Publishable Summary

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Project acronym:	**EURenOmics**
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Funding Scheme:	**Collaborative Project (Large-scale Integrating Project)**

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Periodic report:	**1st ☒ 2nd ☐ 3rd ☐ 4th ☐ 5th ☐**
Period covered:	**from 2012-10-01 to 2013-09-30**

Name, title and organisation of the scientific representative of the project's coordinator:

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Section 1 - Publishable summary

Project title: **European Consortium for High-Throughput Research in Rare Kidney Diseases**

Website: http://www.eurenomics.eu/

Contractors involved (EURenOmics consortium):

The project is coordinated by Prof. Dr. med. Franz Schaefer [Partner 01] UKL-HD, Universitätsklinikum Heidelberg, Im Neuenheimer Feld 672, 69120 Heidelberg, Germany.

Other partners and team leaders:

Partner 02	INSERM	Institut National de la Sante et de la Recherche Medicale
Partner 03	UZH	Universitaet Zuerich
Partner 04	IRFMN	Istituto di Ricerche Farmacologiche Mario Negri
Partner 05	UMCU	Universitair Medisch Centrum Utrecht
Partner 06	UMICH	University of Michigan
Partner 07	HU	Hacettepe Universitesi
Partner 08	Bristol	University of Bristol
Partner 09	RUNMC	Stichting Katholieke Universiteit
Partner 10	UNIMAN	The University of Manchester
Partner 11	UCL	University College London
Partner 12	CSIC	Agencia estatal consejo superior de investigaciones cientificas
Partner 13	UNEW	University of Newcastle Upon Tyne
Partner 14	AP-HP	Assistance Publique - Hopitaux de Paris
Partner 15	HKI	Leibniz-Institut fur Naturstoff-Forschung und Infektionsbiologie eV Hans Knoell Institute
Partner 16	KU Leuven	Katholieke Universiteit Leuven
Partner 17	Oulu	Oulun Yliopisto
Partner 18	GENOMATIX	Genomatix Software GmbH
Partner 19	MTBX	Metabometrix Ltd
Partner 20	Multiplicom	Multiplicom NV
Partner 21	PHG	Philogen SPA
Partner 22	PHC	Philochem AG
Partner 23	CBC	Comprehensive Biomarker Center GmbH
Partner 24	MOS	Mosaiques Diagnostics GmbH
Partner 25	GABO:mi	Gesellschaft für Ablauforganisation :milliarium mbH & Co KG
Partner 26	KIT	Karlsruher Institut fuer Technologie **(terminated June 30th, 2013)**
Partner 27	LMU	Ludwig-Maximilians-Universitaet Muenchen

1.1 Summary description of project context and objectives

Rare kidney diseases impact markedly on the life expectancy and quality of life of affected individuals. The current diagnostic and therapeutic management of rare kidney diseases is highly unsatisfactory. We are typically unable to explain the genetic or molecular abnormality underlying the disease phenotype, to predict the individual risk and rate of disease progression, or to quantitate the risk of relatives to develop the same disorder. Even in patients with known genetic causes, individual risk prediction is limited by considerable phenotypic variability. Molecular disease and progression risk markers are lacking. Effective therapies are largely unavailable; the lack of suitable disease models is a major barrier to progress in therapeutic development.

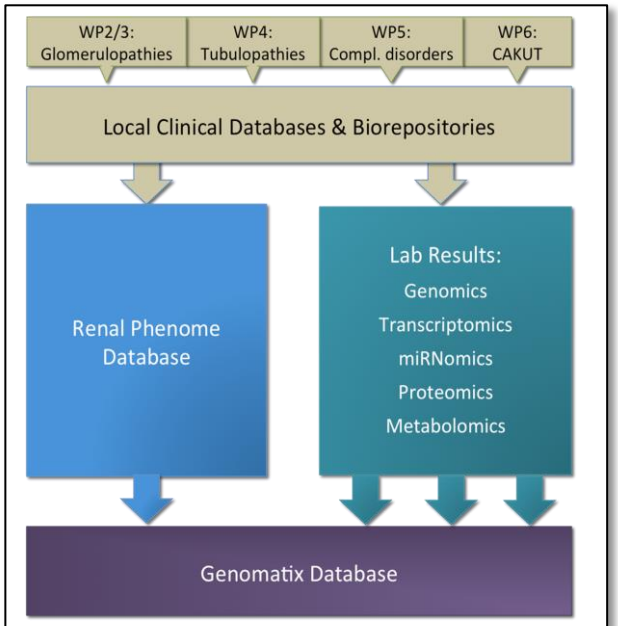
Diseases of the kidney are well suited to high-throughput research approaches due to the opportunity to examine molecular events in the end organ that is manifesting the disease: *kidney biopsy*, a standard diagnostic procedure, provides a unique opportunity to study intrarenal biological processes *ex vivo* using transcriptomic, proteomic and morphological approaches. *Urine* is a readily available non-invasive bioresource 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.

EURenOmics has prioritized five disease groups based on their urgent need and significant potential for diagnostic and therapeutic progress: Steroid-resistant nephrotic syndrome, membranous nephropathy, tubulopathies, complement disorders and malformations of the kidney and urinary tract. The consortium has access to a unique assembly of large existing patient cohorts and biorepositories, encompassing more than 15,000 patients with >10,000 DNA, >3,000 serum, 2,000 urine, 500 amniotic fluid and 3,000 kidney biopsy specimens. Some 30 academic and industry partners have joined EURenOmics to apply a wide range of high-throughput **technologies**, innovative **systems biology approaches** and a plethora of ***in vitro*, *ex vivo* and *in vivo* models** to study disease mechanisms and explore novel therapeutic approaches.

Initial research efforts focus on methodological standardization, involving procedures for acquisition and processing of biospecimens but also the construction of an integrated phenome database for uniform clinical phenotyping. The next objective is to **search for new genes** causing, modifying or predisposing to individual disease phenotypes. Screening strategies encompass **next-generation sequencing** of known genes, regulatory regions, exomes and whole genomes, application of cutting-edge **bioinformatic tools** for data mining, filtering and gene network analysis, and extensive genotype-phenotype analyses utilizing standardized phenotypic information. **Novel epitopes/antigens and antibodies** are searched systematically in those disorders in which an auto-immune pathology is suspected. The protein products of the newly identified gene variants, as well as the mechanisms of auto-antibody formation, are **functionally characterized** by an array of *in vitro, ex vivo* and *in vivo* technologies. **Multi-level -omics profiling** (mRNA, miRNA, peptidome/proteome, metabolome) in body fluids and renal tissues is performed to identify unique molecular disease signatures (**deep phenotyping**), develop a prognostically indicative new **ontology of rare kidney diseases**, discover molecular markers and pathways associated with disease activity and progression, and develop **diagnostic tools and biomarkers**. Finally, *in vitro* and *in vivo* models will be developed that allow **high-throughput screening** of compound libraries **for novel therapeutic agents** reversing or attenuating disease phenotypes.

1.2 Work performed since the beginning of the project and the main results achieved so far

Scientific activities have started in all WPs. **Standard Operating Procedures** for WP- and Consortium-wide use (specimen collection and processing, data transmission formats etc.) have been developed, and various novel methodologies (assays, in vitro and in vivo models) are being established.



Concept of Clinical and -Omics Data Integration

The consortium is working jointly on a central **'phenome' database** integrating clinical information from all patient registries. The structural and functional design of the database has been agreed in a workshop held in April 2013, and the mapping of core variables, as well as annotation of renal phenotype elements to terms of the Human Phenotype Ontology has been initiated. Correspondingly on the WP level, the construction of a Web-based database for MN has started (WP3). Furthermore, the WP7 partners designed, implemented and pre-filled with renal transcriptome datasets the joint -Omics database, which is currently being made available to the investigators.

The development of **targeted NG sequencing assays** capturing all known genes within each disease group has started in WP2 (SRNS panel, 31 genes), WP4 (tubulopathy panel, 39 genes) and WP5 (complement disease panel, 28 genes). The multiplex PCR systems have been optimized by iterative testing and primer redesign. For SRNS the final kit version is already available, and the final kits for WPs 4 and 5 are expected in early 2014. The screening of all patients in the cohorts in year 2 will identify the remaining subset of patients without mutations in any of the known disease genes who will subsequently undergo exome sequencing.

In parallel, **exome sequencing** has been performed in 79 familial cases (WP2: n=26, WP4: n=10, WP5: n=19, WP6: n=24 families). These efforts led to the **discovery** of 3 novel SRNS genes (WP2), 1 novel tubulopathy gene (WP4), 2 new genomic rearrangements in complement disorders and a novel disease gene defining a complement-independent mechanism of atypical HUS (WP5), and 1 novel gene causing CAKUT (WP6). Several additional candidate disease genes are currently undergoing functional characterization utilizing the wide range of *in vitro*, *ex vivo* and *in vivo* methodologies established in the partner laboratories. Moreover, causative mutations in known genes were found in 5 SRNS, 10 tubulopathy, 8 complement disease and 3 CAKUT families.

The WP5 partners substantially advanced the understanding of **variable disease penetrance** in aHUS. Their joint analysis of almost 800 patients revealed a higher aHUS manifestation rate in patients with combined mutations in different complement-regulating genes.

Major progress has also been achieved regarding the objective to identify the **molecular mechanisms of auto-immune disease**. In WP3 the B cell epitope in PLA2R, the target of specific autoantibodies in MN, has been identified. A causative role of mutations in the PLA2R1 gene for disease pathogenesis was ruled out by NG sequencing of 95 MN patients. The WP4 partners have collected sera for further studies from 120 patients with Sjoegren syndrome with renal involvement. In WP5, re-classification of the C3 gomerulopathy cohort by assessment of novel specific autoantibodies to complement proteins has been initiated.

Significant progress has also been made in using insights from the study of rare monogenic disorders to **identify general risk factors** for progressive kidney diseases. WP4 partners have deciphered a mechanism by which allelic variants in *UMOD*, the gene causing familial juvenile hyperuricemic nephropathy, are associated with the risk of chronic kidney diseases and hypertension in the general population.

Finally, the development of *in vitro* and *in vivo* models suitable for **high-throughput compound screening** has been initiated, including cell-based assays as well as Zebrafish and Xenopus models. First compounds with promising biological activity have been identified for genetic SRNS and complement disorders.

1.3 The expected final results and their potential impact and use (including the socio-economic impact and the wider societal implications of the project so far)

The research results expected from EURenOmics will have substantial impact on diagnostic management, risk assignment and preventative and therapeutic strategies in patients with rare kidney diseases, with potential implications also for patients with common kidney and other diseases and the society at large.

Comprehensive exome sequencing of large patient cohorts will profoundly advance the current state of knowledge by **unraveling** the as yet unknown **monogenic and complex genetic causes** underlying the diseases of interest. The newly discovered genes will be included in the panels covered by targeted next-generation sequencing (NGS) arrays currently development by the partners. The advent of these NGS panel kits is considered a major breakthrough as it will render the current sequencing technology with its high cost and time demands obsolete and will bring **rapid and complete genetic diagnostics** into routine clinical practice. For those patients in whom a genetic diagnosis cannot be established, we seek to develop disease-specific RNA, proteome and metabolome profiles and novel auto-antibody assays in the further course of the project.

The availability of such rapid diagnostic tests will constitute a big step towards **personalized medicine**. The need for invasive kidney biopsies will be greatly reduced or eliminated. Exposure to ineffective, potentially toxic and costly medications can be avoided and replaced by rational, risk-adapted therapies tailored to the molecular basis of mechanistically defined disease entities.

In addition to the expected diagnostic progress, the anticipated identification of genetic disease causes and risk factors as well as prognostic biomarkers should greatly facilitate **individual risk assignment** regarding disease occurrence, progression, complications and post-transplant recurrence compared to the currently used clinical, histopathological or imaging criteria. The efficiency of genetic counselling to families with inherited disorders will be much improved, alleviating their psychological burden and distress. Also, accurate risk assignment will form a basis for the development of early intervention and secondary prevention protocols.

Moreover, the partners have started developing *in vitro* and *in vivo* disease models aimed to discover **novel therapeutics**, including alternatives to existing superexpensive drugs. Our preliminary results both with respect to model development and regarding candidate lead compounds to correct hereditary glomerulopathies and complement disorders look promising and will stimulate further efforts to contribute to the IRDiRC goal of establishing new therapies for a significant number of rare diseases by 2020.

Finally, EURenOmics seeks to generate clinical and socioeconomic impact beyond the field of rare kidney disease. The study of the genetic abnormalities and molecular mechanisms underlying the selected disorders is hoped to produce **insights into general mechanisms** of kidney disease progression and even universal risk factors relevant to the general population. Our identification of a mechanism by which genetic variation of a kidney-specific protein involved in a rare kidney disorder predisposes to chronic kidney disease and hypertension impressively exemplifies the far reaching potential of rare kidney disease research.