1 Summary description of project context and objectives.

SYSCILIA, "A systems biology approach to dissect cilia function and its disruption in human genetic disease", is a large scale integrating project funded by the European Community's Seventh Framework Programme under the Health Cooperation Programme (grant agreement no: 241955). It brings together 18 partners from seven different countries. Our highly motivated, multidisciplinary consortium combines the unique expertise and experimental model systems of groups with an extensive track record in the molecular analysis of the major ciliopathies with leading experts in systems biology, bioinformatics and proteomics. Partners in the consortium have complementary expertise, apply state of the art techniques and integrate them within a well established systems biology approach. This combination is excellently equipped to unravel, integrate and employ the great number of variables involved in cilium function and its dysfunction in inherited ciliopathies.

SYSCILIA applies systems approaches to understand the basic biological processes underlying the role of cilia in human disease and to develop models capable of predicting the effects of discrete perturbations or mutations in those protein networks that underpin cilia function. Cilia are ideal organelles for systems biology as they can be regarded as semi-closed systems being both largely spatially and biologically separated from other cellular structures and processes.

The project is organized in 4 scientific components and one management & dissemination component encompassing eleven interdependent workpackages. All scientific WPs "feed" the central resource (WP2) with information, and in turn employ the data from the resource to specify, modify and validate the experimental datasets. The development of a central, shared resource designed to integrate, analyze and disseminate the information from different levels of complexity is almost finalized and will result in a unique, unprecedented tool with both a descriptive as well as predictive value for cilia biology. By its integrated approach this project reaches not only beyond the state-of-the-art in the field but also provides general proof of principle strategies for other genetic diseases.

The systems approach starts to unveil the full regulatory repertoire of this intriguing organelle and facilitates therapeutic approaches to utilize this knowledge. In brief, our proposed studies will set a new world stage in unraveling the role of ciliary proteins in cell biology in health and disease. As a result, our project will provide important novel insights and deliverables for gene identification, protein function prediction, therapy, and diagnostics. We already see that our approach informs other systems-based disorders and as such impacts the scientific community in a broad context.

2 Description of the work performed since the beginning of the project and the main results achieved so far.

SYSCILIA has made important progress towards the **identification of molecular mechanisms which determine ciliary function**, with novel candidate protein networks, pathways, and **ciliopathy-associted genes being identified on a regular basis**. Our systems approach of data generation, integration, model building and model refinement has generated unbiased knowledge on complex composition and mutational load associated to specific protein complexes. This currently accelerates the discovery and functional dissection of new ciliary disease genes, identifies new classes of ciliopathies, improves the accurate diagnosis of ciliopathies, and ultimately will provide targets for therapeutic intervention of ciliary dysfunction.

The most prominent results per workpackage thus far:

WP 1 ("MAPPING THE CILIOME"), generated a solid knowledge base to enable a systems oriented analysis of ciliary function and dysfunction. To this end 211 complexes of ciliary proteins were analysed by SF-TAP analysis, 116 of those are part of the current SYSCILIA ciliary gold standard (http://www.SYSCILIA.org/goldstandard.shtml) and 72 ciliopathy-associated proteins. In addition, 20 ciliary proteins were further analysed by quantitative proteomics methods and 34 baits were used for Y2H screening against retinal, brain and kidney cDNA libraries. Furthermore, a targeted grid was generated that contains constructs for all bait proteins used in SF-TAP analysis, facilitating a rapid, targeted detection of interactions with ciliary components and ciliopathy-associated protein by Y2H. 62 bait proteins were screened against this grid to date. To further characterize the ciliary protein network and to determine alterations induced by mutations, novel approaches were developed to quantitatively compare protein complexes, to dissect the sub-modules structure (EPASIS) and to analyse complexes in their native tissues (ICPL-IP). Furthermore, the ciliary localization and co-localization of more than 30 proteins was determined by different methods (immunohistochemistry, electron microscopy, proximity ligation assay. The proteomic data are currently used as a basis within WP2 to 6 to elaborate an integrated perspective of ciliary function on the molecular level.

In WP 2, the “CENTRAL RESOURCE FOR DATA INTEGRATION” was built and maintained, a robust seamless storing, accessing system for the SYSCILIA project data. A new data integration/viewing toolset (BDT/Quest) has been developed and used for all participants. This toolset takes raw experimental results as flat files, automatically integrates them to a database, interfaces users by web, and therefore, facilitates the workflow in a great measure. The revised socio-affinity indices are embellished by other results in the network. Detailed analysis of the network revealed an array of novel interaction clusters that are currently being validated.

In WP 3 (“CONSTRUCTION, COMPARISON AND APPLICATION OF CILIARY INTERACTOMES”), the strong collaboration between IT partners and the experimental partners of the SYSCILIA consortium led to the successful development of a novel technique to harvest detailed information on protein sub-complex compositions [1]. As one of the results the modular substructure and previously unknown members of the IFT machinery have been identified [Figure 1]. The dissemination of bioinformatically and experimentally acquired knowledge about the ciliome to the scientific community is highlighted by a cooperation with the GO consortium on improving ciliary gene annotations. The practical benefits of this work for the ciliopathy community will be substantial and the SYSCILIA consortium with its mix of partners is in a unique position to do this project.

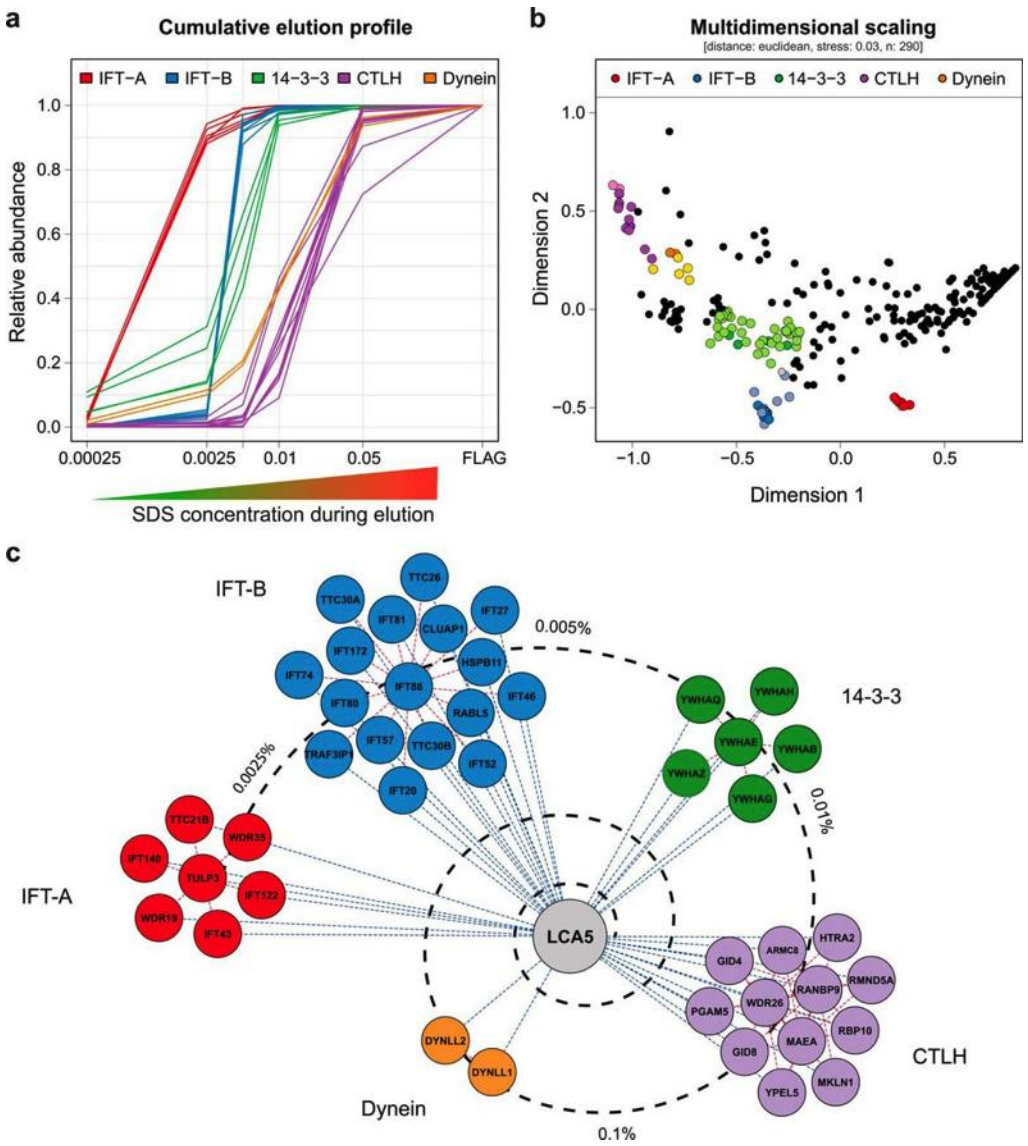

Figure 1: EPASIS of the IFT/lebercilin protein complex [1]

[1] Texier,Y. et al.; Elution Profile Analysis of SDS-induced Subcomplexes by Quantitative Mass Spectrometry; Mol Cell Proteomics; 2014 May [PMID: 24563533]

In WP 4 (“INTEGRATIVE MODELLING AND PREDICTIONS OF CILIARY SYSTEM BEHAVIOUR”) integration of proteomic, disease/phenotype annotations, and resequencing data predicts novel ciliopathy genes and ciliopathy-associated modules. Mapping of pathway annotation data on identified modules reveals known biological pathways to be responsible for specific phenotypes, while others to be related to several ciliopathy phenotypes. Novel ciliary genes predicted by Bayesian integration have been successfully used to identify causal genes in patients. Several predictions are currently in the process of being experimentally validated and will help in the design of new experiments, following our systems biology loop.

In WP 5 (“ASSAY SYSTEMS TO STUDY FUNCTIONAL CILIARY MODULES”), the participants continued their work to examine ciliary functions. Ciliary transport was analyzed by live cell imaging of ciliated human hTERT RPE-1 and murine renal IMCD3 cells expressing GFP::labeled STARD3NL, a ciliary protein candidate found in the siRNA screen in WP7. Fluorescence recovery after

photobleaching (FRAP) experiments were performed in phasmid neurons of different *C. elegans* mutants elucidating the strength of the transition zone diffusion barrier in detail. Transport of rhodopsin-GFP through the inner segment of living photoreceptor cells was monitored by FRAP analysis of mouse retina sections. For this we elaborated a FRAP application implementing adjustments of specimen shifts during long time recordings. To study ciliary polarization, we utilized the epidermis of *Xenopus* embryos as a model as it entails isolated, multi-ciliated cells that establish an anterior-to-posterior fluid flow along the body axis of the embryo. To analyse the mechanism(s) underlying ciliary polarization, we examined localization and function of the nephronophthisis gene product (NPHP) nephrocystin-4 (NPHP4). We uncovered a functional crosstalk between CAMs, and the actin cytoskeleton. NPHP4 (and likely other CAMs) can modulate the local actin environment through an interaction with adaptor proteins such as Inturned, which can recruit actin-nucleating proteins of the formin family. Further, assays to monitor ciliary signaling were systematically implemented.

In WP 6 ("ASSAYS TO DISTORT CILIOPATHY-ASSOCIATED MODULES"), work has focussed on uncovering the ciliary roles of known and novel ciliopathy and related genes using various cellular and animal models, combined with assays for cilium structure/function. Research efforts during year 4 uncovered major new insights into cilium biology and disease gene pathomechanisms, including roles for cilia and ciliopathy genes in autophagy, proteosomal degradation, brain patterning and DNA damage. The methodological pipeline to perform additional functional screening of candidates arising from high throughput discovery workpackages (WP1, WP7) was firmly implemented in *C. elegans* and zebrafish, resulting in the identification of multiple new ciliary proteins and CAM interactors, some of which may be ciliopathy proteins themselves.

In WP 7 *("*SYSTEMATIC RNAi SCREENS TO DISTORT AND IDENTIFY CILIOPATHY-ASSOCIATED MODULES*")* we have used a functional genomics strategy to evaluate the contribution of every human gene to the formation of primary cilia. Our data-set has already identified new disease genes, mechanisms and pathways including unexpected roles for pre-mRNA processing factors (PRPFs) and the ubiquitin-proteasome system (UPS) in ciliogenesis. We have confirmed that this strategy has high specificity for ciliary processes in a series of secondary and validation screens, and our multidisciplinary approach allows the functional annotation of many new genes. We have used our functional genomics data to interrogate WES variant data from existing gene discovery programmes of SYSCILIA partners and collaborators. This has successfully enabled variant filtering and the identification of two new causal genes for ciliopathies. The integrated data-set confirms the clinical utility and validity of "systems medicine" annotation and its ability to make relevant predications about disease causality.

In WP 8 ("ASSESSMENT OF THE INVOLVEMENT OF THE PREDICTED CILIARY MOLECULAR MACHINES IN THE PATHOGENESIS OF CILIOPATHIES") we have used *in vivo* functional approaches in combination with an empirically-trained regression model to assign allele pathogenicity to functional variants identified by targeted resequencing of the coding regions of 785 ciliary genes in 457 ciliopathy patients. We have generated zebrafish pathogenicity data for ~500 variants harbored by ciliopathy patients in ciliary proteome-encoding genes through the use of *in vivo* complementation assays that utilize physiologically relevant gastrulation defect, renal morphology, or laterality readouts. Finally, we have assigned pathogenicity calls to an additional 1,500 rare functional variants within the same ciliary resequencing dataset using a sensitive and specific model developed iteratively and using in vivo pathogenicity results. Combined, our multidisciplinary experimental and

computational approaches have enabled us to achieve functional interpretation of the projected 2,000 variants.

WP 9 ("TRANSLATIONAL SYSTEMS BIOLOGY: CILIOTHERAPEUTICS") is an ambitious plan to implement findings generated by earlier WPs to preclinical and clinical end points. We aim to test candidate approaches based on what is known about ciliopathies as well as results from unbiased screens. We have also developed novel ways to obtain cells from patients (from milk teeth and urine), which is particularly useful given that many ciliopathy patients are children. Our study in which we have used urine-derived renal epithelial cells from a child with Joubert Syndrome to demonstrate that pharmacological treatment with Hedgehog pathway agonist purmorphamine is an exciting avenue for therapeutic development. This reporting period has furthermore seen significant advances in extending our testing of PTC124 *in vitro*. Development of two new murine models for ciliopathies (*Sdccag8* -/-and *Cep290*-/-) coupled with the use of ciliopathy mouse model *Nek8*-/- has also seen the successful application of CDK inhibitors in renal cells derived from these mice. Compound screens in zebrafish have suggested interesting candidate drugs which have been tested in renal explants and will continue development along the pipeline. Zebrafish studies addressing the role of obesity-associated *fto* in ciliopathies offer insight into the obesity-related phenotypes in ciliopathies such as Bardet-Biedl syndrome. Insights into acquired renal ciliopathy phenotypes as a result of long-term lithium use in patients with bipolar disorder represent a broader use of the data generated by SYSCILIA.

The enthusiastic contribution and involvement of all partners has allowed our SYSCILIA consortium to generate **126 peer-reviewed publications**, of which 11% in top-journals such as Cell, Science and the Nature journals with an impact factor >30, and almost 50% in journals with an impact factor >8.

In its final year, SYSCILIA has teamed up with other main European players in the ciliary research field, such as the Ciliopathy Alliance, the Nordic Cilia and Centrosome Network and the French Cilia and Flagella research network, to collaboratively organize the international CILIA 2014 conference, to be held in Paris (November 18-21). This main event will focus on recent developments of us and others in investigating cilia structure and function including trafficking, cilia and development, cilia in human genetic diseases and cilia in infectious microorganisms.

3 Description of the expected final results and their potential impacts and use.

SYSCILIA is the first and largest comprehensive project on Systems Biology of ciliary disease ever conducted. One big problem in the field of rare disease is genetic heterogeneity or classical pharmacological approaches fall short when searching for improved diagnostics and novel therapy. SYSCILIA uses systems tools developed or applied by the project to acquire knowledge on the overarching principles of systems failure in these diseases [Figure 2]. The cilia model systems and associated discoveries will ultimately be employed to accurately diagnose and therapeutically target the growing number of human diseases associated with ciliary dysfunction. The combined output of our work clearly informs multiple aspects of ciliary biology and genetics. For example, we will not only generate and validate both the total protein complement of the ciliary proteome, but also describe the majority of physical relationships of that proteome and how those relationships determine the behaviour of the cilium. Moreover, we will identify novel signalling components of the ciliary proteome (many of which will be likely relevant to disease pathogenesis), which, together with the current knowledge base of the roles of the organelle in vertebrates, will continue to inform our functional work. Thirdly, the combinatorial information from SYSCILIA will be projected to address human genetic disease, since we have both well-validated targets for next-generation medical resequencing, which analysis currently is near-complete, and the means to test the effect of mutations found. Importantly, this work will not only lead to the systematic identification of new disease genes (which can be accomplished, albeit less comprehensively, by independent investigators), but will describe the total mutational load in ciliary dysfunction in humans and imbibe predictive power to the genotype.

Finally, through our consortium, we are generating a plethora of new ciliopathy models and developing new therapeutic paradigms that are based not on the dysfunction of a particular gene/protein, but on the quantitative assessment of an entire functional module. It is our strong expectation that this approach will represent the most efficient means towards drug discovery and that it will overcome the difficulties typically associated with a proteinocentric/single pathway approach to therapeutics. The excellent results from the project are confirming these high expectations, as the positive outcome of our ciliotherapeutic efforts, well ahead of schedule, already urge us to think of ways to implement these results in human trials. It is only through such collaborative structures that we will be able to screen the entire gamut of ciliopathy phenotypes and transition from a genocentric view of genetic disease to a systems-based expression of gene/allele causality and modification.

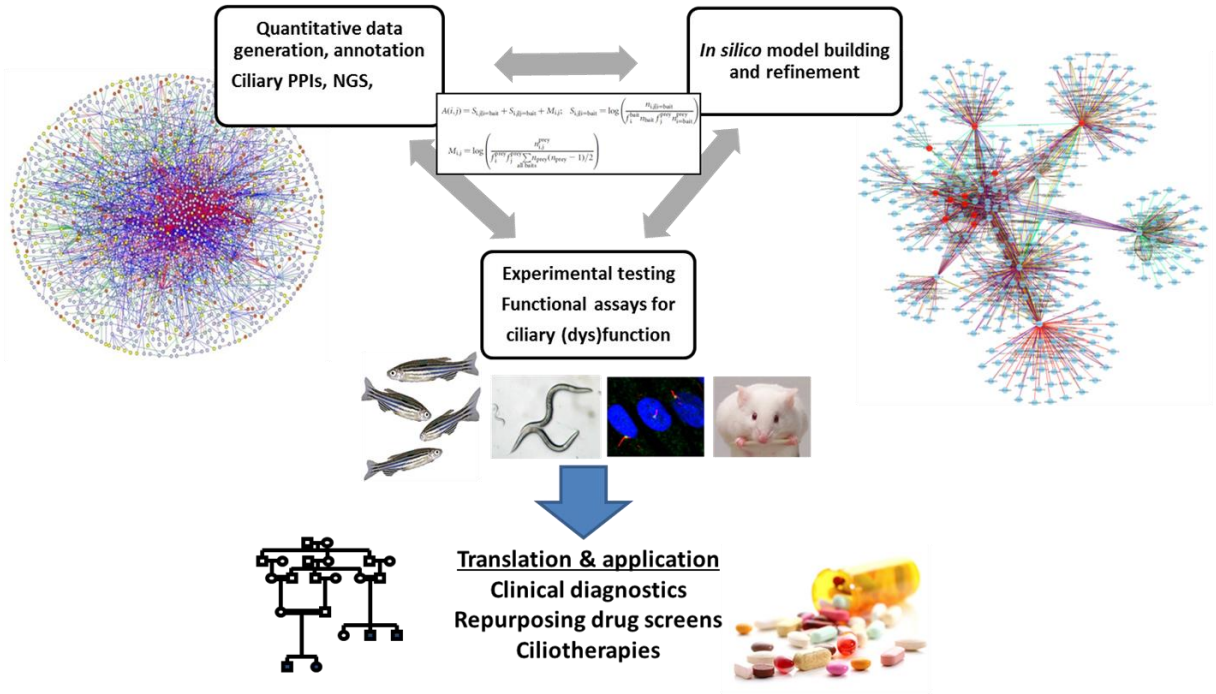

Figure 2: Systems biology → systems medicine

"SYSCILIA is providing proof of principle that an integrated approach to a model organelle (io. cilium) substantiates the utility of a systems biology approach to the analysis of complex biological systems."

More information: www.SYSCILIA.org