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 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. 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 aims to apply 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 will result in a unique, unprecedented tool with both a descriptive as well as predictive value for cilia biology.

As all proposed workpackages are strongly integrated in this resource, their potential to generate biological and medical knowledge of cilia function in a multidisciplinary fashion is similarly unprecedented. The systems approach will unveil the full regulatory repertoire of this intriguing organelle and facilitate 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.

The SYSCILIA consortium at the second annual meeting in London, at the Institute of Child Health.

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

During the first two years of SYSCILIA, work in all areas was focused on establishment of experimental assays and generation and analysis of the first large scale datasets. The computational groups have managed to develop, implement and improve the required systems tools and algorithms to connect to and integrate the wet-lab data into queryable databases. The annual cross-component meeting at the BioQuant building in Heidelberg was introduced, which turned out to be key to the successful translation of data, issues and opportunities between the multidisciplinary groups. This meeting urged the computational post-docs of SYSCILIA to self-organize into the "SYSCILIA IT" team, that closely connects to the other partners, mostly at the post-doc and PhD level. This allowed a very quick and accessible exchange of data and ideas between the partners, and improved the dataflow and output of the project. Overall, with some minor exceptions, all project deliverables were finished in time, and the progress of many deliverables projected further down the line are ahead of schedule.

We have organized two annual consortium meetings, the first one in Mallorca (Spain) and the most recent one in London at the Institute of Child Health. This latter 3-day meeting, with 47 SYSCILIA participants and 4 scientific advisory board members, was organized prior to the first international "CIlia 2012 - Cilia in Development and Disease" conference at the same location, co-organized with the ciliopathy patient organization "Ciliopathy Alliance, UK". This hughely oversubscribed and very successful conference (300+ attendants) was an excellent opportunity to create awareness of our consortium in the ciliopathy field with researchers, clinicians, patients and the industry, and share our unique approach and exciting new achievements with the public.

The most prominent results thus far (per workpackage):

In *WORKPACKAGE 1 ("MAPPING THE CILIOME")*, a robust protocol was set up for cilia isolation, and ciliary interactome descriptions based on native protein complex analysis as well as based on yeast two hybrid binary interaction screens has progressed very well. Combined efforts of six SYSCILIA partners delivered a first very significant set of protein-protein **interaction data for interactomes of 105 ciliary proteins**, including about two-third of the currently known ciliopathy-associated proteins, which was annotated in the central resource. Multiple novel ciliary protein complexes that were derived from these datasets are now under investigation in the experimental workpackages, and the first results have been published, including data on the IFT protein particles, systems analysis of the rhodopsin protein network, and multiple ciliopathy-associated protein modules, including Joubert syndrome, nephronophthisis, Usher syndrome and Meckel syndrome.

In *WORK PACKAGE 2 ("CENTRAL RESOURCE FOR DATA INTEGRATION")* systems for sharing and storing data have been created, a near-complete set of data from the literature has been collected and annotated in the SysWiz software tool, a **combined ciliary database and viewer**. This allows these data and data from multiple other sources (e.g. therapeutic compounds, genes, proteins, interactions), both public domain and proprietary, to be queried by SYSCILIA partners. Regular training sessions to enable optimal use of these extensive datasets are provided. A database of ciliary molecules, interactions and functional data has significantly begun to populate, and a datafreeze was installed to allow **the first collaborative datasets to be analyzed and used for systems modelling** by multiple partners at different levels. A wiki-based web site for secure, password-protected data exchange between all partners is available, including a public domain to view the principle findings within SYSCILIA (SYSCILIA.org).

In *WORKPACKAGE 3 ("CONSTRUCTION, COMPARISON AND APPLICATION OF CILIARY INTERACTOMES")* spatial (3D) interactions of ciliary proteins have been analysed and 79 high confidence interactions, among which multiple new ones, were identified. In addition, **a ciliary interactome containing all data has been built and connected to the public domain databases**, which revealed many connections to non-

ciliary processes. Procedures to filter the data by defining gold-standards for ciliary proteins are currently underway, and will be important to dissect and characterize the ciliary modules. Regarding the functional assessment of the many membrane proteins in the cilium, we have created a pipeline for ciliary membrane protein collection and classification. Detailed evolutionary analysis allowed us to trace the origin of IFT (BBSome, IFT-A, IFT-B) and the order in which they have been added, providing new functional clues of their submodules. **Structural models have been made and are being sorted**, and the newly identified ciliary submodules will be used in WP4 and mapped to phenotypes and diseases.

In *WORKPACKAGE 4 ("INTEGRATIVE MODELLING AND PREDICTIONS OF CILIARY SYSTEM BEHAVIOUR")* the work has been focused on familiarization with the biological system and the available modelling techniques. Importantly, new algorithms have been developed that can capture the the different functions that ciliary proteins have in different cell compartments, which will be a powerful tool to predict the specific properties of the new ciliary protein modules. **Mutagenesis and protein-protein interaction data are now being integrated for the implementation of a biological network model for the prediction of phenotypes**. The aim is to exploit the model to determine the phenotypic impact of gene mutations and explain how single gene mutations produce complex disease phenotypes, improving this way our understanding about ciliopathies.

In *WORKPACKAGE 5 ("ASSAY SYSTEMS TO STUDY FUNCTIONAL CILIARY MODULES")*, ciliary transport was successfully monitored using FRAP-based methods in MDCK cells and in organotypic retinal slices, cell migration could be assessed in zebrafish and modelled in an MDCK-cell based wound healing assay, tubulogenesis can be modelled in 3D cultured of renal collecting duct cell lines, cilium structure/morphology can be efficiently assessed using dye-filling assays of ciliated neurons in worms, and its general function in worms can be evaluated using an olmolarity sensing assay. Motile cilia (e.g. in the brain or lungs) need to be polarized to establish a directional fluid or particle flow. To characterize the polarization process, the motile cilia of the Xenopus epidermiswas used successfully as a model system. Using these assays, **the consortium is now well positioned to efficiently determine how disease genes affect ciliary transport, cell migration, ciliary polarization and tubulogenesis**.

In *WORKPACKAGE 6 ("ASSAYS TO DISTORT CILIOPATHY-ASSOCIATED MODULES")*, work has been conducted towards the genetic inter-relatedness of ciliary/disease genes. This work has been proceeding very well, with many cellular and animal (e.g., mouse, zebrafish, worms) models now established for numerous ciliopathy genes, as well as various IFT gene components. Cilium structure/function assays have been employed, resulting in significant new knowledge. This includes **mTor regulation** by mammalian ciliary signaling, roles for *Nphp* genes in **apical organization** and **DNA damage response signaling**, regulation of **transition zone structure/function** by combinations of nematode MKS and NPHP components, **Wnt signaling** regulation by *Mks1/3*, a role *Ofd1* in **digit number** and **Shh signaling**, further demonstration of genetic associations between ciliopathy genes and **planar cell polarity**, and the role of IFT and CAM components in the **ciliary transport** of Arl13b.

In *WORKPACKAGE 7 ("SYSTEMATIC RNAI SCREENS TO DISTORT AND IDENTIFY CILIOPATHY-ASSOCIATED MODULES")* the firsts results of the **whole-genome reverse genetics siRNA screen** for ciliogenesis and cilia maintenance have very recently become available, as well as the results for the **kinesome-wide screens,** and the results for the **ciliome-wide screens** are expected soon. These results will be integrated in the central resource and included in the SYSCILIA workflow. The first affected cellular mechanisms and modules have already been identified and are currently being validated.

WORKPACKAGE 8 ("ASSESSMENT OF THE INVOLVEMENT OF THE PREDICTED CILIARY MOLECULAR MACHINES IN THE PATHOGENESIS OF CILIOPATHIES") has collated the expected cohort, captured the necessary phenotypic diversity, constructed the database that houses both clinical and, in time, genetic and functional data, and generated high-coverage (99% capture at 10x coverage) **sequencing data for 766 ciliary target genes in 500 patients**. This major effort is now complete and is currently under analysis in the SYSCILIA workflow. Preliminary assessment results indicate that we will soon be able to report a significant number of new ciliopathy genes.

WORKPACKAGE 9 ("TRANSLATIONAL SYSTEMS BIOLOGY: CILIOTHERAPEUTICS") aims to transform preclinical data from SYSCILIA WP1-8 and prepare it for clinical administration in ciliopathy patients. The ultimate goal is to have the Molecular Medicine dictated by the Systems Biology; however these first 24 months of this project we have been testing knowledge-derived candidate approaches. Three SYSCILIA groups have made significant progress in testing **a novel FDA-approved drug called PTC124** in cell-based systems, mouse and human retinas and patient-derived ciliated nasal biopsies. PTC124 holds great potential in treating the 10-20% of patients harboring nonsense mutations by promoting read-through of the premature truncating mutation and generating a full-length gene product with presumable (partial) function. We have tested over 34 wild-type or mutant alleles, with variable read-thorough in 9 nonsense alleles from 8 different ciliopathy genes. In addition, we are performing drug re-purposing screens with FDA-approved drugs on ciliopathy models (zebrafish and cell-based assays). **Candidate pathways are being tested in animals and cell assays, and in particular targeting the AuroraA-HDAC-HSP90 pathway is looking promising.** Working closely with the systems builders in the coming years, we will re-prioritize the pathways to target by allowing the Systems groups guide us towards novel therapeutic approaches suggested by their modeling.

The enthusiastic contribution and involvement of all partners has allowed our SYSCILIA consortium to generate **38 peer-reviewed publications** in the first two years, of which 16% in top-journals such as Cell, Science and the Nature journals with an impact factor >30, and 60% in journals with an impact factor >8.

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

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 will clearly inform 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 will be able to generate a plethora of new ciliopathy models and develop new therapeutic paradigms that will be 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 first two years of 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.**

More information: www.syscilia.org