and rapid progression to ESRD. Truncating mutations conferred the highest Wilms tumor risk (78%) but typically late-onset steroid-resistant nephrotic syndrome. Intronic (KTS) mutations were most likely to present as isolated steroid-resistant nephrotic syndrome (37%), focal segmental glomerulosclerosis on biopsy, and slow progression. We concluded that there is a wide range of expressivity, solid genotype-phenotype associations, and a high risk and significance of extrarenal complications in WT1-associated nephropathy and suggested that all SRNS children should undergo WT1 gene screening.

In the first clinical report of **ADCK4 nephropathy** following the original description of this podocyte limited podocytopathy, we identified 26 patients among 534 consecutively screened cases (**Korkmaz et al. JASN 2016).** The disease presented during adolescence with nephrotic-range proteinuria in 44% and advanced CKD in 46% of patients at time of diagnosis. Renal biopsy specimens uniformly showed FSGS. Whereas 47% and 36% of patients with mutations in WT1 and NPHS2, respectively, progressed to ESRD before 10 years of age, ESRD occurred almost exclusively in the second decade of life in ADCK4 nephropathy. However, CKD progressed much faster during adolescence in ADCK4 than in WT1 and NPHS2 nephropathy, resulting in similar cumulative ESRD rates (>85% for each disorder) in the third

decade of life. We concluded that ADCK4 glomerulopathy is an important novel differential diagnosis in adolescents with SRNS/FSGS and/or CKD of unknown origin. In a subsequent analysis of 28 Turkish patients (**Atmaca M et al. Pediatr Nephrol 2017**), partner 7 reported treatment with CoQ10 supplementation in 8 patients who were diagnosed early in the disease course with asymptomatic proteinuria. Proteinuria was reduced by 70% and eGFR was preserved within 12 months of treatment. Hence, CoQ10 supplementation appears as an **efficacious pharmacotherapy** for this genetic form of SRNS.

Another study focused on **Schimke immuno-osseous dysplasia** (SIOD; caused by mutations in SMARCAL1), a multisystem disorder with early mortality and SRNS progressing to end-stage kidney disease (**Lipska-Ziętkiewicz BS et al., PLoS One 2017**). Hypothesizing that NGS gene panel sequencing may unsurface oligosymptomatic cases of SIOD with potentially milder disease courses, we analysed the renal and extrarenal phenotypic spectrum and genotype-phenotype associations in 34 patients, the largest SMARCAL1-associated nephropathy cohort to date. In 11 patients the diagnosis had been made unsuspectedly through MASTR SRNS gene panel testing. Whereas patients diagnosed by phenotype more frequently developed severe extrarenal complications (cerebral ischemic events, septicemia) and were more likely to die before age 10 years than patients identified by SRNS-gene panel screening (88 vs. 40%), the subgroups did not differ with respect to age at proteinuria onset and progression to ESKD. Also, 10 of 11 children diagnosed unsuspectedly by NGS were small at diagnosis and all showed progressive growth failure. Severe phenotypes were usually associated with biallelic truncating mutations and milder phenotypes with biallelic missense mutations. We concluded that, while short stature is a reliable clue to SIOD in children with SRNS, other systemic features are highly variable and SMARCAL1 should be routinely screened for in SRNS.

Tasks 4b and 5a: Development and implementation of rapid genetic diagnostic tools

A diagnostic kit for SRNS gene testing by NGS has been successfully set up and implemented by Partner 20. The final version of the assay, FSGS MASTR V5, comprises a total of 729 amplicons covering 37 genes in 12 multiplex PCR reactions with the following specifications: 99,5 % of amplicons of the full target list successfully sequenced, uniform amplification: 98,4% of amplicons coverage ≥ 20% of mean coverage and stable amplification of the amplicons.

Altogether, during the whole project, the Multiplicom assay has been tested in **1741 unrelated patients using the MiSeq technology (Illumina)** (Partners 1, 2a and two associate partners) and **166 patients were tested using the Ion Torrent Technology** (Partners 4 and 7).

Mutations were identified in 21 % of patients (392/1907). The most frequently mutated genes were NPHS1 encoding nephrin, NPHS2 encoding podocin and the COL4A3-5 genes encoding type IV collagen chains, with the total number of mutations as follows: ACTN4 (5 family), ADCK4 (20), ANLN(2), CD2AP (2), COQ2 (8), COQ6 (4), COL4A3 (24), COL4A4 (20), COL4A5 (38), CRB2 (4), GLA (2), INF2 (17), ITGB4 (1), LAMB2 (15), LMX1B (17), MAGI2 (2), MYO1E (10) MTTL1 (3), NPHS1 (40), NPHS2 (56), PAX2 (10), PLCE1 (14), SMARCAL1 (12), TTC21B (4), TRPC6 (24) and WT1 (38).

Task 5c: Comparative urine peptidome, metabolome, miRNAome profiling in SRNS subtypes

In this part of WP2, we aimed to identified peptide and miRNA signatures that might allow to identify patients with steroid resistant nephrotic syndrome (SRNS) who will or will not respond to intensified immunosuppressive therapy. Urines were collected from 180 European children with SRNS and 43 healthy controls, and profiling of the peptidome by CE-MS (after elimination of high molecular weight proteins such as albumin, which are highly abundant in active SRNS) and miRNA (by microarray of all known human miRNA) was performed.

We identified several promising candidate peptides and miRNAs that may serve as biomarkers differentiating patients with intensified immunosuppression sensitive disease from genetic and non-genetic multidrug resistant disease. Furthermore, it appears that ISS-responsive SRNS patients in remission can be distinguished from unaffected healthy individuals by their urinary peptidome signature and possibly also by their exosomal miRNA signatures.

The results obtained in these seminal studies are being further evaluated by a detailed assessment of the specific functions of the identified peptides and miRNAs, as well as by prospective validation of the reproducibility and the diagnostic and prognostic value of the identified marker signatures.

Tasks 6, 7and 8: Development of novel molecular therapies for genetic SRNS

To date, therapeutic options for treating hereditary SRNS are limited. In the current contract Partner 2a established the first steps towards the identification of new therapies for hereditary NS linked to mutations in *NPHS2* and *NPHS1*, the two major genes responsible for SRNS in children and encoding for podocin and nephrin, respectively. Some mutants of these proteins are retained in the ER or in cytoplasmic vesicles instead of being localized at the plasma membrane (PM). We thus established podocyte cell lines expressing two types of podocin mutants (localized either in the ER or in cytoplasmic vesicles) or one ER-retained nephrin mutant and set up cell-based assays designed for high-content screening of compound libraries, based on immunofluorescence analyses of the subcellular location of these proteins (collaboration with Biophenics, the Institut Curie Screening Facility and Imagopole, the Pasteur Dynamic Imaging Platform, Paris). Pilot screens using the Prestwick library (1120 molecules) have been performed on podocyte cell lines bearing the R138Q (ER-retained) and the A284V (cytoplasmic vesicles) *NPHS2* mutations and led to the identification of 23 “primary hits” for the podocin^{A284V} screen and of 95 “primary hits” for the podocin^{R138Q} screen that need to be confirmed by visual inspection of the corresponding well-images to reject any image-based bias. Following these steps, primary hits will be confirmed in triplicates in the conditions of the screen and tested in a dose-response experiment when appropriate.

In parallel, Partners 1 and 2a established an inducible podocin$^{/R140Q/-}$ knock-in mouse model of human hereditary nephrotic syndrome. In C57BL/6 mice with Nphs2$^{Flox/R140Q}$/Cre$_{+}$ genotype, hemizygosity for mutant podocin can be induced by tamoxifen injection. Soon after induction the mice develop nephrotic range proteinuria, which peaks after 4 weeks. The animals develop progressive glomerulosclerosis and die within 4 months from renal failure.

Four-week intervention studies were performed by Partner 1 in this model to test nephroprotective effects of candidate pharmacological compounds: the ACE inhibitor Ramipril, the AT1 receptor blocker Candesartan and their combination, the Endothelin-1 type A receptor blocker Atrasentan (based on RAS-independent antiproteinuric action in humans) both alone and in combination with Candesartan, the calcineurin inhibitor Tacrolimus (based on published in vitro findings), the protease inhibitor Bortezomib (based on promising in vitro findings by partner 1), the non-specific chaperone compound 4-phenyl butyric acid, and the vasopressin 2 receptor antagonist Tolvaptan (based on published nephroprotective actions in rodents).

RAS blockade with Ramipril and Candesartan showed excellent antiproteinuric efficacy. The antiproteinuric effect was maximized with combined RAS blockade, which was subsequently used for extended studies and demonstrated to mitigate podocyte loss, glomerulosclerosis and tubulointerstitial fibrosis and to improve renal function and animal survival. Delayed treatment still lowered proteinuria but the beneficial effects on histopathology, renal function and animal survival were less impressive. Treatment withdrawal abolished the antiproteinuric effect.

The other tested compounds were largely ineffective or seven exhibited significant toxicity.

WP3: Membranous Nephropathy

Establishment of a large database of deeply phenotyped patients and bioresources

The Nijmegen, Paris and Manchester groups in collaboration with Prague have assembled the largest cohort world-wide of deeply phenotyped patients with iMN and the related biobank. We have written SOPs for biobanking and build database templates. The database is functioning online since September 1st 2014 (www.mnregistry.eu). We have been able to collect the data of almost 600 patients with MN in our registry. Long-term follow-up will allow analysis of outcome data in relation to baseline characteristics, treatment, and follow-up parameters. This registry can be pivotal in establishing ongoing collaborative studies in the recently installed *European Reference Network for Rare Kidney Disease*. In addition, the

registry will allow continuation of biomarkers discovery studies, using clearly defined phenotypes (i.e. progressors vs non-progressors etc).

The Nijmegen group has performed a proof of principle study to illustrate the potential of using database data. They compared safety and efficacy of rituximab and cyclophosphamide (in collaboration with 04-IRFMN). In total, 100 rituximab treated patients were compared to 103 patients who received cyclophosphamide. The results showed that Rituximab is safer than cyclophosphamide, and its use may be beneficial in some, but not all patients with MN.

Identification of pathogenic epitopes responsible for disease initiation, progression and recurrence in the graft

The Manchester group first described the location of a major, immunodominant epitope in the N-terminal cysteine-rich ricin (CysR) domain of PLA2R that is recognized by 90% of human anti-PLA2R autoantibodies. The epitope was sensitive to reduction and SDS denaturation in the isolated ricin domain and the larger fragment containing the ricin, fibronectin type II, first and second C-type lectin domains (CTLD). Analysis of PLA2R and the PLA2R-anti-PLA2R complex using electron microscopy and homology-based representations allowed us to generate a structural model of this major epitope and its antibody binding site, which is independent of pH-induced conformational change in PLA2R. The Manchester group synthesized novel versions of the native sequences of PLA2R1 immunodominant epitope linked by which retain high binding of PLA2R. These constructs form the basis for new immunoadsorption therapy for removal of anti-PLA2R. Further analysis shows it is the annexin A2-S100A10 complex that PLA2R binds to in the cell.

The Manchester group has developed the first ELISA for detecting anti-THSD7A antibodies in MN cases. Using recombinant extracellular THSD7A protein sequence, they have screened over 1700 MN samples and have identified 22 positive anti-THSD7A cases. Using a shorter N-terminal THSD7A sequence (approx. 50% of the full length) as a competitor, they could show significant inhibition suggesting the dominant epitope is towards the N-terminal. Interestingly, they have identified an epitope common to PLA2R and THSD7A which might be the trigger of autoimmunization.

The Paris group (in collaboration with Gérard Lambeau, Nice) has shown that in their GEMRITUX trial, PLA2R epitope spreading at baseline is a strong predictor of clinical outcome, independently of the titer of PLA2R antibodies. When the ELISA for each epitope will become commercially available (expected soon), this finding will have a major impact on the start of therapy (although they need to be confirmed in other prospective studies). Our proposal is that patients with epitope spreading at diagnosis need early immunosuppressive treatment before the “wait and see period” of 6 months while those with exclusive reactivity with the immunodominant epitope only (CysR) may stay on anti-proteinuric therapy with monitoring of the titer and specificity of PLA2R antibodies.

The Paris group has also described arylsulfatase as an exogenous antigen responsible for MN in a young patient treated with the recombinant enzyme for mucopolysaccharidosis type VI. Ig eluted from isolated glomeruli form the patient’s biopsy specimen specifically recognized arylsulfatase. Enzyme replacement therapy (ERT) could be resumed after tolerance induction therapy. This observation leads to consider the diagnosis of MN in ERT patients when they develop proteinuria and to undertake tolerance induction therapy.

Identification of pathogenic gene variants

The Nijmegen and Paris groups hypothesized that rare genetic variants within the coding region of the *PLA2R1* gene might explain antibody formation. Therefore, full exon sequencing of the *PLA2R1* gene was performed in 96 patients with biopsy-proven pMN. We detected only few novel rare variants. Thus, the pathophysiology of iMN is unlikely to be explained by rare variants (mutations) in the coding sequence, including splice sites, of *PLA2R1*.

The Paris group NGS sequenced *PLA2R1* and *HLA*-loci in 248 patients with MN, 200 ethnically-matched healthy controls (blood donors), and 113 pairs of donors/recipients. Classical HLA-D allelotypes were identified by using PHLAT.

Three independent SNPs in *HLA-D* and 2 independent SNPs in *PLA2R1* could be identified after logistic regression analysis. Three risk allelotypes have been identified: DQA1*05:01 (P=1.2E-16), DQB1*0201 (P=1.2E-16) and DRB1*0301 (P=7.1E-13). These 3 allelotypes define the risk haplotype HLA-DR3-DQ2, with a frequency in cases (29%) three times higher than in controls (9.6%). This haplotype is also at risk for several auto-immune diseases.

The Paris group performed the first detailed analysis of genes involved in complement regulation and showed a common haplotype associated with increased risk of primary MN (odds ratio 2.96 p= 0.022) which was present on 28% of chromosomes of patients with MN and 14% of chromosomes of controls (1000G and ExAC). We found that this risk haplotype carried a deletion of CFHR1 and CFHR3, at variance with AMD and IgAN where this deletion was protective.

Testing of pathogenic effects of epitopes and gene variants

The lead SNP in HLA-D and the 3 risk classical allelotypes are strongly associated with a positive PLA2R status. In a collaborative work with a Beijing group, we found that DRB1*0301 was associated with the level of anti-PLA2R antibodies which suggests that this allelotype plays a key role in the presentation of PLA2R1 T-cell epitope.

We found that the third lead SNP identified in HLA-D by logistic regression analysis was strongly associated with recurrence in the graft when expressed by the donor. These results strongly suggest that recurrence is driven by the donor's genetics, and involves PLA2R antigen presentation by relevant HLA class-2 molecules that fit best with T-cell epitopes on PLA2R. These results which need to be confirmed in a replication cohort (ongoing collaboration with the Mayo Clinic), are of great clinical importance as they will lead to exclude the "at risk" donors, which was not possible yet.

We provided the first evidence derived from genetic studies that in idiopathic MN, activation of the alternative pathway of complement plays a leading role at least in a subpopulation of patients with MBL deficiency.

Analysis of antigen presentation and T-cell response

The Manchester group has identified 5 candidate peptides (T1-5) which they have synthesized and analyzed for solubility in cell culture medium for use in ELISPOT. They are currently running ELISPOT assays for detecting IFNg, IL-4, IL-2, Il-10, IL-17 production in response to peptide challenge. Philochem/Philogen successfully developed a procedure for the purification of soluble intact HLA class II complexes from human plasma, resulting in dozens of peptide sequences identified with high confidence. Among these, were known ligands of HLA DR and DQ, respectively, strongly indicating that at least a subset of HLA class II complexes in blood stays intact. In collaboration with the Paris group, differences between the HLA peptidomes of healthy volunteers and MN patients were successfully characterized.

Revisiting current therapies and testing new therapeutic approaches

The Paris group first demonstrated the efficacy and safety of rituximab in a RCT with a high rate of immunological remission (depletion of PLA2R-Ab) as early as 3 months, and further showed the role of baseline Treg % in predicting response to rituximab. By comparing data of the Paris and Nijmegen groups, we could demonstrate that Rituximab in a dose of 750mg/m^2 is less effective than cyclophosphamide in inducing remission in patients with MN and high antibody levels.

A PLA2R antibody guided therapy approach was successfully used by the Nijmegen group, emphasizing even more the role of this biomarker in patient's monitoring.

Development of proprietary assays for the identified biomarkers and evaluation of their diagnostic and predictive values: towards a new ontology

Manchester, Paris, 23-CBC, 19-MTBX and 24-MOS have laid the grounds for robust Omics studies, and already obtained significant data.

For proteomics, the classification of the MN diagnosis resulted in a sensitivity of 91.3% and a specificity of 80.0% (AUC=0.983 [95% CI: 0.843-1.000]). For the differentiation of MN progressors and non-progressors, 62 peptide biomarkers were used to generate a support vector machine (SVM)-based

classifier, which reached an accuracy of 95.7% with a sensitivity of 92.3% and a specificity of 100.0% after total cross-validation. The Bristol group has also identified a soluble isoform of PLA2R (sPLA2R), that is expressed in podocytes which binds to the membrane bound receptor and inhibits its signaling. There is evidence that the production of both the membrane and soluble isoforms is regulated by methylation of the PLA2R1 promoter.

For metabolomics, the statistical model for differentiating the controls vs iMN was significant (CV-ANOVA $p<0.05$), but it was not possible to differentiate progressors vs non progressors.

WP4: Tubulopathies

Identification of novel genes involved in causing or modifying tubular disorders

- New genes influencing the Mg2+ homeostasis: *ARL15*, *CLDN14*, *TRPM6, FXYD2, HNF1B.*
- Mutations in *SLC34A1* causing idiopathic infantile hypercalcemia.
- Mutations in the peroxisomal protein EHHADH cause an autosomal dominant form of renal Fanconi syndrome.
- Patients with mutations in *HNF1A* develop proximal tubule dysfunction due to defective transcription of megalin, cubilin and ClC-5.
- Association of missense mutations in *CLDN10B* with the new HELIX syndrome encompassing hypohidrosis, electrolyte imbalance, lacrimal gland dysfunction, ichthyosis, and xerostomia.
- De novo mutation in *KCNA1* in a patient with renal Mg2+ wasting.
- Newly recognized polycystic kidney disorder with hyperinsulinemic hypoglycemia caused by a promoter mutation in *PMM2* coding for the phosphomannomutase 2 eznyme.
- Large clinical database of families with familial hypomagnesemia - analysis by NGS.

Development of a novel diagnostic tool for rapid complete screening of mutations and variants in known renal tubular disease genes

TUB MASTR v3 was successfully developed as a diagnostic panel for identification of mutations associated with renal tubulopathies. The TUB MASTR v3 is a high quality, reproducible kit showing high uniformity with ≥ 99,0 % of the amplicons having a coverage at 10% of the mean coverage. Based on the results of TUB MASTR v3 verification testing on 14 patient derived DNA samples, the observed sensitivity of this assay is 100%. The tubulopathies MASTR assay has successfully been used for molecular diagnosis in 384 probands, with documentation of spectacular diagnostic yields.

Use of reference -omics profiling and deep phenotyping to refine ontology and predict global progression processes

- Databases for several tubular hereditary diseases have been analyzed in detail, including Dent disease, Gitelman syndrome, familial hypocalciuric hypercalcemia, Bartter Syndrome type 3 and antenatal Bartter syndrome, EAST syndrome.
- Importance of the *UMOD* gene as risk factor for CKD; selection of common risk variants by protection against urinary tract infections
- Completion of two global, KDIGO consensus conferences on Gitelman syndrome and common elements in rare kidney diseases.
- Detailed phenotypes associated with various tubulopathies including homozygous *UMOD* mutation; distal renal tubular acidosis; Bardet-Biedl syndrome; kidney stone disease in children; familial renal Fanconi syndrome; Bartter syndrome type IV.
- Reference map of miRNAS in micro-dissected tubular segments in mouse kidney, with evidence showing segmental distribution along the nephron and a particularly high expression of miR-200c in CDs compared with PTs.
- Evidence that SNPs in TRPM6 drive the risk of developing hypomagnesemia during chronic PPI use.

- Development of infrared vibrational spectroscopy as a new method for rapid diagnostic and monitoring cystine levels in cystinuria, the commonest inherited cause of nephrolithiasis.

Functional characterization of mutated renal gene products (Functiomics)

- Mechanism linking defective claudin-10 to multiplex epithelium dysfunction in the HELIX syndrome; the biochemical and clinical consequences of homozygous mutations in the *UMOD* gene coding for uromodulin; functional consequences of the C125R mutation of *UMOD* using a novel, knock-in mouse model of autosomal dominant tubulointerstitial fibrosis (ADTKD); pathological mechanisms linking mutations in phosphomannomutase 2 and hyperinsulinism and polycystic kidney disease.
- Functional analysis and classification of the mutations in *SLC12A3* causing Gitelman syndrome, using a mammalian cell-based assay complemented by biochemical analysis.
- Investigation of the splice variants of NCC (*SLC12A3*), with effect of different anti-hypertensive treatments on the abundance and phosphorylation of the three NCC isoforms in urinary extracellular vesicles (uEVs) of essential hypertensive patients.
- Evidence that the transcription factor HNF1beta is regulating the expression of the *KCNJ16* gene that encodes the potassium channel Kir5.1.
- Establishment of flavaglines as a novel class of stimulatory compounds for TRPM6 activity.

Deep phenotyping of the SLC12A3 (Gitelman syndrome) carrier state

Set-up and recruitment for a clinical study to deeply phenotype heterozygous carriers for mutations of the *SLC12A3* gene responsible for Gitelman syndrome. The multicentre study will compare blood pressure and metabolic status in 80 Gitelman syndrome patients, 80 heterozygous individuals and 80 controls. Recruitment has been achieved and the biological centralized analysis were performed and data quality has been reviewed.

Development of preclinical disease models and large-scale in vivo drug discovery screens

- Development of *Xenopus* models of rare renal tubulopathies (alkaptonuria, autosomal dominant polycystic kidney disease type 2, Gitelman syndrome, Dent disease) that will be employed for phenotypic drug discovery. Completion of a comprehensive atlas of renal tubulopathy gene expression during *Xenopus* embryogenesis.
- Development of novel, clinically relevant mouse models of MMAuria using a constitutive Mut knock-in (KI) allele based on the p.Met700Lys patient mutation, used homozygously (KI/KI) or combined with a knockout allele (KO/KI), to study biochemical and clinical MMAuria disease aspects.
- Novel, knock-in mouse model of autosomal dominant tubulointerstitial fibrosis (ADTKD), harbouring the C125R mutation of *UMOD*.
- Demonstration that bone marrow transplantation improves proximal tubule dysfunction in a mouse model of Dent disease, a paradigm of defective receptor-mediated endocytosis and severe proximal tubule dysfunction.

WP5: Complement Disorders

Search for genetic initiators of complement-related disorders

Efforts done in tasks 1 allowed the identification and functional characterization of new genetic initiators of aHUS, IC-MPGN and C3G (including DDD, and C3GN).

A total **of 80 exomes** (WES) have been sequenced in patients with aHUS, IC-MPGN/C3G and their relatives. **DGKE** was identified as a new disease-associated gene in aHUS in two pedigrees. In two additional pedigrees an intronic DGKE mut was identified causing abnormal mRNA splicing. DGKE is the first HUS gene not directly linked to complement system and muts in this gene account for 10-20% of cases with onset before 1 year of age.

In another pedigree WES disclosed a mut in the gene encoding **properdin**.

Finally in a family where the proposita presented with aHUS but did not respond to the anti-C5 monoclonal antibody eculizumab, WES identified a mut in **INF2**, a gene previously associated with FSGS and Charcot Marie Tooth Disease.

In a family with IC-MPGN, WES revealed a **CFH** mut and functional analysis demonstrated that this mut results in a non-secreted protein.

Other candidate genes have been identified by WES and are currently under investigation.

By copy number variation assays, **new genomic rearrangements** were identified:

- An internal duplication in CFHR1 gene in a family with C3G, resulting in a FHR1(123412345) mutant protein with the SCR 1 to 4 duplicated.
- Another internal duplication in CFHR1 was found in a C3G patient, which translated into a serum protein with seven SCRs of which SCRs 1 and 2 are duplicated. Functional studies revealed that both mutant proteins form large multimeric complexes with increased avidity for C3 fragments and increased competition with FH.
- A CFHR5-CFHR2 fusion gene that encodes a 6 SCR FHR5-FHR2 hybrid protein with the first 2 SCRs of FHR5 and SCRs 1-4 of FHR2, was identified in a patient with C3G. The purified hybrid protein formed very large multimers that efficiently competed the regulatory activity of FH on cellular surfaces.
- A CFHR1/CFH hybrid gene was identified in a family with two aHUS patients and subsequently in several other aHUS patients. The resulting FHR1/FH fusion protein contains the first 4 SCRs of FHR1 and the last SCR20 of FH. Functional studies revealed that the hybrid protein acts as a competitive antagonist of FH and activates complement on cell surfaces.
- Another similar reverse hybrid gene encoding a FHR1/FH protein in which the last two SCRs of FHR1 were substituted by SCRs 19-20 of FH was identified in two unrelated aHUS patients.
- A CFH/CFHR3 hybrid gene was found in a patient with aHUS, and encodes a FH/FHR3 hybrid protein consisting of SCR1-17 of FH and SCR1-5 of FHR3. Functional analysis on the hybrid protein purified from patient's serum showed that it has both impaired cell surface decay acceleration and impaired co-factor activity.
- A CFHR2-CFHR5 fusion gene resulting in a 11 SCR hybrid protein with the first two SCRs of FHR2 and the entire FHR5 was found in a pedigree with two siblings with C3G (DDD). Functional studies revealed that the hybrid protein: stabilizes the C3 convertase, reduces FH-mediated decay, forms high molecular mass complexes, interacts with CFHR1, increases complement activation on cell surfaces by enhancing properdin attachment.
- A large deletion from intron 4 of FHR1 to 2 kb before CFHR5 resulting in a plasma protein reacting with anti-FHR1 and anti-FHR5 antibodies, was found in a family with 4 members with C3G and high levels of complement activation products in plasma.
- A CFHR3-CFHR4 fusion gene expressing a plasma FHR3 (SCRs 1-2-3-4)-FHR4 (SCR 9) hybrid protein, was found in a patient with C3G (DDD) and in 7 patients with aHUS.
- A duplication of CFHR4 gene was found in 2 patients, one with C3G and one with IC-MPGN.
- A mutant CFHR1 gene that originates by recurrent gene conversion events between CFH and CFHR1 and encodes a FHR1 protein with the last SCR5 substituted by SCR20 of FH, was found in 9 unrelated aHUS patients. The mutant FHR1 protein strongly competed the binding of FH to cell surfaces, impairing complement regulation.
- A complete heterozygous deletion of CFI gene was found in a patient with aHUS. The deletion encompassed ten other genes.

Sequence analysis of the 5 CFHR genes revealed several muts in CFHR2, CFHR3 and CFHR4 in patients with C3G or IC-MPGN. In addition, expression and functional studies have been done to characterize the impact of 37 different muts in the two components of the alternative pathway C3 convertase, C3 (n=27) and CFB (n=10), and of 29 different muts in the complement regulator FH, found

in patients with aHUS or IC-MPGN/C3G, on complement dysregulation and disease pathogenesis. Gene silencing in cultured endothelial cells demonstrated that loss of DGKE expression induces endothelial cell activation, increases apoptosis and impairs cell migration and angiogenesis. These findings are relevant to clarify the pathogenesis of aHUS associated with DGKE muts and suggest that in these patients the disease results from impaired endothelial cell proliferation and angiogenesis.

Search determinants of disease penetrance and clinical heterogeneity in mutation carriers

The incidence of combined complement gene mutations and the impact of common SNPs in CFH and MCP on disease penetrance were disclosed by a multinational effort in 795 aHUS patients. Studies in three large families with aHUS and with a hot spot CFH mut (p.R1215G) found that the presence of the risk CFH H3 haplotype on the allele not carrying the CFH mut has a significant effect on disease penetrance. In other studies, an extended haplotype, including CFH H3 haplotype, and two SNPs in CFHR1 and CFHR3, increased susceptibility to aHUS. Functional studies on the CFH hot spot p.R1210C mutant revealed that the mutant forms complexes with albumin which impair accessibility to all FH functional domains. These findings may explain why the R1210C mut predisposes to diverse pathologies (aHUS, C3G and age-related macular degeneration). Studies on the c.286+2T>G CD46 variant, which is the most common CD46 mut in aHUS, revealed that this abnormality is not enough to induce the disease.

Genetic screening of 173 patients with IC-MPGN/C3G revealed a comparable distribution and prevalence of gene mut between patients with IC-MPGN and C3G. By unsupervised hierarchical clustering using histology, genetic data, complement parameters and clinical features in IC-MPGN/C3G patients, four different pathogenetic mechanisms underlying C3G and IC-MPGN have been identified. In two independent studies, partners of WP5 provided genetic and clinical characterization of pregnancy-associated aHUS. Another study provided description of prognosis, response to therapies and outcome of kidney transplant in HUS children with DGKE mutations.

A concerted effort has been done by WP5 partners in collaboration with other researchers in EU and US to revise the aHUS mutation database (http://www.fh-hus.org). The new database (www.complement-db.org) includes more than 600 variants in 13 genes with MAF<1% in ExAC data set, which were identified in 3128 aHUS and 443 C3G patients. For each variant MAF, predictive comparisons of wild-type and mutant amino acids, examination of evolution-conserved residues across species, and correlations with functional binding sites are reported in the new database. These tools will help clinicians in interpreting the potential impact of gene variants in the pathogenesis of aHUS and C3G.

Determinants of anti-CFH Abs formation and characterization of C3NeF

Studies have been performed on the determinants of anti-FH antibodies in aHUS. Genetic screening of the polymorphic regions of class I and class II HLA genes has been performed in patients with aHUS and anti-FH antibodies, but no significant association between HLA alleles and the anti-FH Ab-mediated aHUS was found. A multicenter HLA study has been started with more than 100 patients to increase the power of the analysis. In another study, CFHR3 was found to neutralize the adjuvant effect of C3d on B cell receptor stimulation, which might provide a functional explanation for the association between deletion of CFHR1/CFHR3 and anti-FH antibody-associated aHUS. Regarding the characterization of C3NeFs, functional studies on IgG samples from 127 patients with C3G identified Abs that stabilize only the C3 convertase of the AP of complement (true C3NeF) and Abs that stabilize both the C3 and C5 convertases (C5NeF).

Search for novel autoimmune causes of aHUS, MPGN I, DDD or C3GN

In one study, novel autoimmune scenarios including C3 convertase, C3b and Factor B reacting Abs were identified in 5% of IC-MPGN and 23% of DDD patients (34 patients overall). The finding was confirmed in another independent study in 141 patients with IC-MPGN or C3G that identified 7 patients with anti-FB, 3 with anti-C3b and 5 patients with both anti-FB and anti-C3b Abs. Anti-FB Abs selectively enhanced the C3 convertase activity, while combined anti-C3b/anti-FB Abs enhanced the C3 and C5 cleavage. The anti-C3b IgG stabilized the C3bBb and perturbed the binding of CR1 but not of FH to C3b. In another study, anti-CFH Abs were identified in 17 patients with IC-MPGN or C3G. Functional characterization

demonstrated that these Abs induced no perturbations in FH cell surface protection but were able to affect the cofactor activity of FH. Epitope mapping identified the N-terminal domain of FH as the major binding site. In adult patients the anti-FH Abs were frequently associated with monoclonal gammopathy.

Development of tools for screening and diagnosis of aHUS, MPGNI, DDD and C3GN

Complement gene panels for rapid NGS of patients with aHUS and IC-MPGN/C3G have been developed.

A commercially oriented Complement Disorder MASTR assay was developed. Version one comprised 29 genes. Validation of the assay was done in 112 different DNA samples from patients that had been previously sequenced. Due to problems related to sequence homologies in the CFH/CFHR5 genes, a few muts could not be detected. Version 2 of the CD MASTR assay with 12 genes have been obtained and optimized by analyzing 38 patients. The assay is expected to be commercially available in Q4 of 2017.

Four academic NGS panels with 7, 14, 15 and 48 genes respectively, have been developed and used to screen overall 600 patients. The genetic cause was identified in 50% of patients with aHUS and 20% of patients with IC-MPGN/C3G. Notably, the prevalence and distribution of the mut did not differ between IC-MPGN and C3G and C3 was identified as the gene most frequently mutated in patients with IC-MPGN/C3G.

In addition, a multicenter study has been done to standardize the anti-CFH Ab ELISA assay and a publication described the gold standard methodology to detect the Abs. Finally, an analytical strategy for the complete functional characterization of CFH disease-associated variants have been developed.

Establish disease-specific deep transcriptomic and proteomic profiles

Urine and plasma proteomic profiles were obtained from patients with HUS (n=8-18), Ig-MPGN (n=16-27), C3 glomerulopathies (n=18-21) and healthy controls (n=20-21) by capillary electrophoresis coupled to Mass Spectrometry. Comparison of proteomic profiles among the 4 conditions identified 13 and 2 sequenced peptides selectively upregulated in the urines and in plasma, respectively, of patients with aHUS as compared with IC-MPGN, C3G and controls. A great overlap was observed among IC-MPGN and C3G proteomic profiles suggesting common pathogenetic mechanisms. Analysis of miRNA profiles was performed in plasma exosomes from patients with HUS (n=15), IC-MPGN (n=19), C3G (n=24) and healthy controls (n=21). Several miRNA were identified that were up or down-regulated in each condition vs controls. 36 miRNA were identified that are differentially expressed in HUS vs IC-MPGN or C3G.

Discover biomarkers predicting response to Eculizumab and post-transplant recurrence

High levels of plasma sC5b-9 were associated with response to eculizumab among patients with IC-MPGN/C3G of the Italian MPGN Registry. New assays to quantify free eculizumab level during treatment were developed. An ex vivo assay was developed that efficiently marks complement activation at endothelial cell level in aHUS and allowed monitoring Eculizumab effectiveness and/or complement activity during drug tapering in about 50 aHUS patients. Finally, a report on response to eculizumab treatment in 29 patients with secondary HUS has been published.

Among 118 patients with aHUS who received at least a kidney transplant among two cohorts, the presence of mut in CFH, CFI , C3 or CFB was associated with high risk of recurrence (range 50 to 80%).

Develop in vitro, ex vivo and in vivo models for testing new therapeutic interventions

Cell based in vitro and ex-vivo assays, namely FH-dependent hemolytic assays with rabbit and sheep erythrocytes and serum-induced C5b-9 deposition on endothelial cells, were developed. Novel complement inhibitors, an anti-FB mAb and a fusion protein in which the surface recognition domain of CFH was fused to the bacterial complement inhibitor Sbi have been obtained and showed good complement inhibitory activity by the above tests. The anti-FB mAb given to rats by i.v. injection prevented the passive induction of experimental autoimmune myasthenia gravis. Three new moAbs were generated against human C3 fragments and inhibited complement activation; two of them by blocking the cleavage of C3 by the AP C3 convertase and one by impeding formation of the AP C3 convertase. Two mAbs against C5a were also obtained which efficiently inhibit neutrophil chemotaxis.

Conditional knock-in mice carrying the aHUS-associated C3 mut D1115N were obtained. All biochemical, hematological and histology features of this novel mouse model are mirroring that seen in human aHUS. Treatment of the mouse model with an anti-C5 mAb improved mouse survival and prevented weight loss. A knock-in Cfh Δ19-20 mouse model that carries a C-terminus truncated Cfh reproducing the common CFH aHUS-associated genetic abnormalities, was obtained and showed a mixed C3G/HUS phenotype. Knock-in mice carrying the I1157T C3 aHUS-associated mut and the del923DG C3 mut associated with DDD, have been obtained and their phenotypes are under characterization.

WP6: Congenital Malformations of the Kidneys and Urinary Tract

The identification of novel CAKUT genes by next generation sequencing

We used two complementary approaches to address this aim: whole exome sequencing (WES) and targeted gene panel sequencing in ~1300 CAKUT patients from Dutch, French, and German cohorts. We identified multiple novel CAKUT genes: 1) homozygous *ITGA8* mutations cause bilateral kidney agenesis (**Humbert, Am J Hum Genet, 2014**); 2) heterozygous, *de novo PBX1* mutations cause kidney hypoplasia with concomitant deafness, developmental delay, and dysmorphic features (**Heidet, J Am Soc Nephrol, 2017**); 3) heterozygous *GREB1L* mutations cause bilateral kidney agenesis, which is the most severe and lethal form of CAKUT (**De Tomasi, Am J Hum Genet, in press**); 4) homozygous *KIF14* mutations were shown to cause bilateral kidney agenesis in a consanguineous family with four fetuses affected with bilateral kidney agenesis and brain abnormalities (paper in preparation).

We identified numerous novel gene variants with *in silico* predicted pathogenicity. Over 80 CAKUT candidate genes were highlighted that harbor possible pathogenic variants and require further characterization to prove disease causality (**Heidet, J Am Soc Nephrol, 2017; Nicolaou, Kidney Int, 2016**). Importantly, we also showed that the contribution of previously implicated genes to CAKUT risk is significantly smaller than expected and that the disease is far more complex than previously assumed (**Nicolaou, Kidney Int, 2016; Heidet, J Am Soc Nephrol, 2017**). These studies significantly add to the literature since the data indicates that detection of novel and likely deleterious variants in any known genes does not by itself imply pathogenicity, as erroneously reported in many recent publications.

The development and implementation of an NGS-based diagnostic tool for CAKUT

By targeted gene panel sequencing and WES, we provided genetic diagnoses in multiple patients by identifying mutations in known CAKUT genes. This provided new clinical diagnoses after gene testing (e.g. renal coloboma syndrome related to known *PAX2* mutations). Our efforts on CAKUT gene identification and characterization were directly translated to the clinic, by implementation of a CAKUT-specific targeted DNA tests in routine diagnostics. Targeted exome sequencing proved to be an efficient and cost-effective strategy to identify pathogenic mutations and homozygous deletions in known CAKUT genes. CAKUT gene panel analysis improved diagnostics for CAKUT patients significantly, with a rate reaching 18% (**Heidet, J Am Soc Nephrol, 2017**).

The establishment of transcriptional networks driving renal development

We identified direct downstream targets for transcription factors WT1, SOX11, and TCF21. We showed that WT1 activates FGF and suppresses the BMP signaling pathway. A balance between these two signaling pathways is essential to control survival of the early nephron progenitor cells. We generated three *Fgf8* knockout mouse models to investigate the downstream targets. We further identified *Sox11* as a potential candidate for CAKUT and demonstrated that loss of this gene leads to duplex kidney formation and dramatic shortening of Henle's loops in mice. We also showed that R-spondins ensure renal progenitor survival and mesenchyme to epithelial transition by activating canonical β-catenin signaling. Several of these genes have now been included in the CAKUT gene panel testing to identify variants in CAKUT patients in the respective genes.

The functional characterization of novel CAKUT candidate genes and variants, and the generation of in vitro and in vivo models

1) CRISPR-Cas9 knockout mice for ***Greb1l*** demonstrated the crucial role of Greb1l in kidney development (De Tomasi, *Am J Hum Genet*, in press); 2) ***Lgr4*** mutant mice showed disturbed nephrogenesis, suggesting a major role for Lgr4 as a receptor for Rspo1 and Rspo3 in kidney development; 3) ***Sox11*** mutant mice show duplex kidney formation and dramatic shortening of Henle's loops. Characterization showed that Sox11 may be important at early stages of nephron formation, potentially by recruiting cells to the intermediate segment; 4) Deletion of R-spondins (***Rspo1*** and ***Rspo3***) led to loss of renal progenitors, and if deleted only in the cap mesenchyme, a complete block of mesenchyme to epithelial transition; 5) ***Fgf8*** knockout mice were generated by using Cre mouse lines, to investigate the transcription factor targets that were identified by ChIP-Seq. Mesenchyme-specific (Pax8Cre) *Fgf8* knockouts, ureteric bud-specific (Hoxb7Cre) *Fgf8* knockouts, and early renal vesicle-specific (Wnt4Cre) *Fgf8* knockouts were generated. The mesenchyme-specific knockout mice showed smaller kidneys compared to wild-type littermates and were embryonic lethal. We demonstrated that *Fgf8* is crucial for first positioning and maintaining nephron progenitor cells during kidney development.; 6) ***crim1*** mutant zebrafish were created to determine the functional effects of a *de novo* frame-shift variant in human CRIM1, detected in a CAKUT patient; 7) ***rpgr*** mutant zebrafish were generated to investigate a hemizygous RPGR mutation detected in a CAKUT patients; 8) loss-of function mutations in ***KIF14*** cause a kidney-specific cilia defect and disrupt cytokinesis in patient-derived fibroblasts.

The use of amniotic fluid -omics and advanced imaging to predict postnatal renal function in CAKUT

Using 150 amniotic fluid samples, we identified potential amniotic fluid markers, peptides, miRNAs and metabolites, associated to postnatal renal function in CAKUT pregnancies. Validation of the **peptide** markers in combination with clinical parameters in an independent cohort allowed predicting with high precision the postnatal outcome of CAKUT pregnancies. The identification of the 59 amniotic fluid peptide biomarkers is a significant step forward for antenatal prediction of the postnatal renal function outcome in CAKUT fetuses and are potentially of great help for early prenatal counselling and improved clinical management of CAKUT pregnancies. Thereby, we can alleviate the psychological burden imposed on the parents. We aim to further optimize the 59P model for clinical use.

We carried out an untargeted **metabolome**-wide analysis of amniotic fluid samples from CAKUT patients, using NMR spectroscopy, lipid profiling experiments, and HILIC UPLC-MS. Close analysis of the meta-data revealed that gestational time and maternal age at the point of sampling were very different in each group. Given the confounding effect of gestational and maternal age on the patient groups, a univariate approach with adjustment for these confounders was used to investigate metabolic differences in the amniotic fluid from healthy controls and those with normal renal function at 2 years of age, and those with severely altered renal function.

We identified 15 **miRNAs** resisting to a stringent statistical selection including a high predictive value (AUCs>0.77), >1.5 fold change and miRNAs that remained significant after correction for multiple testing. Examination of the top miRNA revealed that has-miR-21-5p shows a very good sensitivity and specificity for postnatal renal function. Validation of amniotic fluid miRNAs and metabolites is ongoing.

We have generated normal values of **Pulse Systolic Velocity in Renal Artery** (PSV RA) and **Total Kidney Volume** (TKV) in normal pregnancies starting from 20 weeks of gestation till 40 weeks. In 74 fetuses we have shown that PSV RA can be used for the diagnosis of a non-functional kidney starting from 25 weeks of pregnancy. Prenatal PSV RA and TKV correlated with postnatal development of the compensatory hypertrophy of the solitary functional kidney. Although advanced prenatal imaging parameters failed to predict renal function outcome at the age of two years, longer follow-up of the patients is required for making final conclusions.

WP7: Integrative Bioinformatics

Development of an integrative biomedical knowledge generation architecture of rare renal diseases

To establish a multilevel data and analysis domain which allows the integration of data from different -omics platforms as well as the implementation of clinical data a basic database concept and standards for data submission had been defined. According to these definitions and standards an integrative EURenOmics data repository was established which is linked to analysis tools specifically designed and standardized for high-throughput data. Processed and quality controlled renal -omics data have been integrated into the analysis tools (EURenOmics analysis pipeline). This includes data e.g. transcriptomic profiles from the European Renal cDNA Bank, murine Chip-data from WP2, identified genes from WP6 (CAKUT panel) as well as publicly available datasets of renal diseases (microarray, RNA-sequencing, GWAS and proteomic results) from different databases as well as data from the GenitoUrinary Development Molecular Anatomy Project (GUDMAP)-Project. The repository and analysis pipeline were described in detail in the recent reports.

Multilevel and hierarchical deep data analysis along the genotype-phenotype continuum

The WP7 research team developed and adapted a series of analysis tools to allow effective integration of renal multi-omics data sets. The unique platform allows the analysis of –omics data sets in a kidney-centered biological context. In the following, we give examples for successful data analyses from several hundred -omics data sets used for biomarker identification and hypothesis generation on GWAS and exome sequencing data.

Identification of urinary biomarkers for progression of renal diseases (collab. with WP2)

By using a renal biopsy transcriptome-driven approach we could identify urinary EGF protein as an independent risk predictor of CKD progression. Urinary levels of EGF protein (uEGF) showed significant correlation with intrarenal EGF mRNA, interstitial fibrosis/tubular atrophy, eGFR and rate of eGFR loss. Prediction of the composite renal endpoint by age, gender, eGFR, and albuminuria was significantly improved by addition of uEGF. Addition of uEGF levels to standard clinical parameters improved the prediction of disease events in diverse CKD populations with a wide spectrum of causes and stages *(**Ju, W et al Sci Transl Med, 2015**)*. Even in pediatric CKD (4C Study; collaboration with WP2) a strong correlation of urinary EGF with the degree of renal impairment could be observed.

Linking tagging SNPs with regulatory information in idiopathic membranous nephropathy (iMN) (collab. with WP3)

Genome-wide association studies (GWAS) by Stanescu et al. have led to the identification of alleles at two genomic loci significantly associated with biopsy-proven iMN, however a direct causative link between the presence of the variants and its autoantibodies is currently missing. We have devised a strategy that integrates a set of less stringently selected genetic variants with multiple types of ENCODE data aiming at expanding from tagging to regulatory SNPs, their cis-transcripts and actual mRNA levels in iMN patients. Using this approach, we detected differentially regulated transcripts identified via tagging SNPs and potentially regulatory SNPs in their LD haplotype block hinting towards a potential mechanistic link between tagging SNP and transcriptional changes. Comparison of eQTL expression analysis from NEPTUNE with the differentially regulated genes in the NEPTUNE cohort identified three genes displaying a significant upregulation of the genes in glomeruli of iMN patients.

Identification of regulatory networks connecting genes with common biological functions in CAKUT (collab. with WP6)

A recent study showed that the contribution of previously implicated genes to CAKUT risk (CAKUT panel genes) was significantly smaller than expected, i. e. that the disease might be more complex than previously assumed (Nicolaou et al., Kidney Int., 2015). To elucidate the functional context within the panel genes and identify new potential CAKUT-associated genes an integrated transcription factor (TF)-gene interactions and regulatory network analysis was applied. By combining a promoter context analysis

with a pathway-analysis we could identify a regulatory network of 128 genes including 25 known CAKUT-genes and 103 potential novel CAKUT-associated genes. Protein-protein-interaction (PPI)-network analysis of the 128 genes indicated highly interconnected biological functions of the identified CAKUT-associated genes. Topological analysis as well as protein subnetworks with common regulatory patterns supported central roles of several genes/proteins within the network (e.g. MAPK1, CDC42, RET, WNT3A, WNT5A).

Human disease and model data integration beyond common ontologies

Murine Chip-data from WP2 and nephron segment-specific rat RNA-Seq data have been integrated in the human-based analysis platform to include specific gene regulation data by cross-ontology analyses. Additionally, data from the GenitoUrinary Development Molecular Anatomy Project (GUDMAP) have been integrated in the EURenOmics analysis tools. Both the rodent genes as well as their homologous human genes have been included and are presented during the analysis process. Using the established –omics-tools different rare diseases were used for cross-ontology data integration.

Glucocorticoid-Receptor-dependent transcripts in steroid-sensitive nephrotic glomerulopathies

Steroids lead to a rapid improvement in steroid-sensitive nephritic syndrome (NS). To identify gene products possibly contributing to podocyte dysfunction in NS, we analyzed human glomerular gene expression datasets for glomerulus-enriched, glucocorticoid receptor-dependent gene transcripts differentially expressed in patients with NS. Candidate genes were screened by in situ hybridization for expression in the zebrafish pronephros. One of the identified glomerulus-enriched products was the Rho-GTPase binding protein, IQGAP2. Immunohistochemistry revealed a strong abundance of IQGAP2 in normal human and zebrafish podocytes. In zebrafish larvae, morpholino-based knockdown of iqgap2 caused a mild foot process effacement of zebrafish podocytes and a cystic dilation of the urinary space of Bowman's capsule upon the onset of urinary filtration and an impaired size selectivity of the glomerular filter *(**Sugano et al, Kidney Int, 2015, Nov;88:1047-56**)*

Transcriptome-based network analysis reveals renal cell type-specific dysregulation of hypoxia-associated transcripts in chronic kidney disease

Dysregulation of hypoxia-regulated transcriptional mechanisms is involved in development of chronic kidney diseases (CKD). It remains unclear how hypoxia-induced transcription factors (HIFs) and biological processes contribute to CKD development and progression. Genome-wide expression profiles of more than 200 renal biopsies from patients with different CKD stages revealed significant correlation of several HIF-target genes with eGFR. These correlations were both positive and negative and in part compartment-specific. To assess the transcriptional impact of HIFs in the different cell lines genome-wide gene expression profiles of stable HIF1α and/or HIF2α knockdowns from glomerular and tubular epithelial cell lines under normoxic and hypoxic conditions have been generated and analyzed. Further analysis revealed common as well as cell group- and condition-specific regulatory mechanisms. Comparison of hypoxia-interconnected pathways with gene expression data of patients revealed increased dysregulation of hypoxia-related pathways with growing loss of renal function (**Shved et al, Sci Rep. 2017; 7:8576**).

Evaluation of Inflammatory and JAK-STAT Pathways as Shared Molecular Targets for Nephrotic Syndrome and ANCA-Associated Vasculitis

Another cross-ontology analysis evaluated the molecular pathways across different rare renal diseases, as this may lead to novel treatment approaches and classification strategies independent of descriptive diagnoses. Data from patients with nephrotic syndrome (NS, n=187) and ANCA-associated vasculitis (AAV, n=80) from NEPTUNE and ERCB were analyzed. In a discovery set, 10-25% of expressed transcripts were differentially regulated in both NS and AAV compared to living donors in the glomerular and tubulointerstitial compartments. The majority of transcripts were cross-validated in a replication study. Functional analysis identified therapeutically targetable networks, including activated inflammatory and JAK-STAT signaling, and decreased differentiation and signaling. Transcripts causally downstream of

STAT1 were used to develop a STAT1 activation score across diseases that correlated with interstitial fibrosis, and urinary EGF, a predictor of CKD progression (manuscript submitted).

Interaction and data integration with RD-Connect

Over the whole project a close collaboration with RD-Connect led to an orchestrated use of data. During the duration of the project all raw data generated in WP7 were submitted to RD-Connect, whereas data, analyzed following internal standards, were used for the EURenOmics database and analysis platform. The EURenOmics analysis pipeline will be continued as a renal-specific –omics tool beside the overall functionalities offered by RD-Connect.

1.4 The potential impact

Impact on understanding of disease mechanisms

The discovery of 40 novel disease-causing genes and genomic rearrangements and several immune-mediated disease entities and their functional characterization has greatly enhanced the understanding of rare kidney diseases. Within the past 5 years, the genes and rearrangements identified with involvement of EURenOmics researchers have increased the number of known genetic abnormalities underlying the disease groups of interest by more than 50%.

Several gene discoveries have led to fundamental novel insights regarding disease causing pathways. E.g., the discovery of DGKE as a monogenic disease-causing aHUS by affecting endithelial function without primary activation of the alternative complement pathway has fundamentally advanced the understanding of this condition. The spectacular discovery that in compound heterozygous patients with certain mutations in the NPHS2 gene, disease manifestation will depend on the types of variants present on the two alleles. The demonstration of "complementary" pathogenicity of mutations in compound heterozygosity is a fundamental insight that adds to the understanding of Mendelian genetics.

An important genetic insight made by researchers in WP5 was the discovery of a number of hybrid genes composed of CFH and CFHR sequences in patients with aHUS, which highlights both the susceptibility of this genomic region to undergo rearrangement and the relevance of the complex homeostatic balance between complement-regulatory CFH and CFH-antagonistic CFHR oligomers and heterodimers. The hybrid gene discoveries highlight the need to use complementary DNA copy number variation assays to disclose genetic abnormalities not revealed by conventional NGS analysis.

Further groundbreaking advances were made in the understanding of autoimmune disease. E.g, in membranous nephropathy, the identification of genetic variants associated with antigen presentation (HLA-D and PLA2R1) and possibly with disease progression (CFHR1-CFHR3 deletion) established a firm link of genetic variation to autoimmune disease. These insights, in conjunction with the identification of the first immunodominant epitope in PLA2R, open new avenues for research into the mechanisms triggering auto-immunity beyond membranous nephropathy. Furthermore, academic and SME researchers of WP3 succeeded in purifying soluble intact HLA class II complexes from human plasma, resulting in the identification of dozens of peptide sequences (the "HLA peptidome"). The results of this untargeted approach open new perspectives for the identification of T-cell epitopes presented by HLA class-II molecules in membranous nephropathy and beyond in auto-immune diseases.

In WP5, great advances in the understanding of immune-complex MPGN and C3 glomerulopathy were made with the identification and characterization of anti-FB, anti-C3b and anti-FH autoantibodies. In addition, functional studies have eliucidated that C3NeFs comprise a heterogeneous group of autoantibodies, with some specifically stabilizing the C3 convertase and others also the C5 convertase leading to both C3 and terminal complement pathway activation.

The research into disorders of kidney development in WP6 has identified key molecular pathways ensuring proper tissue differentiation and nephron endowment, which is directly linked to the risk of developing end-stage renal disease. The genetic, epigenetic and ex vivo functional studies in WP6, complemented by the exploration of developmental gene regulatory networks by the bioinformatics experts of WP7, have unsurfaced a range of novel candidate genes for CAKUT. Moreover, the peptides and microRNAs recovered from amniotic fluid that were found associated with severe structural malformations and poor postnatal outcomes have provided important new clues regarding the transcriptional programs and matrix remodeling processes involved in the physiopathology and disease progression in CAKUT.

Impact on diagnostic efficiency

The gene discoveries achieved by the consortium increased the proportion of patients in whom an unambiguous genetic diagnosis can be made, thus augmenting the potential for diagnostic certainty for new patients presenting with signs and symptoms of a rare kidney disease. Moreover, the development of targeted next-generation sequencing panels covering all known genes associated with a particular

disease group, including the ones discovered during the project, has allowed to establish diagnoses in many patients of the cohorts followed by the Consortium, and transformed the diagnostic efficiency for incident patients. The panels developed by the EURenOmics partners allow to rapidly establish a genetic diagnosis in 75% of hereditary tubulopathies, 60-70% of complement disorders, 25% of steroid resistant nephrotic syndrome patients, and up to 20% of CAKUT cases.

The marketing of the panels will greatly facilitate the access to high-efficiency diagnostics for rare kidney disorders throughout Europe and beyond. The use of conventional Saenger sequencing of individual genes is expected to be largely abandoned within the near future. Compared to the whole exome sequencing approach, the use of targeted NGS gene panels lowers the cost, reduces bioinformatic workload and avoids the ethical dilemmas caused by incidental findings.

Diagnostic efficiency has also been improved for rare renal disorders which are not caused by monogenic diseases. E.g., the researchers in WP3 and WP5 made major contributions to the introduction of auto-antibody screening and monitoring into clinical practice by identifying novel autoantibodies and evaluating their diagnostic and prognostic usefulness. Furthermore, the development of novel ex vivo and in vitro assays of complement activation will be useful to diagnose complement dysregulation in patients with aHUS and immune mediated MPGN/C3G and to evaluate the degree of complement inhibition while complement inhibiting treatment is applied or withdrawn.

Finally, various diagnostic methodologies have been developed or optimized, such as the isolation of microvesicles (exosomes) from urine for miRNA profiling, and the conditions for optimal collection and storage of urine for proteomic and metabolomic studies in disease conditions.

As an immediate clinical impact of the improved diagnostic efficiency brought about by the major advances in genetic and immunological technologies, the need for invasive kidney biopsies in patients with glomerular disorders is expected to decrease sharply.

Impact on risk prediction

One of the most dissatisfying aspects in the care of patients with rare kidney diseases has been our almost complete inability to predict individual disease risks, both with respect to an individual's risk to *develop* the disorder and to the risk of an affected patient to develop a *recurrent or progressive disease course*. Previous attempts to develop genetic or biochemical biomarkers to establish individual disease risk profiles were hampered by small cohort sizes in rare diseases, imprecise disease definitions, the lack of technological prerequisites enabling the application of high-throughput screening paradigms, and inadequate statistical approaches to biomarker detection. EURenOmics was set up to overcome these challenges by an unprecedented European collaborative effort utilizing large, well phenotyped patient cohorts, multi-molecular -omics profiling based on cutting-edge biotechnology platforms, and integrative multiscalar bioinformatic strategies. This approach proved successful in several projects of the consortium, paving the way to personalized disease management based on rational risk stratification.

After identifying low expression of epidermal growth factor (EGF) as associated with increased CKD progression risk by unbiased kidney tissue transcriptome screening, WP7 researchers demonstrated urinary EGF as a prognostic biomarker for rapid CKD progression in several adult CKD cohorts. Subsequently, low urinary EGF was confirmed in a collaborative study within the EURenOmics consortium to be predictive also in children with CKD due to various rare kidney diseases, independently of age, eGFR and other known predictors such as proteinuria and blood pressure. Hence, uEGF has the potential to become a highly useful prognostic biomarker in both rare and common progressive kidney diseases.

The identification of peptide and miRNA signatures closely associated with poor postnatal outcome of CAKUT is expected to lead to valuable prediction tools for prenatal counseling. In a validation study exploring electively aborted foetuses with unexpectedly mild or absent renal malformations on fetopathologiclal examination, the investigators found that informed decision-making based on their proteomic classifier could have avoided 80% of these erroneous pregnancy terminations.

In children with steroid resistant nephrotic syndrome, several peptides were identified which promise to discriminate patients who are responsive to intensified immunosuppression from multidrug resistant

patients and those with hereditary diseases. If confirmed in validation studies, such biomarkers could have major impact on the choice and duration of immunosuppressive protocols in this disease group.

In membranous nephropathy, a condition with a 50% risk of disease recurrence after kidney transplantation, genome-wide association studies identified a common variant in the HLA-D gene of kidney donors that was highly predictive of the risk of post-transplant disease recurrence in the recipient. If confirmed, this discovery could lead to donor genotype-specific selection of donor grafts.

In IC-MPGN and C3 glomerulopathy, the knowledge gained about disease associated auto-antibodies and genetic abnormalities allows to determine the risk of disease progression and the responsiveness to pharmacotherapies with much greater validity than the current classification based on histology and electron microscopy and are expected to lead to individualized treatment including new complement inhibitory drugs.

Impact on therapeutic perspectives

In the course of the past 5 years, EURenOmics researchers made significant progress in developing novel, more efficacious and less toxic therapies for patients with rare kidney diseases, applying precision medicine principles based on clinical and experimental evidence gained in the project.

In the first randomized controlled trial in membranous nephropathy, we demonstrated that the efficacy and safety of the monoclonal B-cell antibody rituximab and cyclophosphamide depend on the prevailing level of PLA2R autoantibodies. The findings provide a rationale for antibody-guided first-line immunosuppressive therapy as one of the first examples of truly personalized therapy in nephrology.

In WP2, the improved diagnostic yield achieved by comprehensive NGS panel screening for genetic forms of SRNS and the evidence from large cohort studies that genetic entities are universally resistant to any immunosuppressive therapy prompted us to modify treatment algorithms to completely avoid such ineffective and potentially toxic therapies in the rapidly growing fraction of patients with a genetic disease origin. Limiting immunosuppressive therapy to patients without an identifiable genetic disorder sharply increases the likelihood of treatment response.

Furthermore, the discovery of new genetic disease causes followed by systematic cohort screening unexpectedly led to a therapeutic option for up to 10% of hereditary SRNS cases, i.e. those with disorders caused by impaired mitochondrial COQ10 synthesis. For these patients, oral COQ10 supplementation provides a cheap, safe and efficacious therapy. in a group of children with early-stage disease, chronic COQ10 supplementation reduced proteinuria substantially and in a sustained manner. This result nicely exemplifies how genetic research may lead to unexpected therapeutic options even in hereditary diseases.

The integrative bioinformatic studies using Omics-based systems biology approaches (functional enrichment, activation network analysis) yielded several potentially druggable targets: IQGAP2 was identified by cross-species analysis in podocyte pathology as a key mechanism in steroid-sensitive nephrotic syndrome, and STAT1 activation as a common pathway to tubulointerstitial fibrosis and disease progression in autoimmune glomerulonephritis, providing a rationale for drug repurposing approaches in this group of disorders.

Although the development of models suitable for high-throughput compound screening was more complex and time consuming than originally anticipated, significant progress in this field was made towards the end of the EURenOmics project. Cell-based high-throughput screening for compounds correcting defective cellular trafficking of mutant podocyte proteins led to the identification of numerous hits, which are currently undergoing further evaluation. Likewise, a high-throughput in vivo zebrafish screening system was successfully established and used to screen for compounds inhibiting cyst growth in a cystic nephropathy model with promising results. Since the Prestwick library, a collection of approved drugs, has been used in both projects, the final list of phenotype rescuing compounds may lead to rapidly available pharmacotherapies using the repurposing approach.

Strategic impact on Rare Disease Research

EURenOmics was committed to contribute to the **IRDiRC** aims and adhered to the policies of this international initiative. The core activities of the EURenOmics consortium directly served the main objectives formulated by IRDiRC. The discovery of 40 new disease genes and genomic rearrangements, the development of NGS gene panels for rapid and accurate diagnosis of all hereditary kidney diseases, the demonstration of efficient therapies for hereditary podocyte disorders and membranous nephropathy by drug repurposing (RAS blockers, COQ10, Rituximab), and the experimental identification of compounds or drug classes attenuating the effects of specific podocyte, tubulopathy and ciliopathy gene mutations or blocking the complement system are concrete milestones and toward the IRDiRC goals.

The **European Reference Network for Rare Kidney Diseases (ERKNet)** was approved by the European Commission in December 2016 and started its activities in March 2017. The Network comprises 38 expert centers for rare kidney diseases, including 9 EURenOmics consortium members. While ERKNet is primarily focused on promoting excellence in clinical patient care, there are numerous areas of common interest, in particular the development and extension of registries and biorepositories for patients with rare kidney diseases, the use of the diagnostic tools developed by the EURenOmics partners throughout the ERKNet centers, and the facilitation of clinical trials with innovative therapies. Hence, ERKNet is expected to contribute in many ways to the sustainability of the research accomplished in the EURenOmics project. In turn, the availability of a large and highly integrated clinical network incorporating almost all clinical EURenOmics partners will provide an excellent infrastructure for new research opportunities and a superb platform for future funding applications.

Socio-economic impact and wider societal implications

A look at the demographics of the rare kidney diseases studied by EURenOmics illustrates the profound socio-economic impact of the performed research. Europe-wide approximately 10,000 new cases of membranous nephropathy, 10,000 CAKUT, 10,000 tubulopathy, 2,000 steroid resistant nephrotic syndrome, and 1000 patients with complement mediated kidney disorders are diagnosed each year.

The collaboration of academic researchers and industry partners in EURenOmics led to diagnostic products (NGS gene panel kits, biomarker ELISAs, prototypes of peptide classifiers), proprietary technologies (e.g., high-throughput in vivo screening technologies), and new indications for established drugs as well as candidate therapeutic compounds. The progress achieved in each of these fields will have major socioeconomic impact.

The marketing of three NGS gene panels by EURenOmics partner Multiplicom/Agilent is imminent. NGS gene panels are currently the most cost-effective diagnostic instrument in patients with genetic diseases. As compared to the sequential testing of genes performed previously, the turnaround times for genetic diagnosis have dramatically decreased with use of these gene panels. Moreover, it is expected that the total **costs of diagnostics will decrease** with the use of gene panels early in the diagnostic trajectory, as has been shown for other disorders. Even more importantly, access to tools allowing rapid diagnostic work-up will foster **precision medicine,** eliminating the futile administration of ineffective and potentially toxic therapies.

Moreover, the identification of patients at risk for rapid disease progression based on the **biomarkers** discovered in the project (e.g. urinary EGF in adults and children with CKD, amniotic fluid peptides and miRNAs in fetal CAKUT, urinary peptide classifiers for disease progression in membranous nephropathy and thin glomerular basement membrane disease) will lead to **individualized disease management**, with intense monitoring and nephroprotective interventions in high-risk patients but less frequent examinations in patients with low risk of disease progression. Likewise, we expect that pharmacological interventions can be increasingly tailored according to evidence based on risk stratification algorithms. E.g., costly B-cell depleting monoclonal antibody therapy will be avoided in patients with SRNS in whom a genetic diagnosis is ascertained, and used selectively in patients with progressive membranous nephropathy who have low anti-PLA2R antibody titres. As another example, the knowledge gained by EURenOmics researchers concerning new genetic and immunological causes of complement disorders and the resulting diagnostic tools (NGS gene panel, antibody tests and functional complement assays)

will lead to refined, rational indications for treatment with super-expensive complement inhibitors (currently 450k € annual treatment cost for Eculizumab), **with substantial cost savings for European healthcare systems.**

The potential **market size** for the diagnostic products will be several folds larger than the incidence figures given above since it will include all clinical conditions for which the diagnoses covered by EURenOmics are part of the differential diagnostic spectrum. Also, the biomarker tests indicative of CKD progression risk could become valuable tools also for common diseases leading to CKD. Finally, such tests would be likely to be applied repeatedly in an individual to monitor disease activity during treatment.

Most importantly, we expect that the advent of personalized medicine in the management of rare kidney diseases will eventually delay progression to end-stage kidney disease in a a sizeable number of cases. Currently, some 500 patients with membranous nephropathy, 1250 with CAKUT, 800 with SRNS, and 650 patients with complement disorders progress to end-stage kidney disease each year in Europe alone, altogether representing 6-7% of the total annual burden of new patients requiring renal replacement therapy (RRT). At an annual RRT cost of 50,000 € per patient, 16 million € would be saved each year if progression to end-stage kidney disease could be prevented in just 10% of the patients suffering from the rare kidney disease studied in this project. This figure would be amplified if some of the direct or indirect outputs of this project (monitoring tools, new therapies) prove useful also in common kidney diseases. These cost savings would add to the major **reduction of secondary morbidity** and **improvement in quality of life** achieved with an extended preservation of kidney function.

In conclusion, from a macroeconomical point of view we expect substantial returns from this itment in rare disease research, both with respect to the strengthening of European biotechnology and pharmaceutical industry by generating **innovative diagnostic and therapeutic products** and in view of the public cost savings related to a more **cost-efficient use of healthcare resources** throughout the European Union.

Main dissemination activities and exploitation of results

A Dissemination Task Force was implemented to ensure continuous, professional release of information about the project's results.

The Consortium's **website** served as a primary dissemination resource for information regarding the individual projects of the consortium, its members, workshops and conferences organized by Consortium members, and the up to date list of scientific publications arising from the project. During the funding period the EURenOmics Website (www.eurenomics.eu) has been visited more than 35.000 times by nearly 28.000 different users from 167 countries.

During the second half of the project, EURenOmics was also represented on **Twitter** (@EURenOmics). Here, the project mainly disseminated information on recent scientific results including new publications as well as news about rare kidney diseases and interesting meetings and conferences. @EURenOmics follows 2.147 Twitter users and has 2.199 followers. To date, @EURenOmics tweeted 1.208 times and got mentioned 254 times.

Furthermore, **video interviews** with the Coordinator and work package leads have been released in early 2017. Distribution channels are the EURenOmics website, Twitter, LinkedIn and YouTube. Each video has been streamed approximately 200 times to date.

The Consortium has been highly successful in disseminating research results to the medical and scientific community. To date, 318 peer-reviewed **publications** acknowledging EURenOmics funding appeared in scientific journals (Figure 1), thereof 1 in the New England Journal of Medicine, 5 in The Lancet, 5 in Nature Genetics, 1 in Nature Medicine, 1 in Nature Cell Biology and 2 in Nature Communications, 2 in Science Translational Medicine, 4 in the Journal of Clinical Investigation, and no less than 75 in the two top nephrology journals JASN and Kidney International. Eight review articles were published in Nature Reviews Nephrology, the specialty's leading review journal, and one in Physiology reviews. The cumulative impact factor was 2,249 (mean 7.1 per publication).

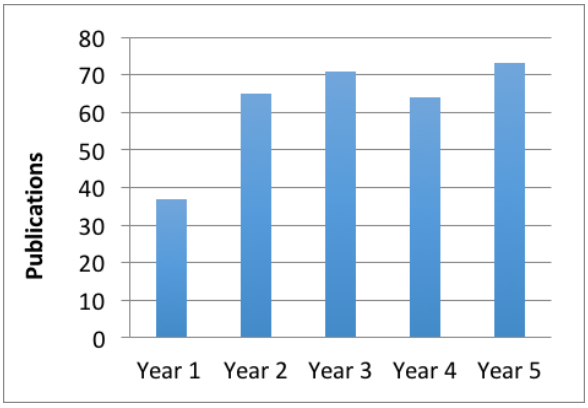

Figure 1: EURenOmics publications during funding period

In addition to the excellent publication record, the Consortium's research results were disseminated at multiple national and international scientific **congresses and symposia**. The partners disseminated their research findings at numerous scientific and health research meetings. A total of 619 oral presentations were given (597 at scientific meetings and 23 to a wider public) and 128 posters were shown; 564 presentations were held at international, and 237 at national meetings. Seventeen scientific meetings (congresses, conferences, symposia and workshops) were organized and 17 press releases were generated. These included several events of public interest beyond rare kidney disease, such as two **Workshops on Drug Development in Orphan Diseases** organized by partner 4 in Bergamo, Italy, which led to important position papers.

A major opportunity to discuss and disseminate the project's results was the **Rare Disease Research Outreach Day** jointly organized by RD-Connect, NeurOmics and EURenOmics in Berlin in May 2017 in conjunction with the joint annual meetings of the consortia. The aim of this event was to bring together stakeholders from the rare disease community to share the value of the tools created and the knowledge generated, to discuss how their utility can grow beyond the original scope, and to interact with other organizations and infrastructures in order to make an even larger impact in the field of rare diseases. In addition to representatives from all workpackages and projects, stakeholders from patient advocacy groups and industry as well as policy makers attended, and the day provided lots of time for discussion and interaction.

The European Reference Network for Rare Kidney Diseases (**ERKNet**) started its activities in March 2017. ERKNet comprises 38 expert centers for rare kidney diseases, including 9 EURenOmics consortium members. EURenOmics and ERKNet share numerous areas of interest. In order to inform ERKNet members about the research accomplishments of the EURenOmics consortium and develop future research perspectives and funding strategies building on the outcomes of the EURen-Omics project, a joint **Dissemination and Exploitation workshop** was held on July 11, 2017 in Paris. More than 30 representatives of the two organizations explored possibilities for future collaborations and sustaining the outcomes of EURenOmics, both at a project and community level. The direct exchange with ERKNet members greatly helped in developing joint strategies for research covering the entire spectrum from basic research to clinical research and care. The meeting was followed up by conference calls and has led to a commitment of both consortia to jointly contribute to the content planning of the emerging European Joint Programme for Rare Disease Research (EJP).

EURenOmics interacted with the relevant **stakeholders** in every phase of the project. This included contacts with the professional societies (pediatric and adult nephrology) and 17 patient organizations, research societies, medical charities and action groups active in Europe and beyond.

These contacts guaranteed dissemination of the research results to the wider community, but also information of the researcher about patients' views including the ethical aspects of the work. Several meetings with representatives of patient advocacy groups were organized during the funding period. In 2016 Daniel Renault, president of the European Federation of Renal Genetic Diseases (FEDERG) and

member of the Ethics Advisory Board of EURenOmics founded the **Renal ePAG** (European Patient Advocacy Group), an umbrella organization of 40 national patient advocacy groups for rare kidney diseases. Through their active involvement in ERKNet, the Renal ePAG members participate in the dissemination network for EURenOmics results.

Future research and outlook

Many collaborative projects initiated by the Consortium partners are currently still ongoing and will be finalized over the next few months. Moreover, the partners have identified important new research topics that they would like to tackle in the foreseeable future.

In clinical research, it is felt imperative to further **expand the collaborative patient registries** and databases formed by the consortia. The emerging core patient registry of the European for Rare Kidney Disease Reference Network (ERKNet) will provide an ideal platform to identify and link new patients to the disease-specific registries procured by the EURenOmics partners. It will also be important to increase the possibilities for data exchange by ensuring maximal interoperability of the existing databases (observing the FAIR principles) and linking them to integrative rare disease platforms as much as possible within the limits given by informed consents and date protection regulations. The same holds true for information about materials held in biorepositories.

The systematic application of diagnostic panels (including NGS gene panels and exome sequencing for suspected genetic diseases and antibody screening for immune disorders) and capture of the information together with standardized clinical phenotyping should greatly enhance our capacity to establish unambiguous diagnoses in an individual patient and to **describe the phenotypic spectrum** for a given diagnosis at a cohort level.

Moreover, expansion of the clinical databases should enhance our ability to perform **efficacy and outcomes research** on real-world data. This is a major clinical need since in the rare disease setting most questions aiming at the optimization of therapeutic protocols cannot be answered in randomized trials. Biostatistical methodology has advanced sufficiently to address such questions in an appropriate manner. In addition, for those research questions that will require randomized interventions, the availability of large ongoing patient registries will allow to initiate ‘pragmatic clinical trials’ from within the registry cohorts. This will greatly reduce the cost and time efforts related to clinical trials.

The identification of novel molecular pathways and respective **biomarkers** associated with kidney disease progression has been a major output of EURenOmics research. An important next step will be to validate the clinical usefulness and explore the added value of the biomarkers identified. It may be possible to develop much improved prediction models by combining several biomarkers with established risk factors and, wherever applicable, genetic information. At the current time such approaches appear promising in SRNS, membranous nephropathy, ic-MPGN/C3 glumerulopathy, and CAKUT. Prospective cohort studies will be required to address these issues.

Furthermore, the partners will continue to analyze the wealth of Omics data generated in the course of the project to seek for further biological signals informating of disease risk and progression. In several projects parallel measurements of the miRNome, the proteome and the metabolome have been performed on the same samples. These profiles will allow **cross- and multi-omics approaches** with the aim of identifying critical molecular pathways more consistently than achieved by individual Omics profile analysis.

While exome sequencing performed in families with assumed hereditary nephropathies and negative targeted gene panel screening yielded 40-45% detection rates, the underlying disease remained obscure in the remaining cases. This raises the question whether the unexplained families might suffer from structural genomic variations (e.g. DNA copy number variations) or alterations in the regulatory, non-coding part of the genome (e.g. transcription factor binding sites in promoters, enhancers, intronic sequences affecting splicing, long non-coding RNAs, microRNAs, etc.). With the advent of **Whole Genome Sequencing** and the rapidly increasing availability of powerful bioinformatic tools and reference genome databases, applying this technology to the unexplained cases in the Consortium will be the logical **next step in genetic research**.

The partners interested in immune-mediated glomerulopathies, tubulopathies and complement disorders will continue to **explore the interface of genetics and autoimmunity** by further investigations into antibody target epitopes and their variation in disease. Another promising use of the genomic information collected in EURenOmics will be further investigations into the **genetic and epigenetic causes of disease predisposition**, including the study of the functional effects of common variants in rare disease genes. Research in these areas has the potential to generate fundamental insights on common health issues from studies in rare disease populations.

The **therapeutic compound screening** projects have progressed at different pace across the projects. Several cell-culture and lower-vertebrate disease models suitable for high-throughput screening were established towards the end of the EURenOmics project; compound screening has been started with promising results in a few sub-projects. These efforts are planned to be continued or initiated in the near future and are expected to generate a bulk of hits in need for validation and effect replication in higher organisms. Hence, therapeutic compound screening and advanced testing of candidate compounds based on the pioneering work of the EURenOmics Consortium will be a major future research focus of the groups. **Collaboration with pharma industry** will be sought in this venture.

In the field of renal malformative diseases, research is strongly moving towards the integrated functional characterization of *in silico*, *in vitro* and *in vivo* models of kidney development to obtain a better understanding of the complexities of kidney development and their alterations in disease. In this context, a major research task in the near future will be the improvement of *in vitro* culture conditions for **renal organoids** to exploit the huge potential of the **inducible pluripotent stem cell technology** in developmental nephrology research. This line of research will increasingly focus on the long-term aim of developing **artificial organs**.