PI3K-C2α regulates Polycystin-2 ciliary entry to prevent kidney cyst formation

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Objective: PI3K-C2α is a regulator of vesicle recycling at the base of the primary cilium and is required for the targeting of ciliary components. Here we sought to understand whether PI3K-C2α is required for targeting of Polycystin-2 to primary cilium and the consequent regulation of kidney cyst formation.

Methods: Homozygous mutation of Pik3c2α, the gene encoding for PI3K-C2α, is embryonic lethal. Hence, the function of PI3K-C2α in kidney cilium has been studied both in vitro, in Pik3c2α-silenced IMCD3 cells, and in vivo, in Pik3c2α-heterozygous mice, using an Ischemia/Reperfusion model of renal injury.

Results: PI3K-C2α resides at the recycling endosome compartment surrounding the primary cilium base where it controls the activation of Rab8, a key mediator of cargo protein targeting to the primary cilium. Consistently, partial reduction of PI3K-C2α is sufficient to impair elongation of the cilium both in Pik3c2α-silenced IMCD3 cells and in kidney tubules of Pik3c2α+/− mice. Importantly, absence of PI3K-C2α impairs the Rab8-dependent transport of Polycystin-2 to cilia and produces an overactivation of proliferative pathways regulated by ciliary Polycystins, such as the mTOR and MAPK pathways. Both defects can be rescued by transfection of constitutively active Rab8, in line with defective Polycystin signaling. Heterozygous deletion of PI3K-C2α in mice causes an overall deregulation of proliferative signals in response to Ischemia/Reperfusion-induced renal damage, and this condition predisposes to cyst development.

Conclusion: These results indicate that PI3K-C2α is required for the transport of ciliary components like Polycystin-2 and that reduction in PI3K-C2α levels is sufficient to enhance susceptibility to cystic kidney disease.

KIF13B interacts with a number of centrosome-targeting regions in its central and C-terminal region, respectively. Within the central region of KIF13B we identified by bioinformatics analysis two conserve beta-sandwich fold domains that were identified in mammalian kinesin-3 members KIF13A, KIF13B, KIF1B and C. elegans KLP-4 and KLP-6, as well. Immunoprecipitation of ectopically expressed proteins and mass spectrometry analysis indicated that KIF13B interacts with a number of centrosomal proteins, likely via these beta-sandwich fold domains. Depletion of KIF13B in RPE cells using siRNA caused significant elongation of primary cilia, altered IGF1 signaling and decreased expression of Wnt5a.

Our results identify KIF13B as a cilia-associated kinesin involved in regulating ciliary length and signaling, and indicate that KIF13B interacts physically with the centrosome/transition zone. We hypothesize that KIF13B is recruited to the centrosome to regulate its kinesin activity and downstream signaling events and are currently investigating this further.

Reference
Control of initial steps of ciliogenesis by protein kinases

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Cilia are microtubule-based organelles present on the surface of most vertebrate cells. The formation of the primary cilia requires the mother centriole, which is the older of the two centrioles, to convert into the ciliary basal body. Although recent research has begun to shed light onto the molecular composition of the cilium, the regulation of ciliogenesis is just beginning to be understood. To get insight on the molecular mechanisms that regulate basal body formation, we performed a high-throughput RNA interference screen to identify protein kinases required for ciliogenesis. Of the novel kinases we identified, we are characterizing the function of the microtubule-associated/affinity regulating kinase 4 (MARK4), and tau-tubulin kinase 2 (TTBK2). We show that MARK4 and TTBK2 are both required to initiate axoneme extension and to promote the removal of the inhibitory protein complex composed of CP110/Cep97. Together, our data indicate that cilia formation is a highly regulated process, which requires the concerted action of protein kinases that regulate the transition from the mother centriole into the basal body.

9+2 to 9+0 axoneme conversion in Leishmania

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Eukaryotic cilia/flagella typically exhibit two characteristic ultrastructures reflecting two main classes of function; a 9+2 axoneme for motility, and a 9+0 axoneme for sensation/signalling. The key difference between these two ultrastructures is whether a central pair is nucleated at the basal plate of the centriole/basal body transition zone. We aimed to determine whether basal bodies are precommitted to nucleate one ultrastructure of cilium or can nucleate either, and whether transitions between the two ultrastructures of cilia/post-assembly is possible. We analysed axoneme ultrastructure change using Leishmania mexicana, a unicellular eukaryotic parasite which has a long motile 9+2 flagellum in the sand fly vector and a short immotile 9+0 flagellum when infecting mammalian macrophages. Using parasites expressing GFP proteins marking different axonomal structures, light and electron microscopy we analysed the transformation of axoneme ultrastructure by direct observation during macropage infection and in vitro proteolysis. This analysis showed that immature pro-basal bodies are ‘bipotent’ and are not committed to form either a 9+2 or 9+0 axoneme; that during the maturation process they become determined to one pathway of assembly. Axoneme ultrastructure was also flexible, 9+0 axonemes can form by both de novo extension from BBs and restructuring of existing 9+2 axonemes by removal of the central pair.

Atomic models of microtubule doublets and dyneins in cilia revealed by cryo-electron microscopy

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Microtubule doublets (MTD) in cilia consist of the A-tubule (13 protofilaments) and the B-tubule (likely 10 protofilaments). While 3D molecular arrangement of ciliary proteins is our interest, our knowledge about how dyneins, radial spokes and other proteins bind to MTD was limited, due to the limitation of resolution. We have been investigating the conformation and localization of inner and outer dynein isoforms (Bui et al. (2012) JCB 198, 913) and their conformational change induced by ATP (Molassaghi et al. (2010) NSMB 17, 761), radial spokes (Pigino et al. (2011) JCB 195, 673) by cryo-electron tomography (resolution 30Å). We newly employed single particle cryo-EM technique and improved resolution to 20Å. This enabled us to locate tubulin isoforms and describe interaction and binding of dyneins and other proteins precisely. The arrangement of tubulin dimers in MTD demonstrates difference from the reconstructed microtubule, indicating specific building mechanism of MTDs. Microtubule binding domains (MTBD) of outer arm dyneins bind to the three adjacent protofilaments, between alpha and beta-tubulins within one dimer. The orientation of the stalk changes -5 degrees during the power stroke caused by rearrangement of the linker domain of dynein with respect to the AAA-ring as we reported (Ueno et al. (2014) Cytoskeleton 71, 412). MTBD of inner arm dyneins bind to one protomofilament of the B-tubule, with the exception of dimeric dynein f. The interface of N-terminal tails of inner dyneins and the A-tubule spans over two protofilaments. We will extend our discussion of how this architecture is maintained during the bending motion.

The regulation of mechanosensory motile cilium formation

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In contrast to the progress in understanding ciliogenesis and cilium function, we know less about the pathways for generating ciliary diversity. Drosophila has a variety of sensory neurons with ciliary dendrites that are structurally and functionally specialised for receiving different sensory modalities. For instance, chordotonal (Ch) neurons have mechanosensory ciliary dendrites and are required for proprioception and hearing. Time-course gene expression profiling of differentiating Ch neurons allowed us to characterise the roles of two transcription factors for ciliogenic gene regulation: the well-known cilia gene regulator, Rfx, and a factor of the Forkhead family (Fd3F), which appears to be a diverged homologue of FOXJ1. Fd3F and Rfx cooperate to regulate a cohort of genes required for ciliary motility - in Drosophila this is a specialisation unique to Ch neuron cilia and is essential for the hearing mechanism. Analysing the target genes of Fd3F has led to the implication of new factors in the assembly of axonemal dynein complexes. Two of these are also mutated in human primary ciliary dyskinesia. Further analysis of these genes in Drosophila and mouse suggests that ZMYND10 may be linked to an emerging chaperone pathway, while HEAT2 appears to have a distinct function related to transport.

Cilia-autonomous regulation of tubulin transport by IFT

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Objective: The assembly of the axonem, the structural scaffold of cilium and flagella, requires translocation of a vast quantity of tubulin into the growing cilum, but the mechanisms that regulate the targeting, quantity, and timing of tubulin transport are largely unknown. Methods: GFP-tagged α-tubulin was expressed in Chlamydomonas reinhardtii and its transport in cilia was analyzed using TIRF microscopy. Results: GFP-tagged α-tubulin entered Chlamydomonas cilia as a cargo of IFT and by diffusion. IFT-based transport of GFP-tubulin occurred at a low frequency in full-length steady-state cilia and was strongly increased during cilary growth when IFT trains carried more tubulin. Cells possessing both non-growing and growing cilia selectively targeted GFP-tubulin into the latter indicating that cells regulate tubulin influx individually for each cilium. The preferential delivery of tubulin boosted the concentration of soluble tubulin in the matrix of growing cilia. Cilia length mutants showed abnormal kinetics of tubulin transport, suggesting that cilary length control involves a regulation of the occupancy of IFT trains by tubulin cargoes.

Conclusions: Tubulin is a bona fide cargo of IFT. We propose that IFT functions as a tubulin pump concentrating soluble tubulin in growing
The results from the phase I/II clinical trials showed improvements in the RPE65 gene causing inherited retinal degeneration were given a unilateral sub-retinal injection of AAV/RPE65 into the worse-seeing eye. Objective and subjective visual testing were performed at baseline and following intervention. Subsequently, 11/12 subjects received injections in the contralateral eye, in a Phase II follow on study. These results have initiated the first Phase III clinical trial for retinal disease. The methods and technology used in this study have enabled gene transfer to cells involved with ciliopathies. Unilateral subretinal, intra-ocular, and retrograde ureteral injections of AAV vectors were carried out in cohorts of mice. Intra-ocular injections were carried out at embryonic day 12; ocular and renal injections were performed in adult mice. The transgene cassette consisted of a CMV-promoted EGFP and/or a luciferase transgene. Transgene expression was evaluated qualitatively and quantitatively over time. Cellular specificity of expression was evaluated histologically. Delivery of the vector was well tolerated locally and systemically and the animals showed no alteration in behavior or general well-being.

Results: The results from the phase I/II clinical trials showed improvements in one or more visual function endpoints for all subjects. There was no evidence of inflammatory response as a result of surgery or exposure to the vector. In respect to the animal studies for ciliopathies, the cellular specificity and efficiency of transduction differed for each vector/delivery approach. After subretinal injection, vectors delivered high levels of transgene product to ciliated photoreceptor cells. In the otocyst, vectors transduced targeted cochlear hair cells efficiently. Following a transurethral approach, transgene expression in the kidney was visible in the tubule cells of the collecting ducts.

Conclusions: Initial phases for retinal gene therapy have shown safety and efficacy, which have given rise to the first Phase III clinical trial for retinal degenerative disease, which is ongoing. AAV vectors allow efficient transduction of specific subsets of cells with primary cilia. AAV vectors can efficiently transduce photoreceptors, ciliary hair cells, and renal tubular epithelial cells, and could be used to develop treatments for retinal and cochlear degeneration and inherited kidney disease, respectively. These vectors could also be employed to treat syndromes such as Usher Syndrome, Bardet-Biedl Syndrome and polycystic kidney disease.

Defects in primary cilium biogenesis underlie the ciliopathies, a growing group of genetic disorders. We describe the first whole genome siRNA-based reverse genetics screen for defects in biogenesis and/or maintenance of the primary cilium, obtaining a global resource for investigation and interventions into the processes that are critical for the ciliary system. In total, we identified 83 candidate ciliogenesis and ciliopathy genes, including 15 components of the ubiquitin-proteasome system. The validated hits also include 12 encoding G-protein-coupled receptors, and three encoding pre-mRNA processing factors (PRPF6, PRPF8 and PRPF31) mutated in autosomal dominant retinitis pigmentosa. Combining the screen with exome sequencing data identified recessive mutations in screen candidate genes as novel causes of ciliopathies, emphasizing the utility of our screen for ciliopathy gene discovery. Our findings emphasize the relevance of global, unbiased functional and genetic screening approaches in understanding ciliogenesis complexity, and in identifying loss of function in unanticipated pathways of human genetic disease.

Cilia are microtubule-based membrane protrusions conserved across evolution involved in cell motility, fluid flow and sensing. The diversity in functions is generally attributed to a core conserved microtubule-based structure, the axoneme, decorated by different structures, membrane and signaling systems. Here we study four classes of cilia that represent very diverse motility and sensory functions within a single organism, the fruit fly. We uncover that the base of the cilium, the basal body and transition zone, is much more diverse than previously thought, showing large variation in number, length, ultrastructure, and connection to other cellular structures. We further demonstrate that basal body diversity is imparted by differential regulation of evolutionarily conserved core components. The tissue specific regulation of core basal body and transition zone genes suggests mechanisms that generate tissue specific phenotypes in human ciliopathetic syndromes.

A novel form of PCD that impacts nodal, but not tracheal cilia J. Keyton1*, E. Adams2, K. Riley1, N. Powles-Glover1, K. Shinohara1, J. Lucas2, P. Lackie1, D. Norris1

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Cilia in the embryonic node, drive a leftward fluid flow (termed nodal flow) that establishes the left-right axis. We identified lms in a genetic screen for mouse left-right patterning mutants - the embryos exhibited disturbed situs. Mapping and sequencing revealed a novel mutation in the axonemal dynein heavy chain locus Dnah1: loss of function gives rise to immotile cilia in mice; many human primary ciliary dyskinesia (PCD) patients with DNAH11 mutations have hyper-motile cilia. To our surprise, unlike the previously characterised Dnah11+/- mutant, lms+ chiral ciliary beat frequency (CBF) was normal. However, the number of lms homozygotes at weaning was lower than Mendelian ratios would predict. Age of death analysis identified a 50% reduction in embryos between E14.5 and E18.5, consistent with death from embryonic cardiac failure. Expression analysis of early molecular markers of left-right asymmetry revealed randomised or bilateral activation of the normally left-sided Nodal Cascade. As this suggested an early, primary patterning defect, we analysed nodal flow by particle image velocimetry (PIV); rather than the wild-type leftward flow, the absent flow in Dnah1+/-, we observed a chaotic fluid flow in lms nodies. We therefore assessed nodal CBF and ciliary amplitude by DIC microscopy; lms cilia beat at 1.5x normal frequency, but with an abnormal motion. In summary, lms is a novel form of PCD, impacting nodal but not tracheal ciliary beating. We would predict that equivalent mutations in humans might underlie situs defects and congenital heart disease in the absence of respiratory disease.
O16 Ultrastructural studies of Intraflagellar Transport trains in Chlamydomonas reinhardtii suggest a revision of the current model for IFT trafficking in the flagellar compartment

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Intraflagellar Transport (IFT) is the molecular process responsible for the active bidirectional trafficking of structural and functional components that occurs in the flagellar compartment of eukaryotic cells. Flagellar components undergo a constant turnover at flagellar tip and multiple evidences indicate that flagellar elongation, maintenance and reabsorption depend on the correct balance between anterograde and retrograde trafficking. IFT particles are formed by >220 polycomplexes assembled into two subcomplexes, A and B, and are moved bidirectionally along the outer surface of axonemal doublets as linear rows of IFT particles, for which we proposed the term “train”. Anterograde IFT trains are moved by kinesin II and carry to flagellar tip the retrograde motor cytoplasmic dynein 1b, responsible for retrograde IFT. In a previous study carried out on Chlamydomonas flagella we identified two types of IFT trains we named long and short trains, each characterized by a specific ultrastructure and a definite internal repeat, and proposed that long, less compact trains could represent anterograde IFT while the short, more compact trains could be retrograde. To challenge such model, we monitored by transmission electron microscopy the IFT trains expressed both in wt regenerating flagella and during flagellar reabsorption induced in the ts mutant pf1-12. We also progressed in our electron tomographic 3D modeling of short IFT trains. Our data suggest that long IFT trains are not the only anterograde IFT component. Rather, anterograde IFT is contributed also by a subclass of short trains that is expressed in a flagellar length-dependent fashion.

O17 Uncoupling flagellum formation and maintenance

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Objective: The role of intraflagellar transport (IFT) in the construction of cilia and flagella is well established in numerous species. In contrast, its involvement in flagellum maintenance has only been shown in Chlamydomonas. Here, the protist Trypanosoma brucei was used as a model to investigate the role of IFT in flagellum maintenance. This organism has the advantage to maintain the existing flagellum whilst assembling the new one during the cell cycle. Knocking down the expression of any IFT protein inhibits flagellum formation in trypanosomes. Nevertheless, 25% of the cells still possess a flagellum of variable length. It was initially proposed that this was a consequence of RNAi that targets mRNAs and not proteins. Therefore IFT would still be active in these flagella.

Methods: The IFT88\textsuperscript{GFP} and IFT140\textsuperscript{GFP} strains were transfected with a construct allowing endogenous tagging of IFT81, another IFT protein, with YFP. IFT was monitored at different stages of RNAi and cells were also analysed by electron microscopy.

Results: New flagella of intermediate length are still assembled at early time points of RNAi but IFT trains are less frequent and appear smaller in the IFT88\textsuperscript{GFP} strain. Later on, the new flagellum is not formed but the old flagellum, assembled before RNAi was triggered, remains in place. Nevertheless, IFT is not detected anymore, in agreement with the dramatic reduction in the number of IFT trains observed by electron microscopy. In the IFT140\textsuperscript{GFP} strain, an excessive amount of IFT material is detected in their old flagellum and photobleaching experiments revealed that this material does not traffic. Therefore IFT is either missing or arrested in IFT-8 and IFT-A mutants respectively.

Conclusion: In both cases, absence of active IFT is not accompanied by flagellum shortening meaning that IFT is not necessary for flagellum length maintenance. Proteomic comparison of flagella is in progress and suggests modification of the flagellum content in the absence of active IFT.

O18 The Intraflagellar Transport Protein IFT27 promotes BBSome exit from cilia through the GTPase ARL6/BBS3

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Results: Upon disengagement from the IFT-B complex, the IFT27/RabL4 subunit directly and specifically recognizes nucleotide-empty ARL6. IFT27 stabilizes nucleotide-empty ARL6 against aggregation, supporting a role for IFT27 in promoting nucleotide exchange on ARL6.

Immunocytochemistry on IFT27-depleted cells reveals hyperaccumulation of ARL6 and BBSome in cilia. Direct measurements of ciliary entry and exit rates show that IFT27 promotes BBSome exit out of cilia with no influence on entry, thus placing the site of IFT27 action within cilia. While the BBSome is normally associated with IFT trains inside cilia, most of the BBSome is dissociated from IFT trains in IFT-depleted cells. A putative BBSome cargo, the Hedgehog signaling intermediate GPR161, accumulates inside cilia of IFT27 and ARL6 knockout cells.

Conclusions: Our data suggest that upon disassembly of IFT/BBSome trains at the ciliary tip, the IFT27 subunit transiently detaches from the IFT complex to participate in GTP loading onto ARL6, which then triggers formation of a retrograde BBSome coat for trafficking of the BBSome and its associated cargoes out of cilia. In other words, the disassembly of an anterograde IFT/BBSome train promotes the trigger for assembly of the future retrograde IFT/BBSome train.

O19 Developing stem cell therapy for retinal dystrophies

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Inherited retinal disease leading to death of the photoreceptor cells affects around 1 in 3000 people and includes the retinal ciliopathies which disrupt the function of proteins associated with the photoreceptor cilium. We are interested in the potential for repair of the retina and preservation of vision via cell transplantation. This approach could be broadly applicable across a range of inherited retinal dystrophies. Our work has identified an optimal donor cell population from the developing retina, that following subretinal transplantation in adult mice generates large numbers of new functional rod photoreceptors [1]. Although retinal neurogenesis ceases soon after birth, successful integration and differentiation of new rod cells can be achieved within the adult retina by transplanting immature post mitotic cells already committed to a photoreceptor fate. This same type of photoreceptor precursor cell can be isolated from 3D embryonic stem cell-derived retinal differentiation cultures and successfully transplanted into mouse models of retinal disease [2]. Thus, pluripotent stem cell lines offer the potential to generate unlimited quantities of photoreceptor precursor cells for retinal repair and restoration of vision. Current and future challenges for the development of photoreceptor cell replacement therapies for retinal disease will be discussed.

References
Cilia and flagella are cylindrical organelles that protrude at the surface of numerous eukaryotes including most human cells. They are composed, from base to tip, of the basal body (9 triplet microtubules), the transition zone (TZ, 9 doublet microtubules) and the axoneme (9 doublet microtubules ± 2 central microtubules). New flagellar sub-units are added at the distal tip by intraflagellar transport (IFT), a dynamic process where IFT motors drag IFT particles in both anterograde and retrograde directions. IFTs concentrate in a pool at the base of the flagellum, whose localization depends on the organism. In Trypanosomatidae, immunofluorescence assays reveal the association of this pool to the TZ, split apart from the cytoplasm by the transitional fibres. In Trypanosoma brucei, we undertook to investigate about the role of the TZ in flagel lum expansion and maintenance of IFT in mature flagella, by depleting the role of RP2, a candidate protein located at the TFs [Stephan et al., Traffic 2007]. We constructed a strain impaired in RP2 production by tetracycline-inducible RNAi and obtained the expected phenotypic: clinical growth was affected from the third day of induction. In parallel, diminution of IFT concentration at the flagellum base suggests that RP2 is involved in IFT recruitment during flagellum formation and/or RP2 takes part in a ciliary gate that hinders IFT leaking towards the cytoplasm. We are currently studying these hypotheses by live microscopy using fluorescent fusions of IFT proteins, RP2, and other ciliary gate candidates, dissecting the molecular structure and mechanisms involved in flagellum construction and homeostasis.

**POSTER PRESENTATIONS**

**P1**

**Phenotypic variability in Meckel-Gruber syndrome**

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**Objective:** Meckel-Gruber syndrome (MGS) is a clinically and genetically heterogeneous disease with severe multisystem manifestations, associated with mutation in primary cilia-related genes. It is marked by a characteristic triad of major abnormalities: brain malformation (typically encephalocele), hepatofibrolenticular cysts and polydactyly. The aim of the study was to present a comprehensive analysis of autopsy findings in fetuses with MGS and to emphasise the phenotypic variability in this ciliopathy.

**Methods:** We retrospectively examined the clinico-pathological findings in ten fetuses with MGS. The autopsies were performed after medical interruption of pregnancy due to fetal malformations.

**Results:** All cases had bilateral and symmetrical enlargement of the kidneys with abdominal distension and lung hypoplasia. Nine fetuses had classical clinical triad. Polydactyly was absent in one case. Brain abnormalities consisted of encephalocele in nine cases (with molar tooth sign in two cases) and Dandy-Walker malformation in one case. Additional anomalies included microphthalmia (n = 1), aniridia (n = 2), cleft palate (n = 3), lobulated tongue (n = 3), micrognathia (n = 3), umbilical hernia (n = 1), genital abnormalities (n = 3), club foot (n = 5), cardiac septal defects (n = 1) and polysplenia (n = 2). Microscopically, the renal parenchyma demonstrated diffuse multicystic dysplasia. The liver showed fibrosis of the portal areas with variable degree of ductal proliferation and dilatation.

**Conclusion:** Our data confirmed that MGS may demonstrate variation in phenotypic expression. However, occipital encephalocele or other central nervous system malformation and fibroelastic changes in the kidney and liver are constant findings. Thus, meticulous autopsy is necessary to carefully assess all the various possible anomalies associated with MGS.

**P4**

**Identification of Polycystin-2 and CFTR common targets**

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Autosomal-Dominant-Polycystic-Kidney-Disease type-II (ADPKD-II) is caused by mutations in the Polycystin-2 (PC2) encoding gene. The dysfunction of this Ca2+-conducting channel leads to the formation of fluid-filled renal cysts (1). Through poorly known mechanisms, cystogenesis entails an overstimulation of Cystic-Fibrosis-Transmembrane-conductance-Regulator (CFTR), a key Cl-channel in epithelia’s ion/fluid transport (2). To study PC2-CFTR crosstalk we used the zebrafish embryonic node (Kupffer’s Vesicle, KV) as a model system. Both proteins are required for KV proper function (3,4).

**Objective:** To determine common gene targets of PC2 and CFTR specific knockdown.

**Methods:** foxj1a:gfp transgenic zebrafish embryos (5) were injected with antisense morpholinos against pc2 (augMO-pc2) or cftr (augMO-cftr). This strain offers a KV specific GFP-reporter at 10-11 somites stage. KV cells were isolated by Fluorescent-Activated-Cell-Sorting (FACS) and tested for its quality (Agilent 2100 Bioanalyzer, Affymetrix). Transcriptomes were assessed with the Zebrafish Gene 1.1 ST Array Strip (Affymetrix).

**Results:** ~2 ng of each morpholino were required to efficiently reduce the PC2 and CFTR expression. In agreement to the literature (3,4), the augMO-pc2 induced curly-up tail and the augMO-cftr prevented the proper KV lumen expansion. In both cases laterality defects were observed. We have novel information on differentially transcribed genes that we are validating by qPCR.

**Conclusions:** Among the PC2- and CFTR-knockdown overlapping targets, we found genes encoding proteins involved in the Calmodulin-mediated Ca2+-signalling. These could be involved in the PC2-CFTR crosstalk.

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**References**

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**P5**

The study of a total and two hypolhamic-specific BBS10 knockout models highlights the importance of systemic inactivation in the obese phenotype in Bardet-Biedl Syndrome

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The autosomal recessive disorder, Bardet-Biedl syndrome (BBS) is an iconic ciliopathy, clinically characterized by obesity, retinopathy, polydactyly and renal dysfunction. To date, 19 BBS genes have been identified, with BBS10

**Conclusion:** Our data confirmed that MGS may demonstrate variation in phenotypic expression. However, occipital encephalocele or other central nervous system malformation and fibroelastic changes in the kidney and liver are constant findings. Thus, meticulous autopsy is necessary to carefully assess all the various possible anomalies associated with MGS.
being one of the most commonly mutated genes in human patients. The historical origin of the BBS-induced obesity has been associated with leptin resistance correlated with hyperleptinemia as well as decreased signaling in the appetite-governing arcuate nucleus neurons (ARC) of the hypothalamus. The ARC controls energy homeostasis, food intake and energy expenditure, through the detection of peripheral hormones by POMC and AgRP/NPY expressing neurons. POMC neurons are anorexigenic while NPY/AgRP are orexigenic, and both are ciliated cells. Several hormone receptors have ciliary localization, as the leptin receptor, and inactivation of the BBS proteins results in their mislocalization and signaling impairment. The present work aims at investigating the origins of obesity in the BBS by comparing the phenotype of a Bbs10 total knockout (Bbs10<sup>−/−</sup>) with that of two Bbs10 hypothalamic-specific KO mice: namely the POMC (Bbs10<sup>−/−POMC-Cre<sup>−/−</sup></sup>) and the AgRP (Bbs10<sup>−/−AgRP-Cre<sup>−/−</sup></sup>). Bbs10<sup>−/−</sup> mice develop obesity, together with other BBS cardinal traits, but surprisingly, both Bbs10<sup>−/−POMC-Cre<sup>−/−</sup></sup> and Bbs10<sup>−/−AgRP-Cre<sup>−/−</sup></sup> display a lean phenotype. Further characterizations of these mice highlighted the activation of compensatory mechanisms in response to the specific BBS10 inactivation probably forestalling the obese phenotype. These results indicate the complexity of the BBS-related obese phenotype, and support the need for an integrative approach that would include the contribution of other peripheral organs to better understand the origins of obesity in BBS.

### P6

**Abnormalities in primary ciliary of osteoblasts of adolescent idiopathic scoliosis patients**

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Adolescent Idiopathic Scoliosis (AIS) is a common complex genetic disease and one of the most prevalent childhood deformities. AIS is clinically characterized by a 3D spinal deformity with unknown cause. The aim of this study is to identify rare genetic variants and associated biological pathways involved in AIS onset and its progression. We recently completed exome sequencing of 69 AIS cases vs. 70 matched controls in a French-Canadian AIS cohort using SOLID S technology. Results showed two gene families with significant enrichment for single nucleotide variants (SNVs) involved in cilia signalling pathways. To investigate the functional effect of these SNVs, we explored biomechanical responses on osteoblasts from AIS patients carrying these SNVs. More specifically, we applied a sinusoidal/oscillatory fluid flow at 2Pa, 0.5Hz for 90min using parallel plate flow chamber. Preliminary qPCR results of the cells post flow showed a significant increase (±10-folds) in the expression of cyclooxygenase-2 (COX2) in AIS cells versus control ones. Interestingly, microscopy imaging of the same cells revealed a 30% decrease in the length of cilia (p<0.001) of AIS cells when compared to control ones. Of note, increased COX2 expression has been reported as a flow-modulated response for bone cells. Our results suggest an increased sensitivity of AIS bone cells to fluid flow due to the elongation of cilia. Although cilia abnormalities have never been investigated in idiopathic scoliosis, ciliary structural and functional abnormalities in AIS osteoblasts might be involved in the onset and progression of the disease. More extensive experiments are currently underway to confirm the role of cilia in AIS pathogenesis.

### P7

**Mutations of IFT81, encoding an IFT-B core protein, as a rare cause of a ciliopathy**

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Objective: To identify ciliopathy-causing genes in a very large cohort of patients with symptoms consistent with cilia dysfunction.

Methods: 1,036 index cases with nephropathies-related ciliopathies were screened for mutations in all genes encoding components of IFT-B and 572 unrelated individuals with early onset retinal dystrophies or multisystemic ciliopathies were subjected to targeted ciliome resequencing.

Results: Homozygosity for IFT81 mutations were identified in two consanguineous sporadic cases. The first individual harbored a splice site change predicted to result in an intron exon skipping; the second carried a 4 bp deletion resulting in a loss-of-stop with extension of the deduced protein by 10 amino acids. The spectrum of IFT81-related disease expression included nephronophthisis, retinal dystrophy, cerebellar atrophy, and polydactyly. Fibroblasts from one affected individual showed no difference to control cells with regard to IFT81 localization or binding to IFT25, but a statistically significant decrease in ciliated cell abundance was noted. GLI2 expression and ciliary localization were impaired suggesting altered sonic hedgehog signaling.

Discussion and conclusion: Mutations in all components of IFT-A complex have been reported to cause ciliopathy phenotypes. In contrast, only two peripheral IFT-B members, IFT172 and IFT80, were known to be involved in these conditions. The identification of mutations in the IFT-B core protein IFT81 in two unrelated patients out of 126 individuals with ciliopathy further elucidate the role of this complex in human disease and show that defects in the IFT-B core are an exceedingly rare finding supporting the view that it is indispensable for ciliary assembly in development.

### P8

**Genotype-Phenotype correlations in Joubert Syndrome in the Era of Next Generation Sequencing**

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Objective: To provide extensive genotype-phenotype correlations for Joubert syndrome (JS), a ciliopathy characterized by a distinctive hindbrain malformation ("the molar tooth sign"), ataxia and cognitive dysfunction.

Methods: Phenotypic data was collected from the University of Washington JS cohort and all known JS genes were sequenced in 429 individuals (364 families) using the MIPS capture technique and next-generation sequencing.

Results: Core JS diagnostic features (hypotonia, ataxia, cognitive dysfunction, oculo-motor apraxia) were present in >80% of individuals, while abnormal breathing pattern was reported in 60%. Frequently associated features included retinal dystrophy (31.4%), renal disease (20.9%), coboloma (17.7%), polydactyly (15.3%), liver fibrosis (15.2%) and encephalopathy (8%). Liver fibrosis and coboloma were strongly associated with each other (Odds Ratio 7.0, 95% Confidence Interval = 3.0-13.2), while retinal dystrophy and renal disease were weakly associated (O.R. 2.2, 95%CI = 1.7-5.6). Additional clinical features included other brain abnormalities (n = 73), seizures (n = 49), cleft palate (n = 16), hearing loss (n = 14) and psychiatric problems (n = 45). The genetic cause was identified in 60% of families, with 5 genes accounting for the majority of patients (COSRF42, CEP290, CC2D2A, AHI1, TMEM67). Bi-allelic causal
mutations in B9D2 and C2CD3 were identified in 2 families each. Bi-allelic mutations in 2 different genes were identified in 4 families and heterogeneous mutations (in addition to the causal mutation) were present in 62 individuals. Significant (p<0.001) genotype-phenotype correlations were observed: CEP290 with renal disease and retinal dystrophy; TMEM67 with liver fibrosis and coloboma.

Conclusion: This study provides a comprehensive description of the phenotypic spectrum, genetic makeup and genotype-phenotype correlations of a large JS cohort.

P9
Investigation into the Importance of genes encoding cilary proteins in congenital heart disease using whole exome sequencing
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Congenital Heart Disease (CHD) is the most common congenital defect. Many families with left-laterality defects and complex CHD have an unknown genetic aetiology. Many ciliopathies, including Primary Ciliary Dyskinesia (PCD), are associated with intracellular defects. The role of primary cilia in cardiac morphogenesis remains unknown, although cardiac cilia have roles that are distinct from the definition of laterality at the embryonic node. We hypothesise that defects in genes important in the assembly and function of cilia are responsible for some inherited forms of CHD.

This research project aims to recruit families with a recurrence of CHD and to perform Whole Exome Sequencing (WES) to identify putative pathogenic variants and to delineate novel genetic causes of CHD. Twelve families have now been recruited and WES has been carried out in seven of these families using paired-end sequencing. Data analysis follows a standardised pipeline to call and then filter variants in order to assess their pathogenic potential. Variants are prioritized on the basis of known or suspected function of the encoded protein, and publicly-available RNA expression data.

Variant filtering has allowed the identification of a limited number of candidate variants in recruited families. Of particular interest, a likely causative homozygous variant within a PCD gene has been identified in two siblings affected with heterotaxy, thus confirming a link between ciliopathies and CHD. The function and interactions of identified genes will be assessed, using cellular techniques and animal models, to provide insights into the pathogenesis of CHD.

P10
DNA Methylation and Cilopathies: a way to be explored
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Objective: Since the molecular basis underlying cilopathies such as Bardet-Biedl (BBS) or Alström (ALMS) syndromes is not fully understood, we hypothesised that changes in pattern of DNA methylation, due to its role in embryogenesis and differentiation, could be a mechanism that explains the pathogenesis of these diseases.

Methods: CpG islands search was performed by Methyl Primer Express software v1.0 (Applied Biosystems) in the promoter region of ALMS1, BBS1, BBS2, BBS5, BBS7, BBS9, BBS10 and BBS12 genes. In order to quantify the degree of methylation, we carried out MS-qPCR using SYBR Select Master Mix (Applied Biosystems). Blood lymphocyte DNA samples from seven patients with ALMS were selected.

Results: Regarding to bioinformatic analysis, all genes harboured at least one CpG island. Some of them included one or more sequences compatible with x-box motifs, which can be recognized by transcription factors of RFX family that are known to be involved in the regulation of ciliary genes transcription.

We selected a CpG island in the ALMS1 gene containing 67 cytosine residues potentially methylated for performing MS-qPCR. A mean efficiency ranging from 90 to 96% was reached for each amplicon in which the CpG island was divided. Unfortunately, no methylation was detected in the enrolled patients.

Conclusion: Although the results of this preliminary study were negative, limitations due to sample size, sample type and experimental approach have to be taken into account. However, we consider it worth exploring this mechanism in BBS and ALMS using different techniques such as methylation arrays, which could provide more accurate data.

P11
Performing whole-exome sequencing in Bardet-Biedl syndrome
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Objective: Bardet-Biedl syndrome (BBS) is a rare disease characterized by a high genetic heterogeneity, accounting for 75% of affected families. As part of the next-generation technology, whole-exome sequencing (WES) allows all exons of the genome to be sequenced at once. Here we show the use of WES as a useful approach in BBS families in which mutations in predominant genes have been discarded.

Methods: We studied the exome of 15 unrelated patients clinically diagnosed with BBS. For this purpose, we used the Nimblegen SeqCap v3 (64Mb) kit for exome capture, followed by the use of Illumina HiSeq 2000 sequencer, with a mean coverage per sample >50X. Once the sequencing data alignment and variant calling were made, we carried out a filtering strategy to identify candidate mutations responsible for the disorder, which need to be confirmed by direct sequencing. Segregation analyses were performed when possible.

Results: This approach, focused on evaluating mutations in genes involved in the pathology (all BBS genes, and also ALMS1 gene), has allowed us to diagnose 4 families, identifying 5 potential disease-causing mutations representing 3 different known genes (BBS2, BBS3 and ALMS1). 4 of these changes were novel mutations and segregation studies confirmed the carrier state in parents. In the remaining families, we have selected some candidate genes which are under evaluation.

Conclusion: We consider this is a good and economically worthwhile strategy when predominant mutations have been discarded in the analysed population. Moreover, it allows us to reduce diagnostic time and offers the possibility to identify novel candidate genes.

P12
PDZD7 connects the Usher protein complex to the intraflagellar transport machinery
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Objective: Several Usher syndrome (USH)-associated proteins are known to localize to the connecting cilium of photoreceptor cells. The unconventional myosin MYO7A (USH1B) was long accepted as the transport molecule responsible for the cilary localization of USH proteins. However, based on the typical location of several of the USH proteins along the ciliary
axoneme, the involvement of the main ciliary trafficking machinery, intraflagellar transport (IFT), seems apparent. The USH-associated scaffold protein PDZD7 is known to interact with SANS, Usherin, GPR98 and Whirlin, all of which can be found in the connecting cilium. Here, we report that PDZD7 provides the physical link of the USH-protein network to IFT-complex members. Tandem affinity purification (TAP) studies revealed a potential interaction between PDZD7 and several IFT molecules, including IFT25 and IFT27. TAP analyses of the other USH proteins were negative for IFT proteins, suggesting that this interaction is unique for PDZD7. In addition, a dedicated yeast two-hybrid screen of 200 (predicted) ciliary proteins revealed an interaction between PDZD7 and IFT57. The interaction between PDZD7 and selected IFT subunits was substantiated by co-immunoprecipitations. In accordance with these results, mRFP-tagged PDZD7, expressed in ciliated hTERT-RPE1 cells, localizes not only at the basal body, but also at the axoneme of a subset of cells. Pending further validation of the interaction between PDZD7 and IFT-8 proteins, these first results suggest PDZD7 as a functional connection between USH-proteins and the IFT machinery. Future studies should reveal whether PDZD7 is involved in IFT of USH proteins in vivo.

P15

Prostaglandin-D2 synthase localises to centrioles and primary cilium, and interacts with TOPORS, implicated in retinal cilopathy
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Objective: Prostaglandin-D2 synthase (PTGDS; MIM#176803) is a novel protein-partner of TOPORS (TOPORS; MIM#609507), a ubiquitously expressed nuclear and cilary protein, implicated in retinitis pigmentosa. This study investigated the localisation of PTGDS and its potential mechanism-of-association with TOPORS.

Methods: Yeast two-hybrid screens, using TOPORS as bait, were performed against human retinal CDNA libraries. Validation and interaction-characterisation were performed in yeast, and by co-immunoprecipitation (co-IP) from HeLa cell extracts. Co-localisation studies were performed in hTERT-RPE1 cell line, and in murine retina cryo-sections. PTGDS expression was validated by RT-PCR.

Results: Co-IP demonstrated PTGDS was found in endogenous protein complexes with TOPORS, whereas in yeast PTGDS interacted most strongly with TOPORS’ residues 1-380, comprising the RING-domain conferring its E3-ubiquitin-ligase activity. PTGDS co-localised with TOPORS, and cilorial markers in dividing cells, and was observed at basal body and along ciliary axoneme in ciliated cells. In mouse retina PTGDS was observed in several cell layers, partly overlapping with TOPORS in the photoreceptor layer. In human retina, RT-PCR studies demonstrated expression of several PTGDS isoforms.

Conclusion: PTGDS, a novel component of the primary cilium, could be involved in centriolar-ciliary homeostasis. This putative role of prostaglandin synthases, is additionally supported by independent findings on the role of prostaglandin-E2 in ciliogenesis. Results suggest TOPORS could regulate PTGDS levels at the cilium by marking it for degradation by the ubiquitin-proteasome system, providing a basis for understanding the retinal cilopathy associated with TOPORS mutations.

P16

Comparative genomics reveals novel genes associated with sensory cilia
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Objective: To identify evolutionarily conserved ciliary genes that facilitate sensory-specific roles.

Methods: Using reciprocal BLAST analyses, the genomes of organisms that do not make cilia (the plant Arabidopsis thaliana and the yeast Saccharomyces cerevisiae) and that retain motile but not sensory cilia (the moss Physcomitrella patens) were subtracted from the genomes of organisms that have retained sensory cilia (the worm Caenorhabditis elegans and the alga Chlamydomonas reinhardtii).

Results: These analyses revealed a list of 272 genes that are found exclusively in organisms with sensory cilia but not motile cilia. Importantly, nearly 10% of the genes on this list have previously been implicated in sensory cilia-specific roles, thus providing numerous internal positive controls that demonstrate this list is enriched with sensory-specific ciliary genes. A subset of uncharacterized candidate genes are currently being studied in C. elegans, which retains cilia exclusively on a set of neurons termed ciliated sensory neurons (CSNs). We are currently generating a number of promoter- and gene-to-green fluorescent protein (GFP) fusion constructs in order to determine the expression and localization patterns of the proteins encoded by these genes. Two of these candidate genes, which are found in worms (C. elegans) and algae (C. reinhardtii) but not in moss (P. patens) have been termed wam-1 and wam-2, respectively. Expression of wam-1 appears to be localized exclusively in the support cells of ciliated dopaminergic neurons, while expression of wam-2 has yet to be fully characterized.

Conclusion: Our analyses have successfully revealed novel genes involved in ciliary sensory-specific processes in animals.

P17

Coordination of TGFβ/BMP signaling is associated with the primary cilium
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We previously showed that canonical TGFβ signaling is regulated in part by the primary cilium, and that ciliary TGFβ signaling is upregulated in stem cells differentiating into cardiomyocytes [1]. Ciliary signaling was shown to be associated with clathrin-dependent endocytosis at the ciliary
Rab23 is a novel role in the trafficking of Kif17 to the primary cilia

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Rab GTPases are regulators of the intracellular vesicular transport and interorganellar protein trafficking of both endocytic and secretory pathways. The small GTPase Rab23 is an antagonist of the Sonic hedgehog (Shh) signaling pathway during mouse embryonic development, but its exact role and mode of mechanism have remained elusive. Since modulation of Shh signaling depends on normal functioning of primary cilia, and EvIII's overexpression (Rab23's RabGAP) led to decreases in primary ciliogenesis, Rab23 likely has a role at the cilia. We found Rab23 wild-type and constitutively active Rab23Q68L, mutant enriched at the primary cilia. In testing the effect of Rab23's manipulations on the ciliary localization of several known ciliary cargoes, ciliary localization of a kinesin-1 motor protein Kif17 turned out to be disrupted by overexpression of dominant negative Rab23S23N and in Rab23-depleted cells. In addition, Kif17's ciliary mislocalization could be rescued after expression of a non-degradable wild-type Rab23 and FRAP experiments showed that Rab23 silenced cells exhibited reduced recovery of ciliary Kif17 after photobleaching. Co-immunoprecipitation studies further revealed that Rab23 exists in a tripartite complex with Kif17 and Importin II (Kif17's ciliary import carrier), implying that Kif17's binding requires to regulatory proteins like Rab23 for its ciliary transport. Although a ciliary-cytosplasmic gradient of nuclear Ran is necessary in regulating Kif17's ciliary import, both small GTPases Rab23 and Ran appear to have independent roles in the ciliary entry of Kif17. Our findings have uncovered a hitherto unknown effector of Rab23 and demonstrated how Rab23 could mediate Kif17's trafficking to the primary cilia.

P19

Pericentrin interacts with KASH domain-containing protein Syne-2

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Objective: Pericentrin, a highly conserved protein of the pericentriolar material, serves as a multifunctional scaffold for numerous proteins and plays an important role in microtubule organization. Mutations in the human PCNT gene are associated with a range of diseases including primordial dwarfism and ciliopathies. In the mouse retina Pericentrin colocalizes with several proteins responsible for transport processes at the connecting cilium between the two photoreceptor compartments. In order to get more insights on the function of Pericentrin in the retina we try to identify new cilia as well as centrosomal interaction partners.

Methods: Identification of Pericentrin interaction partners was done by Tandem Affinity Purification, Yeast two-hybrid with a self-constructed cDNA library from mouse retina and GST pull-down.

Results: We were able to show that Pericentrin interacts with Klarsicht/ANC-1/Syne-homologue (KASH) domain-containing protein Syne-2. Furthermore, we found a partial colocalization of Pericentrin and Syne-2 in the ciliary region of mouse photoreceptors. Pericentrin is localized at the basal body of the connecting cilium while Syne-2 seems to be localized in the inner segment of photoreceptors.

Conclusion: Yu et al. (2010) suggested an interaction between Syne-2 and dynein/dynactin as well as kinesin complexes as the molecular motor of nuclear migration in the mouse retina. The interaction between Pericentrin and Syne-2 could play an essential role in interkinetic nuclear migration and may provide us new insights in the photoreceptor cell migration progress in general.

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P21
Remodeling Cildb, a popular database for cilia and links for ciliopathies
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Cildb (http://cildb.cgm.cnrs-gif.fr/) is a multi-species knowledgebase gathering high throughput studies, which allows advanced searches to identify proteins involved in centrosome, basal body or cilia biogenesis, composition and function. Combined to localization of genetic diseases on human chromosomes given by OMIM links, candidate ciliopathy proteins can be compiled through Cildb searches. Since its creation in 2009, Cildb has been updated twice and the latter version displays a novel BioMart interface, much more intuitive than the previous ones. We describe here the novelties of the new version V3.0 and give an example on advanced search on centrosomal proteins to illustrate the evolutionary conservation viewed through Cildb.

P22
Fluid flow-induced bending of the primary cilium triggers a distinct intracellular calcium flux in mesenchymal stem cells
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Objective: Primary cilia are proposed to form a regulatory microdomain in mesenchymal stem cells (MSCs) where they have also been shown to be necessary for loading-induced osteogenic lineage commitment. The specific mechanisms mediating this are unclear. This study aims to characterise subcellular ciliary calcium signalling in response to mechanical load and identify the mediating channels in MSCs.

Method: Genetically encoded calcium indicators were transfected into murine MSCs. To analyse calcium within the ciliary micro-domain, we linked a sensor to a ciliary specific GTPase (Arl13b). Oscillatory fluid flow was applied to model physiological mechanical loading. High speed real-time imaging of the sensors’ was acquired. Ionomycin was added to the medium as a positive control. Immuno-cytological analysis of calcium was performed in all but the GBF1-ASAP1 interaction, exclusively at the Golgi/TGN. The GBF1-rhodopsin-Arf4 interaction, nearly exclusively at the Golgi/TGN. The GBF1-GEF activity was essential for the GBF1-rhodopsin-Arf4 interaction, since its selective inhibitor Golgicide A (GCA) caused a significant decrease in all but the GBF1-ASAP1 interactions. GCA specifically inhibited rhodopsin delivery to the cilia, but, in contrast to BFA, it had no effect on the Golgi morphology. GST-pulldowns of purified rhodopsin with DCB-HUS domain, whereas GDP-bound Arf4 interacts with DCB-HUS domain, whereas GDP-bound Arf4 interacts with DCB-HUS domain, whereas GDP-bound Arf4 interacts with DCB-HUS domain.

Conclusion: Our data indicates that under fluid flow, there is a discrete calcium response which is believed to coordinate the recruitment and organization of the two apical complexes: the Crumbs (Crb/Pals-1/PATJ) and the PAR (Par3/Par6/aPKC) complexes. In this study, we investigated the role of Crumbs in ciliogenesis.

Methods: The depletion of crumbs2 (Crb2) and/or crumbs3 (Crb3) via transient siRNA transfection in ARPE-19 cells (human pigmented retina epithelium) leads to strong inhibition of ciliogenesis which underscores the involvement of Crb2 and Crb3 in the formation of the primary cilia.

Results: Crb2 and Crb3 share many protein interactions and mechanisms of compensation could exist. However our data revealed the contrary since we showed that primary cilia formation requires a threshold level of both Crb2 and Crb3. To decipher the mechanism that underlies this requirement we have focused on Crb2, which is predominantly expressed in ARPE-19 cells. Using both optical imaging and electron microscopy we showed that Crb2 is involved in cilia initiation but not cilia maintenance. Furthermore we uncovered that Crb2 acts at a very early stage of ciliogenesis, by affecting the localization of centriolar and peri-centriolar markers such as PCM-1.

Conclusion: Crb2 allows the efficient organization of the centrosome and associated proteins and the primary vesicle formation to promote ciliogenesis. Taken together, our data show that Crb2 is essential for the primary ciliogenesis by a still unknown mechanism.

P25
Integrative approaches to investigate the structure and assembly of Trypanosoma brucei BILBO1, a multidomain cytoskeletal protein at the flagellar pocket collar
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Objective: This project aims to identify the function of polarity protein complexes in the formation of the primary cilia. We focus specifically on the two apical complexes: the Crumbs (Crb/Pals-1/PATJ) and the PAR (Par3/Par6/aPKC) complexes.
Trypanosoma brucei is a protist parasite and the causative agent of Human African Trypanosomiasis (sleeping sickness). At the base of its single flagellum is a bulb-like structure called the flagellar pocket (FP). The FP is the site of all endo-/exo-cytosis and thus essential for the survival of the parasite. At the neck of the FP is an electron-dense cytoskeletal structure termed the flagellar pocket collar (FPC), which currently has only one known protein component, BLB01. Bioinformatic analysis indicates that there are four structural domains in the 67-kDa protein, including a globular N-terminal domain, two central EF-hand motifs followed by a long coiled-coil domain, and a C-terminal leucine zipper. T. brucei BLB01 (TbBLB01) by itself forms insoluble oligomers in vitro, which makes it intractable to any single conventional structural study method. We recently carried out structural dissection of TbBLB01 using integrative structural biology approaches including NMR, crystallography, EM, and various biophysical methods. The high-resolution structure of its N-terminal domain reveals a variant ubiquitin-like fold with a conserved surface patch; mutagenesis of this patch causes cell death in vivo. We further found that the EF-hand motifs change their conformation upon calcium binding, the coiled-coil domain forms an antiparallel dimer, and intermolecular interactions between adjacent leucine zippers allow TbBLB01 to form extended filaments in vitro. These filaments were additionally shown to condense into fibrous bundles through lateral interactions as demonstrated by our EM studies. Based on all these experimental data, we propose a mechanism for TbBLB01 assembly into the flagellar pocket collar.

P26
Transition zone: the sequential assembly of its components parallels its dual role in basal body anchoring and ciliary function
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Objectives: The assembly of cilia can be followed step by step in the unicellular Paramecium, thanks to its predictable spatio-temporal pattern of basal body (BB) duplication and ciliary growth. In order to dissect the process of transition zone (TZ) assembly, we compared the behaviour of several proteins.

Methods: Combination of EM, immunocytochemistry, protein tagging and RNAi knockdowns.

Results: Two proteins, FOR20 and OFD1, present at the level of the terminal TZ plate in ciliated BB, are recruited during early BB assembly, are required for building the nascent BB tip where a cap is detected and are necessary for BB anchoring. After anchoring and before ciliation, a structure similar to the TZ, defined as the pro-TZ, is detected at the BB tip. At that stage, OFD1 and FOR20 are detected at the level of the proximal pro-TZ plate. In contrast, two other proteins involved in the ciliary barrier function, MKS2, and in the axonemal building, IF5T7, are detected only at ciliated BB and recruited at time of ciliation in correlation with the extension/mutation of the pro-TZ into TZ. The depletion of these proteins does not affect BB docking.

Conclusion: 1. The assembly of the transition zone proceeds stepwise (building of a cap on nascent BB; differentiation of a pro-transition zone on anchored non ciliated BB; maturation of the transition zone during ciliation).

2. These steps correlate with the recruitment of proteins required for BB anchoring and ciliary growth/function respectively, highlighting the dual role of the TZ in the process of ciliation.

P27
MARK4 contributes to cilia formation by regulating the degradation of inhibitory OFD1 from the centriolar satellites
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Cilia formation starts at the mother centriole and requires the activity of the microtubule affinity regulator, MARK4, which promotes axoneme extension during the initial phase of ciliogenesis. The defective axoneme extension in MARK4 depleted cells could be in part rescued by co-depletion of the inhibitory complex CP110/Cep97. Whether MARK4 only influences CP110/Cep97 or influences additional inhibitory components is not known. Interestingly, MARK4 has been recently shown to regulate the position and movement of autophagic vesicles within the cell. Recent studies also revealed that autophagy promotes ciliogenesis by inducing the selective degradation of the centriolar satellite pool of OFD1, an inhibitor of centriole elongation and axoneme extension. Here, we investigated whether MARK4 is functionally linked to autophagy to promote cilia formation. Analysis of RPE1 cells stably expressing YFP-LC3 (Suppl L2) shows that autophagosomes exhibit a perinuclear clustering upon MARK4 depletion. Quantitative immunolocalization analysis shows that in MARK4 depleted cells, OFD1 levels at the centriolar satellites are higher than in control cells. This could be reverted by partial knock down of OFD1. By co-depleting MARK4 and OFD1, the cilia loss phenotype of MARK4 knockdown was partially reverted. Our results suggest that MARK4 acts in ciliogenesis by regulating the movement and position of the autophagosomes that degrade OFD1 at the centriolar satellites.

P28
Nucleoporins but not septicans at the transition zone of cilia in Paramecium and Tetrahymena
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The transition zone (TZ) of locomotory cilia participates in docking young basal bodies to the surface, stabilizes the basal body-membrane connection, forms a diffusion barrier and modulates the intraciliary transport. In eukaryotic ciliated cells, nucleoporins and septins were suggested to be present in the TZ of locomotory and primary cilia. Here we present data suggesting the presence of nucleoporins, at both nuclear pores and ciliary TZ, in Tetrahymena and Paramecium. Using the monoclonal 414, specific to FG-containing nucleoporins, and anti-tubulin antibodies, we detected labeling at the distal part of basal bodies and as a spotted pattern around nuclei. In previous studies on Tetrahymena, GFP-tagged septins (Sep1, Sep2 and Sep3) localized to mitochondrial/ER compartments but neither to basal bodies, cilia nor nuclei. Tetrahymena cells with knocked out septins display disrupted nuclear (Mac and Mic) membranes, abnormal mitochondria, but unaffected ciliary TZ. Similarly, in Paramecium, silencing of the SEP2 gene revealed affected nuclear membranes and mitochondria but normal TZ, and the mAb 414 and DAPI showed a normal labeling of the ciliary base, but patches of missing nuclear pores in the macronucleus.

Our studies support the conclusion that some nucleoporins are present in both nuclear pores and ciliary TZ. In addition, the nuclear pore proteins seem to interact with septins, yet the lack of effect of septin silencing on the localization of the mAb 414 in the ciliary TZ suggests that nucleoporins may have different binding partners in nuclear pores and ciliary TZ.

P29
OFD1 and VFL3/CCDC61 in basal body positioning and docking in Paramecium
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Objectives: Ciliogenesis is conditioned by a correct positioning/anchoring of the basal body at the cell surface. In Paramecium we have shown that three conserved proteins FOR20, centrin 2 (CEN2) and centrin 3 (CEN3) participate in this process, with FOR20 and CEN2 being also involved in the transition zone assembly. We established a chronology in basal body assembly: CEN2 is required for FOR20 recruitment, the latter being necessary to recruit CEN3. Our goal now is to integrate other molecules in this cascade.

Methods: We used a combination of electron microscopy, immunocytochemistry, GFP protein tagging and RNAi knockdowns to study the function of OFD1 and VFL3/CCDC61 in Paramecium. OFD1 is a well-studied
protein which is involved in human development whose mutations in human males can impair basal body docking. In contrast, only studies in Chlamydomonas indicate that VFL3 could be involved in this phenomenon.

**Results:** As in human, the depletion of OFD1 in Paramecium induces defects in basal body docking, these defects being similar to those observed upon inactivation of FOR20, CEN2 and CEN3: 1) like FOR20 and despite its distal location on anchored basal bodies, OFD1 is recruited early during their assembly; 2) while the recruitments of OFD1 and CEN2 proceed independently, the two molecules are required for the recruitment of FOR20. We also present preliminary results indicating that VFL3/CCDC61 is crucial for maintaining both basal body polarity and positioning and for the recruitment of CEN3, but neither for CEN2 or OFD1.

**P30**

**Gene discovery for motile cilia disorders: mutation spectrum in primary ciliary dyskinesia and discovery of mutations in CCDC151**

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We present a stratification of the genetic basis of primary ciliary dyskinesia (PCD), based on screening >230 individuals for gene mutations using various approaches including whole exome sequencing. PCD is a genetically heterogeneous recessive ciliopathy, characterized by chronic lung disease and laterality and fertility defects arising from cilia and sperm motility. Most PCD is caused by loss of the ciliary outer dynein arm motors (ODA) essential for motility, arising from mutations in ODA subunits or ODA docking and targeting proteins. Gene panel resequencing of candidate ciliopathy genes in affected children from a consanguineous Bedouin-Arabic family has recently revealed a homozygous protein truncating variant in CCDC151 (c.925G>T; p.Glu308?). Parallel exome sequencing combined with autozygosity mapping in a consanguineous UK-Pakistani-origin family highlighted a large autozygous region on chr 19p13 harbouring a homozygous CCDC151 protein-truncating variant (c.1256C>T; p.Ser419*). Sanger sequencing of CCDC151 in 150 more PCD cases identified another individual carrying c.925G>T. Transmission electron microscopy of respiratory cilia from individuals carrying CCDC151 mutations showed loss of ODA. Consistent with laterality defects in these individuals, we find Cdc151 expressed in vertebrate left-right organizers. Both homozygous zebrafish and mouse Cdc151-deficient mutants display situs defects associated with complex heart defects. Immunofluorescence analysis in patients shows that CCDC151 mutations abolish assembly of CCDC151 into respiratory cilia, and furthermore cause a failure in assembly of the ODA component DNAH5 and ODA docking proteins CCDC114 and ARMC4. We conclude that CCDC151 mutations appear to cause PCD by disruption of the axonemal ODA docking complex machinery.

Many human diseases are linked to mutations in genes encoding membrane trafficking proteins, among them the X-linked centronuclear myopathy (XLCNM) and ciliopathies. We use yeast Saccharomyces cerevisiae as a model system to study these human disease trafficking genes. It is a good model because yeast and human cells present similar intracellular organization and its membrane trafficking and metabolism are well characterized. We first studied the MTM1 gene, responsible for XLCNM, which codes for a myotubulin, a lipid phosphatase required for endosomal sorting. The heterologous expression of wild-type MTM1 or XLCNM patient mutants in yeast allowed comparing their in vivo trafficking and their cellular function in trafficking. The yeast results showed that the phosphorylation activity is not defective in patient mutants, further confirmed in the mouse Mtmt KO model for myopathy [1]. Ciliopathies are complex genetic disorders that result from defects in the formation and/or function of the primary cilium. They include disorders such as Bardet-Biedl syndrome (BBS), Joubert syndrome (JBTS) and Alström syndrome (ALS) [2]. Cilia are involved in development and in signaling cascades. Despite, the identification of a large number of genes involved in ciliopathies (http://www.syscilia.org/goldstandard.shtml), the molecular and cellular mechanisms by which they cause the disease are largely unknown. In collaboration with the laboratory of Hélène Dollfus, we aim at understanding the cellular roles of membrane trafficking genes involved in ciliopathies by using the yeast model system. Indeed, organization of the primary cilium depends on membrane trafficking since many genes linked to ciliopathies are involved in membrane trafficking [3].

**References**


**P32**

**Role of outer dense fiber of sperm tails 2-like (ODF2FL) protein in ciliation in mammalian cells and in zebrafish**

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**Background:** The centrosome is a subcellular organelle which plays an important role within the cell as the microtubule organising centre (MTOC) and many other cellular functions. In a quiescent cell, centrioles can migrate to the apical surface of the cell and nucleate hair-like projections called cilia and flagella. The stages of ciliogenesis require a number of candidate ciliopathy genes in affected children from a consanguineous Bedouin-Arabic family has recently revealed a homozygous protein trunca
Objective: INF1 is a novel member of the formin family of cytoskeletal regulatory proteins. We previously identified a connection between INF1 expression and microtubule acetylation and more recently have found that INF1 expression induces Golgi dispersion. Given the dependence of ciliogenesis on microtubule acetylation and Golgi-dependent trafficking we wished to determine the effects of INF1 expression on cilia formation.

Methods: Transient transfection was used to express a series of INF1 derivative in NIH 3T3 cells and the effects on cilia formation, cilia length and Golgi dispersion were monitored by immunofluorescence. Anti-acetylated tubulin was used to visualize cilia and anti-Giантин was used to visualize Golgi morphology.

Results: INF1 expression inhibited ciliogenesis in the majority of NIH 3T3 fibroblasts, however, inhibition of ciliogenesis was not connected to INF1-induced Golgi dispersion. A minority of INF1-expressing cells did form cilia and these were greatly elongated, the longest exceeding 70mm. A series of INF1 deletion derivatives were used to show that both inhibition of cilia formation and the induction of cilia elongation were dependent upon both the FH2 and microtubule-binding domains of INF1.

Conclusion: The effects of INF1 expression on ciliogenesis were separate from its effects on Golgi-dispersion suggesting that Golgi untethering does not always inhibit ciliogenesis. The morphology of the elongated cilia formed in INF1 expressing cells is consistent with defects in dynein function.

Objective: Cerebellar development involves a spurt of proliferation in external granule layer (EGL) in response to Shh, causing granule neuron precursors (GNPs) to proliferate. These cells subsequently differentiate into granule neurons in the inner granule layer (IGL). F3, a CNTN family molecule, can interact with NrCAM to switch GNPs from proliferation to differentiation. We aim to identify the role of NrCAM in the sonic hedgehog response in GNPs.

Methods: GNPs were extracted from wildtype and NrCAM mutant P5 cerebella using Percoll gradient centrifugation. Proliferation response to Shh was measured using EdU in presence/absence of F3-Fc. GNPs treated with shh/SAG were stained with antibodies against Ar13b and smo to look for differences in cilia size and smo occupancy after different treatment times.

Results: NrCAM-/- and wildtype GNPs both proliferated equally in response to Shh. F3 was found to block the proliferation response in wildtype but not in NrCAM-/- GNPs. F3 also failed to affect proliferation in SmoA1 GNPs with a constitutively active smo suggesting that the F3-NrCAM mediated block lay upstream of Smo. NrCAM was detected in wildtype cilia and smo localization was affected in NrCAM-/- GNPs. No differences in cilia length were observed.

Conclusion: Our results suggest that NrCAM affects Shh-mediated proliferation by controlling smo movement into the cilia.

Agensis of the Corpus callosum (AgCC) is a frequent brain disorder found in over 50 human congenital syndromes including ciliopathies. Here, we report a severe AgCC in Ftm/Rpgrip11 knock-out mice, which provide a valuable model for Mieckel-Gruber syndrome. Ftm encodes a protein of the ciliary transition zone, which is essential for ciliogenesis in some, but not all cell types in mice, including neuroepithelial cells in the developing forebrain. We show that AgCC in Ftm-/- fetuses results from a mislocalization of guidepost cells in the dorsomedial telencephalon and not from a reduction of callosal neurons in the cortex. This abnormal distribution of the guideposts primarily results from patterning defects in the medial telencephalon, which relies on Gli3 processing defects. These patterning defects, mispositioning of dorsomedial guideposts and AgCC can all be rescued by reintroducing Gli3R in Ftm-/- embryos, provided by the Gli3D699 allele, which produces only the short repressor isoform of Gli3. This rescue of AgCC of Ftm-/- mutants by one allele of Gli3D699 confirms and completes our knowledge on the pre- eminent role of Gli3 in CC formation.

Furthermore, Gli3D699 also rescue AgCC in Rfs31/-/- mutant, another ciliary mutant deficient for a transcription factor controlling several ciliary genes. Rescuing the CC formation in two independent ciliary mutants by a single molecule, namely Gli3R, highlight the crucial role of primary cilia in maintaining the porp level of Gli3R required for CC morphogenesis.

These data demonstrate that Gli3 processing is the major outcome of primary cilia function in CC formation.

Objective: The embryonic node is a ciliated pit-like structure that is conserved in the human-mouse lineage, in some cases to the binding site mouse. Our work has revealed the two regions to be non-homologous, but identified FOXA2 binding regions upstream of this gene. Both FOXA2 controls expression and microtubule acetylation and more recently have found that INF1 deletion derivatives were used to show that both inhibition of cilia formation and the induction of cilia elongation were dependent upon both the FH2 and microtubule-binding domains of INF1.

Conclusion: The effects of INF1 expression on ciliogenesis were separate from its effects on Golgi-dispersion suggesting that Golgi untethering does not always inhibit ciliogenesis. The morphology of the elongated cilia formed in INF1 expressing cells is consistent with defects in dynein function.

Objective: FOXA2 controls Pkd1l1 expression in the mouse node during left-right determination.

Methods: Pkd1l1 and Foxa2 expression was compared through whole-mount in situ hybridization and β-galactosidase staining in 7.5-9.5 dpc mouse embryos. Bioinformatic analysis was used to characterise FOXA2 binding regions upstream of Pkd1l1 in the human-mouse lineage. Finally, luciferase assays were used to test the functionality of these potential regulatory regions.

Results: Our expression studies indicate that a regulatory relationship might exist between FOXA2 and Pkd1l1. Furthermore, Shh- and Foxa2-/-ShhcreER² embryos display abnormal Pkd1l1 expression. Indeed, previous studies identified FOXA2 binding regions upstream of Pkd1l1, in both human and mouse. Our work has revealed the two regions to be non-homologous, but conserved in the human-mouse lineage, in some cases to the binding site level. Although bioinformatic analysis predicts lower binding affinities for the conserved mouse-identified region in human and vice versa, luciferase assays indicate that the conserved regions are capable of driving gene expression.

Conclusion: Our study implicates FOXA2 in the regulation of Pkd1l1 and characterises two FOXA2 binding regions upstream of this gene. Both regions are conserved across the human-mouse lineage to varying degrees. Furthermore, in both mouse and human, the two regions are able to activate gene expression.


Methods: S Bhattacharya, Cilia, Development and Disease, MRC Harwell, Didcot, UK;²Department of Molecular Genetics and Microbiology, College of Medicine, University of Florida, Gainesville, FL, USA;³Radcliffe Department of Medicine, University of Oxford, Oxford, UK

Results: Our expression studies indicate that a regulatory relationship might exist between FOXA2 and Pkd1l1. Furthermore, Shh- and Foxa2-/-ShhcreER² embryos display abnormal Pkd1l1 expression. Indeed, previous studies identified FOXA2 binding regions upstream of Pkd1l1, in both human and mouse. Our work has revealed the two regions to be non-homologous, but conserved in the human-mouse lineage, in some cases to the binding site level. Although bioinformatic analysis predicts lower binding affinities for the conserved mouse-identified region in human and vice versa, luciferase assays indicate that the conserved regions are capable of driving gene expression.

Conclusion: Our study implicates FOXA2 in the regulation of Pkd1l1 and characterises two FOXA2 binding regions upstream of this gene. Both regions are conserved across the human-mouse lineage to varying degrees. Furthermore, in both mouse and human, the two regions are able to activate gene expression.
The C-terminal anchored golgin giantin (golgb1) plays a crucial role in Golgi structure and acts as a tether for COPI vesicles at the cis- and medial Golgi. Recently, our lab has shown that in addition to its known roles in membrane trafficking, giantin is required for ciliogenesis in vitro. A functional knockout of giantin exists. The osteochondrodysplasia rat, that arose from a spontaneous insertion in the golgb1 gene. Homozygous embryos depicted severe craniofacial defects and oedema, caused by defects in extracellular matrix composition. However, ex vivo cultured skin fibroblasts from these animals showed no major ciliogenesis defects. To further elucidate the role of giantin in extracellular matrix and cilia function we have used morpholino knockdown in the zebrafish. Knockdown resulted in a curly tail down and tail tip up phenotype with severe cardiac oedema, hydrocephalus, and craniofacial defects in these structures. Furthermore, defects in cilia function were observed. In the neural tube cilia number was reduced and visible cilia were longer. Addition of giantin to culture failed to respond to Wnt5a stimulation. In most vertebrates organ asymmetries arise in early development. Organ asymmetries are thought to arise through one of two processes: first, asymmetric expression of Nodal pathway genes in tissues that will originate internal organs. We have recently published that charon/dand5 transcription is an early flow target in zebrafish by being expressed in the LRO, first symmetrically and later asymmetrically to the right where the fluid flow is weaker. We showed that absence of flow originates symmetric charon expression [1]. Pkd2 has been reported as part of a mechanosensor complex that senses flow and induces a calcium inward flux in kidney cells. We recorded flow by particle analysis, quantified Pkd2 expression and MO to stop cilia movement [1] and a pkd2-MO. We also co-injected both. Methods: We injected two morpholinos in zebrafish embryos: a dnah7-MO to stop cilia movement [1] and a pkd2-MO. We also co-injected both. We recorded flow by particle analysis, quantified Pkd2 expression and correlated these with charon/dand5 expression patterns by in situ hybridization and qRT-PCR.

Results: 50-60% of injected embryos have symmetric charon/dand5 expression when flow is low and homogeneous or when Pkd2 is absent from LRO ciliary membrane. We will show if charon/dand5 quantification by qRT-PCR is also similar. When both flow and Pkd2 are impaired, 80% of embryos showed symmetric charon/dand5, which indicates a synergistic effect.

Conclusion: Although no flow and no Pkd2 have virtually the same phenotype, there seems to be a positive epistasis when both are affected.

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References
Methods: Using both in vivo and in vitro methods we analyzed specification, migration, proliferation and differentiation of cranial neural crest cells (CNCC) when C2CD3-dependent ciliogenesis was impacted.

Results: Our studies suggest that whereas disruptions of C2CD3-dependent ciliogenesis did not affect CNCC specification or proliferation, it did affect CNCC migration and differentiation. Migrating talpid2 CNCCs were more disperse than control CNCCs and their migration was impaired. Furthermore, talpid2 CNCC derived cartilages are larger relative to controls.

Conclusions: Taken together, these findings suggest that the avian talpid2 mutant is a bona fide, novel model for OFD and that aberrant CNCC migration and differentiation could contribute to the pathology of C2CD3-dependent human OFD.

P43
Evidence for a role of the ciliopathy protein MKS1 in cell polarity
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Mutations in the protein MKS1 cause severe developmental disorders such as Meckel-Gruber syndrome (MKS). Dysfunctional MKS1 caused a panel of cellular defects ranging from abortive centriolar migration to ciliary instability and defective ciliary signaling [1-3]. Most analyses converge to the conclusion that in vertebrates, the depletion of MKS1 leads to impairment of Hh and Wnt signaling pathways [4-9]. Accordingly, MKS1 has been shown to be localized at the transition zone and as such, involved in the ciliary membrane composition [6]. By combining two complementary cell models - a mammalian epithelial cell line and the unicellular Paramecium, we identified a new function of MKS1 in cell polarity.

We show that MKS1 displays a typical pattern of membrane-associated protein, being localised to exocytotic vesicles, the plasma and the ciliary membrane and to cell junctions during epithelial differentiation. Based on RNAi experiments of MKS1 which leads to impairment of ciliary sensory functions, defective vesicle transport and plasma membrane distension, we propose that MKS1 knockdown impairs interactions between actin and membranes.

We finally show how MKS1 depletion interferes with epithelial differentiation and cell organogenesis in 3D cultures.

References:

P44
Transgenic tools for proteomic analysis of ciliary transport
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Vision begins as photons are captured by photoreceptor cilia and light is converted into electrical signals that are then sent to the brain. As the photoreceptor cilium is not able to make its own proteins, all polypeptides needed for converting photons into electrical signals are synthesized in the cell body. How these molecules move from the cell body to cilia is still unclear. Opsin is one of the best-characterized transmembrane proteins. Our goal is to understand the mechanism of opsin transport in photoreceptor cilia. In this project, we use a combination of genetic and proteomic approaches in the zebrafish model. As the first step, we are constructing a transgenic line that expresses EGFP-opsin fusion from an inducible promoter specifically in photoreceptors. For this purpose, we will make two transgenic lines: 1. A line that specifically expresses Cre recombinase in photoreceptors, and 2. A line that conditionally expresses EGFP-opsinC44 (EGFP fused with the 44 C-terminal residues of opsin, which are sufficient to mediate ciliary transport) from the heat-shock promoter. A lox-mCherry-STOP cassette is inserted upstream of EGFP-opsinC44. In the long run, we plan to purify photoreceptors via FACS sorting, pull down EGFP-opsinC44 using the EGFP tag, and analyze opsin C-terminal binding proteins by mass spectrometry. In parallel, we will perform analysis of opsin transport in cilia mutants. We hope this will allow us to formulate a general model of how transmembrane proteins, such as GPCRs and TRP channels, are transported into cilia.

P44
A novel kinesin involved in flagellar attachment and positioning in Trypanosoma brucei
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Objective: Kinesins are motor proteins that transport cargo along microtubules using ATP and fulfill important roles in cilium and flagella. KINS is an orphan kinesin, i.e. it does not belong to any of the known kinesin families, and it is found only in trypanosomatids.

Methods: The function of this protein has been investigated by inducible RNA interference (RNAi) followed by phenotypic characterisation, in the protist Trypanosoma brucei, which possesses a single attached flagellum. Its localisation was determined by expressing a hybrid YFP::KINS protein.

Results: In procyclic cells KINS is localised in the flagellum (probably the axoneme), with a strong fluorescent signal at the distal tip. Depletion of KINS results in cells with a mispositioned and partially detached flagellum. The flagellum of the cells is still beating, but the cells are unable to swim. Nevertheless they divide normally indicating that they have adapted to the partially detached flagellum, probably by modifications of the intracellular organisation. Preliminary data indicate that the filament of the Flagellum Attachment Zone is smaller in length in cells depleted of KINS.

Conclusion: KINS is a flagellar kinesin involved flagellum positioning/attachment, which is not essential for cell survival in procyclic cells in culture. We hypothesise that the cells have adapted their intracellular organisation to allow replication and cell division. We now investigate the relationship between KINS and other proteins known to be involved in flagellum attachment.
P45
FPC4: a new cytoskeletal component in *T. brucei*
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*Tryptansoma brucei* is the causative agent of African sleeping sickness. It possesses a single flagellum, which additional to its mobility role, is an important sensory and signaling organelle [1,2]. The flagellum exits the cell from a membrane invagination, called the flagellar pocket (FP), region where endo- and exocytosis occur. A ring-shaped structure, called the flagellar pocket collar (FPC), encloses the FP defining the flagellum exit point. BILBO1 is the first FPC protein identified and is essential for the FPC and FP biogenesis [2]. A *T. brucei* gDNA yeast two-hybrid (Y2H) screen identified several BILBO1 protein partners, including FPC4 a protein we are characterizing at the molecular and functional level.

Our anti-FPC4 antibody and endogenous FPC4-myc cell confirmed that FPC4 localizes both on, and close to the FPC. RNAi down-regulation of FPC4 has no impact on cell proliferation. However, over-expression of FPC4 leads to filament formation and to a mild, but reduced growth defect. Co-immunolocalization demonstrated that BILBO1 relocates to the FP4 filaments indicating BILBO1-FPC4 interaction and the role of FPC4 in FPC structure. Y2H shows that BILBO1 interacts with the C-terminal domain of FPC4 and we are currently identifying the minimal interaction domains, with the long-term objective of a drug screen to block this interaction. Expression of FPC4 in a heterologous system (human cells) suggests that the N-terminal domain binds to microtubules (MT). This was confirmed by in vitro MT binding assays and is ongoing. We are also characterizing FPC4 function in bloodstream form.

Our preliminary data suggest that FPC4 could be a FPC-microtubule linker involved in FPC segregation during the cell cycle.

References

P46
An essential role of *Plasmodium berghei* kinesin 8 in axoneme assembly and male gametogenesis
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Objective: The male gamete of the *Plasmodium* parasite is the only developmental stage that possesses a flagellum. Very little is known about the identity and function of the proteins involved in the parasite’s flagellum assembly. Assembly is intracytoplasmic, IFT independent and extremely fast. To understand this essential step of the parasite life cycle, we focused on a male gamocyte and gamete specific kinesin (Kin8), initially identified by proteomic analysis.

Methods: Kinesin 8 knock-out parasites were constructed, cloned and their ability to form male gametes in vitro, to fertilize female in vivo and to pursue their life cycle was assessed. The ultrastructure of the male gametocytes/gametes was studied in detail.

Results: Kin8 mutants lines produce male and female gametocytes similar to WT parasites but male gametogenesis was severely impaired as male gametocytes were not able to release male gametes (no exflagellation). The ultrastructure analysis revealed a default in axoneme assembly: elongated microtubules were seen in longitudinal sections but the classical 9+2 axoneme structure was never observed. Nevertheless, Kin8 KO mutants were able to form some ookinetes in vivo and could be transmitted by the mosquito to a new host.

Conclusion: We characterized a kinesin essential for male gametogenesis and axoneme assembly in *Plasmodium berghei*, providing new insights into *Plasmodium* flagellar organization. Further characterization and the protein localization are ongoing.

P47
Recruitment of IFT proteins during flagellum construction in *T. brucei*
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Introduction: *Trypanosoma brucei* is a protozoan parasite responsible for sleeping sickness in Central Africa. It is transmitted by the bite of the tsetse fly. During its complex life cycle, it undergoes significant morphological changes including extensive variations in flagellum length (3 to 30 µm). The flagellum is an essential organelle for parasite survival as it is involved in morphogenesis, movement, division and adhesion of the trypanosome. Intraflagellar transport (IFT) refers to the movement of protein complexes between the membrane and the microtubules. Like in other eukaryotes, IFT is essential for the construction of the trypanosome flagellum. Studies in the green alga *Chlamydomonas* revealed that the total amount of IFT proteins injected in the flagellum defines its final length: the higher the amount, the longer the flagellum. Moreover, the total amount of IFT proteins would be injected at once.

Methods: We have studied the distribution of IFT proteins during the development of *Trypanosoma brucei*. Expression and localization of IFT proteins was analyzed by immunofluorescence with antibodies against two IFT proteins (IFT22 and IFT172).

Results: The analysis of fluorescence intensities revealed that the total amount of IFT proteins in the flagellum is directly proportional to its length in all stages examined, as observed in *Chlamydomonas*. However, the IFT protein concentration per unit of flagellum length is constant at any steps of flagellum formation. These results were confirmed by monitoring protein trafficking in live cells expressing the TdTomato::IFT81 fusion protein.

Conclusions: Our results lead to a new model where IFT proteins would be progressively recruited to the flagellar compartment during elongation of the organelle. This raises the question of the regulation of IFT injection to the flagellum that will be addressed by studying the formation of very long or very short flagella in several development stages in the tsetse fly.

P49
Leishmania IFT140 mutants show normal viability but lack external flagella: a tool for the study of flagellar function through the infectious cycle
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A striking feature of the parasitic protozoan *Leishmania* is the dramatic remodeling of the flagellum during the infectious cycle; in the sand fly midgut the promastigote form has a long, motile flagellum, while within the phagolysosome of the mammalian macrophage the flagellum barely protrudes past the surface of the amastigote form. The flagellum functions in promastigote motility and likely in cell morphogenesis, division and maintenance of flagellar pocket structure for both forms. The flagellar pocket is the sole site of endo- and exocytosis in trypanosomatids and a sensory role for the amastigote flagellum has been proposed but a function is not established. Intraflagellar transport (IFT) is required for flagellar assembly and viability in the infected cell. *Leishmania* and *T. brucei* are known to lack an IFT complex. We asked whether IFT is essential for viability in ‘long’ promastigote and ‘short’ amastigote flagella in *L. donovani* strain Bob by targeting a core retrograde pathway gene *IFT140*. Using a plasmid segregation knockout approach [1], viable knockout promastigotes were readily obtained. ΔIFT140 lack external flagella by light and scanning electron microscopy, and have defective axonemes by transmission electron microscopy, which was reversed by complementation. This is the
first example of a *Leishmania* mutant lacking flagella while retaining normal viability and growth. Thus, ΔIFT140 allows for further studies of promastigote flagellar function. In addition, LeBob can generate amastigotes in vitro and infect mammalian hosts allowing us to probe the effect of ΔIFT140 on the short flagella of amastigotes and parasite virulence.

Reference


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**P50**

**KOUNCIL: Kidney-Oriented Understanding of Correcting Ciliopathies**

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**Objective:** Nephronophthisis is an autosomal recessive renal ciliopathy that constitutes the leading monogenic cause of end-stage renal disease in children. The KOUNCIL consortium is a collaboration between the UMC Utrecht, the Radboud UMC Nijmegen and UC London aimed at elucidating the genetic etiology and pathophysiological mechanisms underlying nephronophthisis and identifying drugs that prevent or delay renal insufficiency. Our goal is to improve genome diagnostics, genetic counselling and therapeutic options for nephronophthisis patients.

**Methods:** We employ next-generation sequencing to identify novel disease genes in 100 nephronophthisis patients included within the AGORA biobank project. The functional effect of novel mutations is assessed using in vitro and in vivo models. Genotypic and phenotypic patient characteristics are registered in a nephronophthisis database, facilitating correlation analyses and identification of early phenotypic markers. Newly identified nephronophthisis-genes are incorporated into diagnostic next-generation sequencing panels of ciliary genes. We use a systems-biology approach to identify and functionally characterize nephronophthisis-associated protein modules. Finally, we use high-throughput repurposing screens in zebrafish embryos to identify FDA-approved drugs that halt renal failure.

**Results:** With this approach, we expect to uncover the causal mutation in 60-90% of nephronophthisis patients. KOUNCIL members were involved in the recent identification of three novel genes (IFT172, WDR34 and WDR60) for nephronophthisis-related disorders. Clinical guidelines and new diagnostic tools for nephronophthisis are developed and implemented in diagnostics. We expect to identify drugs that can lead to novel therapies for nephronophthisis.

**Conclusion:** The KOUNCIL study is designed to advance understanding of renal ciliopathies and improve clinical care for nephronophthisis patients.

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**P51**

**Urine-derived Renal Epithelial Cells (URECs) as a source of biomaterial from ciliopathy patients for functional studies and diagnostics**

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**Objective:** Many ciliopathies are diagnosed in infants and children. Obtaining blood and/or skin biopsies for diagnostic and research purposes requires a visit to the clinic and a specialized sample collection skill. We sought alternative non-invasive sources of biomaterial from patients.

**Methods:** We have generated an approved Standard Operating Procedure to isolate and culture primary renal collecting duct cells from urine (URECs).

**Results:** We show that URECs are approximately 30% proximal tubule cells (mevalon positive) and 70% collecting duct cells (AQP2 positive) from the patient. The cells grow on coverslips for immunocytochemistry, can be frozen and frozen for 15-20 passages without immortalization. We demonstrate that whereas URECs from healthy individuals ciliate well, URECs from ciliopathy patients do not. URECs can form 3D spheroids to study patient-specific tubulogenesis defects. siRNA and overexpression “rescue” experiments in URECs make these cells amenable to functional studies complementing diagnostics. As a proof of principle we show how URECs can be used to test the relevance of genetic variants of “uncertain significance” obtained from whole-exome sequencing. Lastly, we have used patient URECs to explore pharmacological intervention on a patient-specific basis.

**Conclusions:** This protocol expands the toolkit available to clinical geneticists and researchers alike in a child-friendly manner. Urine culture offers a non-invasive option for genetic and functional testing and does not require the family to go to the clinic for sample donation. We contend that UREC culture will facilitate personalized medicine for the ciliopathy community and beyond.

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**P52**

**Identification of human mutations in TRAF3IP1 in patients with nephronophthisis and retinal degeneration**

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**Nephronophthisis (NPH) is an autosomal recessive inherited cystic kidney disorder.** It represents the most frequent genetic cause of end-stage renal disease in the first three decades of life. NPH is characterized by the dysfunction of sensory cilia which explains the complexity of the NPH phenotype. It can be associated with retinitis pigmentosa (Senior-Loken syndrome), mental retardation and ataxia (Joubert syndrome), skeletal anomalies (Jeune syndrome), or situs inversus.

To date, recessive mutations causing NPH have been identified in more than eighteen different genes (NPHP1-NPHP18). Their gene products localize at the primary cilia-centrosome complex, along the cilium as intraflagellar transport proteins and are important in signaling pathways downstream of cilia including Wnt signaling. Shh signaling and the DNA damaged response pathway.

Using whole and targeted exome sequencing, we identified novel protein altering mutations in TRAF3IP1 in patients presenting with NPH, retinitis pigmentosa, skeletal defects of the pelvis, hexadactyly and hepatic fibrosis. TRAF3IP1 encodes IFT54 which is involved in the anterograde transport along the primary cilia. Besides its known function in cilia we demonstrate that TRAF3IP1 act as a key regulator of cytoplasmic microtubule organization. Mass spectrometry analyses as well as pull-down experiments demonstrated that mutations in TRAF3IP1 lead to an altered binding to actin and microtubule associated proteins. Immunofluorescence stainings using patient fibroblasts as well as mIMCD3 TRAF3IP1 knock-down cells confirmed the observed defects in microtubule organization. Furthermore, sphere formation assays as well as the pronephros of elipsa zebrafish embryos showed defects in epithelialization.

Altogether our findings demonstrate that NPH causing mutations of TRAF3IP1 affect both ciliary and non-ciliary functions of TRAF3IP1 which can provide an explanation for kidney tubules morphogenesis defects as well as the other disease phenotypes e.g. retinal, skeletal and hepatic defects.
P53
Multidisciplinary nephrogenetic outpatient clinic combined with diagnostic exome sequencing for improved diagnostics and treatment
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Single gene disorders are estimated to account for ~30% of children and ~10% of adult patients attending renal outpatient services. For mutation detection by exome sequencing, deep phenotypeing, reverse phenotypeing and family history information are important. A multidisciplinary nephrogenetic outpatient clinic for children and adult patients with (genetic) kidney diseases has been established by a team of (pediatric) nephrologists and a clinical geneticist in the Radboudumc. Clinical exome sequencing for a broad spectrum of isolated- and syndromic renal (ciliary) disorders has been developed. The approach consists of a two-tier analysis in which the first step is to screen for pathogenic variants in genes that are known to be mutated in renal diseases (170 genes) or (renal) ciliopathies (125 genes). If causative mutations are not identified in the first step, the complete exome data set can be analysed with informed consent. The first results with the renal disease gene panel in 35 unrelated patients with undiagnosed renal disease led to pathogenic mutations in the CC2D2A, CLCN5, NPHP1 and UMOD gene, detected in four cases and in six cases likely pathogenic variants needed follow-up studies. Further analysis of the complete exome data set in 13 patients, revealed possible pathogenic mutations in two cases. While variant and copy number variation analysis in the rest of exome is expected to further increase diagnostic yield, we can already conclude that the combination of the multidisciplinary outpatient clinic with diagnostic exome sequencing provides a powerful tool for detecting causative mutations of renal disease.

P54
A study of new NEK8 mutations in patients with severe renal cystic hydropydalsplasia and ciliopathy-associated defects
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NEK8/NPHP encodes a NIMA (Never-In-Mitosis A) protein essential for cell cycle control. NEK8 is composed of kinase and RCC1 domains, the latter involved in centrosomal localization. It localizes into the nucleus and at the inner compartment in the primary cilium. Using ciliary gene-enriched exome sequencing, we identified recessive NEK8 mutations in 3 cases with severe overlapping phenotypes including renal cystic (hydropydalsplasia, situs inversus, cardiopathy and paucity of bile ducts. Two patients who died early after birth carried missense mutations in the kinase and/or RCC1 domains. A homogenous splice mutation was identified in a fetus with Meckel-like phenotype. Analyses of patient fibroblasts and IMCD3 cells expressing mutated NEK8-GFP revealed that the mutations affect NEK8 nuclear and ciliary localization. The number of ciliated cells was reduced and ciliary localization of NEK8 protein. ANK6/NEPK16 was lost, demonstrating the key role of NEK8 in cilium function. Surprisingly, in patient fibroblasts, NEK8 accumulates at the Golgi that appeared dispersed into the cytoplasm suggesting a role in vesicular trafficking. Cell cycle defects in response to siRNA-mediated knockdown were rescued by RNA expression of WT NEK8 but not by the mutated forms, further demonstrating pathogenicity of the mutations. Altogether, we demonstrate that human NEK8 mutations alter developmental ciliary and non-ciliary processes, thus leading to multisystemic defects.

P55
Ciliome resequencing: A lifestyle for molecular diagnosis in LCA
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Aims: Leber congenital amaurosis (LCA) is the earliest and most severe retinal dystrophy. It occurs as non-syndromic or syndromic. 26/37 LCA genes are important to ciliary function and account for < 1/3 of cases. These cases develop- or are at risk to develop- skeletal, renal and/or neurologic symptoms. Here, we assessed efficiency of ciliome resequencing (CR) as a tool for molecular diagnosis and patient care.

Patients and methods: The DNA of 60 unrelated young children with LCA was screened for mutations using a custom 5.3 Mb Agilent SureSelect Target Enrichment library which captures 32,146 exons of 1,666 genes selected from cilia databases. Segregation analysis of rare candidate variants was performed by Sanger sequencing.

Results: Biallelic disease-causing mutations in known genes were identified in 17/60 patients (30%): CEP290 (n = 4), CRB1 (n = 4), RPRG1P1 (n = 2), LCAS (n = 1), JCB1 (n = 2), FT140 (n = 2), AH1 (n = 1), AM51 (n = 1). In addition, 3/60 patients harbored biallelic mutations in three novel genes which screening in additional non syndromic and syndromic LCA cases allowed identifying additional mutations in 2/3 of them.

Conclusions: The identification of mutations in known and novel genes in 33 % of the cases, makes targeted sequencing an interesting alternative to exome resequencing. The identification of mutations in several genes responsible for syndromic LCA in young children with no overt extracocular expression demonstrates the importance of NG5-based molecular diagnosis to set-up a rational and efficient follow-up of patients.

P56
X-box promoter motif searches: from C. elegans to humans to novel candidate ciliopathies
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Objective: Ciliary defects are known to cause severe genetic disorders, collectively called ciliopathies. We attempt to identify genes involved in human ciliopathies by making use of the evolutionarily conserved X-box promoter motif recognized by ciliogenic RXF transcription factors.

Methods: We use bioinformatics tools to identify potential RXF target genes across species. We currently focus on genes associated with human dyslexia, the most common learning disorder. In human cell culture systems we test the functionality of X-box motifs, determine target gene expression levels in dependence to RXF and cilia development, and visualize their subcellular protein localization by immunocytochemistry.

Results: Following the methodology of X-box searches developed in C. elegans, we have identified many X-box containing genes in the human genome. Luciferase gene reporter assays show that the dyslexia candidate genes DXY1C1, KIAA0319 and DDCD2 possess a functional X-box motif. Analogous to expression profiles of known cilia markers, DXY1C1, KIAA0319 and DDCD2 gene expression increases during the first 24 h after induction of ciliogenesis. Furthermore, expression levels of DXY1C1, KIAA0319 and DDCD2 change in response to siRNA-mediated
knockdown of RFX factors. In line with a proposed ciliary function, the proteins of DYNC1C1 and DCCD2 localize at the cilium.

**Conclusions:** The evolutionarily conserved X-box promoter motif can be used to identify potential ciliary genes across species. Human dyslexia candidate genes DXY1C1, KIAA0319 and DCD2 possess a functional X-box motif and are regulated by RFX factors. Human cell lines thus provide an easily accessible and rapid way to test X-box functionality.

**PS7**

Identification of ciliogenic compounds using pancreatic cancer cells as a screening model

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**Objective:** Primary cilia regulate intracellular signaling pathways by sensing the extracellular environment. Abnormalities in the structure and/or function of cilia results in deregulation of these pathways leading to clinically significant diseases like cancer. In the present study, we demonstrate the use of cancer cells in the development of a cell culture-based semi-high throughput screening method to identify ciliogenic compounds that may serve as potential anti-cancer agents. Using this method, we have identified compounds that enhance ciliation in pancreatic cancer cells.

**Methods:** A screening platform was developed by culturing cancer cells in a 96-well plates followed by immunostaining for cilia. Quantification of cilia was accomplished by analyzing images captured by INCell Analyzer, an automated machine used for image acquisition. Ciliogenic compounds were identified based on percentage of ciliated cells. The hits were further assessed in cancer cells. Their ability to decrease the proliferation of cancer cells was analyzed by spheroid assay. Western Blots were performed to identify the pathways through which ciliogenesis was induced by these compounds.

**Results:** 26 such compounds were identified which enhanced ciliation in pancreatic cancer cells. Based on their targets, they were classified into 14 different classes. Most of these compounds effectively decreased the rate of proliferation in pancreatic cancer cells. Two of these compounds namely Gefitinib and Rapamycin suppressed the expression levels of DYNC2LI1 protein localization. Mutations in both DYNC2LI1 and DCCD2 quitously expressed and interacts with DYNC2H1.

**Conclusion:** Via development of a semi-high throughput screening platform, 26 ciliogenic compounds were identified which may serve as anti-cancer agents by virtue of their ability to induce cilia in cancer cells.

**PS8**

Meckel-Gruber syndrome patient cells exhibit alterations in cell-substrate interaction, deformation response, and gene expression consistent with defects leading to liver fibrosis

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**Objective:** Meckel-Gruber syndrome (MKS) is a lethal ciliopathy characterised by CNS malformations, cystic kidneys, polydactyly, and liver fibrosis. Most of these can be explained by disruption of cilium-dependent signalling pathways. However, the aetiology of liver fibrosis is less well-understood. We hypothesised that alterations in cell-substrate interaction, ECM organisation, dysregulated secretion and ECM organisation are upstream of fibrosis in MKS.

**Methods:** We used a combination of imaging, biophysics and RNAseq expression analysis to study alterations in cell adhesion and matrix composition in neonatal fibroblasts isolated from patients with mutations in MKS2 (TMEM67), MKS3 (TMEM216) or MKS1 (KIAA0319) and in age-matched controls.

**Results:** We found that MKS cells exhibit striking differences in spreading morphology on ECM substrates in comparison to controls. While MKS2 cells spread faster than controls on all substrates examined, MKS3 cells showed a substrate-specific increase in spreading on collagen IV. We also observed differences in the morphology and distribution of focal adhesions, which appeared more mature and pervasive. Of the ~3000 genes with altered expression levels in MKS cells, those associated with ECM components as well as fibrosis-implicated upstream effectors of ECM organisation and cell-substrate signalling were highly over-represented. These differences were consistent with those reported in other studies of liver fibrosis. Finally, MKS cells were less resistant to deformation and fragmentation under externally applied pressure.

**Conclusions:** We propose that a combination of defective regulatory signalling and excess ECM deposition, together with changes to cortical integrity and/or plasma membrane tension may contribute to the fibrotic pathology observed in patients.

**P59**

Identification of mutations in DYNC2LI1, a member of the mammalian cytoplasmic dynein 2 complex, expands the clinical spectrum of Jeune/ATD ciliopathies

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Ciliopathies are caused by defects in formation, maintenance and function of the primary cilium and underlying genes affect the dynein motor, intraflagellar transport complexes, or the basal body. In a patient of non-consanguineous parents presenting an intermediate phenotype between asphyxiating thoracic dystrophy and Ellis-van Crefeld syndrome we performed exome sequencing. Variants were selected based on potential ciliary function as identified in a yeast two-hybrid screen with NEK1, a basal body protein involved in short rib-polydactyly type Majewski (SRPSII). We identified compound heterozygous nonsense (p.R208X) and missense (p.T221I) mutations in DYNC2LI1 segregating in the family. DYNC2LI1 is ubiquitously expressed and interacts with DYN1CH21 to form the dynein 2 complex important for retrograde intraflagellar transport. The hypothetical protein caused by the nonsense mutation lacks the coiled-coil domain involved in protein interaction and dimerization. The mutation p.T221I affects a highly conserved nucleotide triphosphate hydrolase domain responsible for GTPase driven dynein protein localization. Mutations in both DYNC2LI1 interacting partners DYNCH21 and NEK1 are associated with ATD and SRPSs. We screened further patients of our short stature cohort and identified in two siblings heterozygous mutations in DYN1CH21 (p.M11T) and its interaction partner DYNCH21 (p.K495T). The DYN2CH1 mutation was previously reported by El Hakayem et al. compound heterozygous with a splice site mutation in a patient with SRPSII. Our results might indicate a possible digenic diallelic inheritance in our patients. This is the first report of mutations in DYN1CH21 as part of the dynein 2 complex further expanding the clinical spectrum of ciliopathies.

**P60**

A reference to assess cilium phenotype in ciliopathy patients

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**Objective:** The cilium presence and constitution of human fibroblasts are accessible and measurable cellular markers for ciliary homeostasis. Fibroblasts are most commonly generated from skin biopsies and comparison of the cilary phenotype of patients vs controls can be used to
identify or evaluate a potential ciliary defect. Subtle differences are difficult to detect, but critical to establish a genotype-phenotype correlation. The aim of this study is to establish the parameters to detect subtle differences in cilium phenotype, and develop a reference for cilium phenotype that can be used to improve diagnosis and prognosis of ciliopathies.

**Methods:** We derived fibroblast cell lines from skin biopsies from ciliopathy patients and healthy controls. Ciliogenesis was induced by adding culture medium containing 0.2% FCS prior to immunocytochemistry. The cells were stained with antibodies against different ciliary proteins to study cillum presence, length, morphology, and intraflagellar transport proteins.

**Results:** Fibroblasts from three controls and five patients with different ciliopathies were studied so far. We found major and subtle differences in cillum frequency and phenotype in patients and subtle differences in controls, partly influenced by the culturing conditions. This suggests that there is a significant natural and experimental variation that needs to be taken into account when evaluating the statistical relevance of such findings.

**Conclusion:** We detected major and subtle differences in cillum phenotype between patients. In addition, we found a natural variation in the cillum phenotype of control fibroblasts. By including more samples, we intend to improve and expand this reference for ciliopathy-associated cillum phenotypes.

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**P61**

**Phenotypic variability of CCDC103 mutation in British Pakistani children with Primary Ciliary Dyskinesia (PCD)**

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**Objectives:** PCD is an autosomal recessive condition that affects the structure and function of motile cilia in the respiratory tract, middle ear and reproductive organs. The estimated prevalence is 1:15,000, but as high as 1:2265 in the British Asian population.

Mutations in the CCDC103 gene have recently been identified as PCD disease-causing in Pakistani individuals. It is found to be an essential gene for dynein arm assembly and ciliary motility.

**Methods:** We present eleven British Pakistani children found to be homozygous for a missense mutation in CCDC103, resulting in the amino acid substitution His154Pro.

**Results:** Nasal Nitric Oxide screening test results were normalised, mean 290ppb (range 22-857), compared to usual values in PCD (<100ppb). Ciliary beat frequency was also often in the normal range 10.6Hz (range 0-16.3). Seven had a defect of the ciliary inner and outer dynein arms demonstrated in ciliated nasal cells by electron microscopy. This defect was partial and distinct from the near complete absence of dynein arms seen in children with mutations in LRRC6 and ZMYND10.

A further four children (3 siblings) presented with a phenotype suggestive of PCD but electron microscopy studies were inconclusive on repeat testing. Genetic testing revealed the same CCDC103 homozygous mutations, making the diagnosis of PCD possible based on genetic analysis.

**Conclusion:** We report a high prevalence of the CCDC103 His154Pro mutation in the British Asian PCD community and the phenotypic variability of CCDC103 in order to raise awareness of the potential benefit of genetic testing as a diagnostic aid in non-typical PCD cases.

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**P62**

**Expression of the oncogenic, constitutive active Platelet-Derived Growth Factor Receptor alpha D842V mutant induces loss of primary cilia**

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**Objective:** The receptor tyrosine kinase (RTK) Platelet-Derived Growth Factor Receptor α (PDGFRα) localizes to the primary cilium, and expression of the constitutive active PDGFRα D842V mutant has been linked to gastrointestinal tumor formation. We set out to investigate whether expression of D842V causes loss of primary cilia in cultured cells.

**Methods:** Plasmids encoding GFP-tagged wild type and D842V mutant PDGFRα were expressed in hTERT-RPE1 cells. To promote ciliogenesis, transfected cells were grown under serum-free conditions for 12 hours and in the presence of various inhibitors. Cilia numbers of GFP-positive cells were assessed by immunofluorescence microscopy with antibodies against cilia markers. In parallel, cells were analyzed by western blotting to evaluate expression level and activity of the tagged receptors.

**Results:** Cells expressing the D842V mutant contained significantly fewer cilia than cells expressing wild type PDGFRα. Cilia numbers of D842V-expressing cells were restored to control levels upon treatment with the RTK inhibitor crenolanib, but not with other RTK inhibitors such as AG1296 or imatinib. Western blot analysis confirmed that D842V activity is inhibited by crenolanib, but not by AG1296 and imatinib. Treatment with inhibitors against AKT, ERK1/2 and HDAC6 failed to restore cilia numbers in D842V-expressing cells, suggesting that the D842V mutant induces cillum loss by an alternate pathway.

**Conclusions:** Our results indicate that the D842V mutant promotes loss of primary cilia by a mechanism that depends on its kinase activity, but not on activation of AKT, ERK1/2 or HDAC6. We are currently investigating the mechanism by which D842V induces loss of cilia.

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**P64**

**Number of nexin links detectable at standard electron microscopy of normal human nasal cilia and at nexin link deficiency**

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**Objective:** We described 11 years ago 3 patients with missing nexin links as a possible cause of primary ciliary dyskinesia (PCD). The assumption was substantiated last year by finding a mutation in these patients. Here we counted the number of nexin links per cilium in normal cilia and at deficiency of nexin links.

**Methods:** We counted the nexin links, inner (IDA) and outer (ODA) dynein arms and microtubuli in each of, if possible, 50 cilia in 41 patients with normal cilia, 4 patients with deficiency of nexin links only and 4 with deficiency of nexin links and IDA.

**Results:** In the control group the median number of nexin links was 4.5 per cilium, range 3.4 - 5.2. In the second group the mean number of nexin links per cilium was 1.1 - 1.4, in the third group 0.8 - 1.2, per patient. The median number of IDA was in the control group 4.2, range 3.3 - 5.2. In groups 2 and 3 the numbers were 3.0 - 3.5 and 0.2 - 1.0, respectively, per patient. Numbers of ODA were normal in all groups.

**Conclusion:** It is possible to reliably count the number of nexin links in nasal human cilia and to distinguish cases with missing nexin links from normal controls.

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**P65**

**C2 domains as protein-protein interaction modules in the ciliary transition zone**

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**Objective:** We described 11 years ago 3 patients with missing nexin links as a possible cause of primary ciliary dyskinesia (PCD). The assumption was substantiated last year by finding a mutation in these patients. Here we counted the number of nexin links per cilium in normal cilia and at deficiency of nexin links.

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**Conclusion:** It is possible to reliably count the number of nexin links in nasal human cilia and to distinguish cases with missing nexin links from normal controls.
is a type II C2 domain with a canonical β-sandwich structure. However, it does not bind Ca²⁺ and/or phospholipids and thus constitutes a new type of protein-protein interaction module. Judging from the large number of C2 domains present in nearly all ciliary transition zone proteins identified, the structure presented here seems to constitute a cilia-specific module present in multi-protein transition zone complexes.

P66

Cep164, but not EB1, is critical for centriolar localization of TTBK2 and its function in ciliogenesis

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Cilia 2015, 4(Suppl 1)P66

Objective: Primary cilia play crucial roles in sensing and transducing external signals. They extend the axoneme from the mother centriole-derived basal body. A centrosomal protein CP110 blocks axoneme extension and is removed from the mother centriole at the onset of ciliogenesis. TTBK2 is recruited to the mother centriole in response to serum starvation and is required for CP110 removal and ciliogenesis. To elucidate the mechanism of centriolar recruitment of TTBK2, we examined the roles of two TTBK2-binding proteins, EB1 and Cep164, in TTBK2 recruitment. Methods: The EB1- and Cep164-binding sites of TTBK2 were determined by co-precipitation assays, using their deletion and point mutants. The knockdown/rescue experiments of TTBK2 were performed using hTERT-RPE and IMCD3 cells.

Results: TTBK2 interacts with EB1 via the SRIP and SKIP motifs in the C-terminal region of TTBK2 and the EBH domain of EB1. EB1 meditated TTBK2 localization at the plus-ends of microtubules but was not essential for its centriolar recruitment and ciliogenesis. TTBK2 interacts with Cep164 via the C-terminal proline-rich motif of TTBK2 and the WW domain of Cep164. The Cep164-non-binding mutant was not recruited to the centriole, and depletion of Cep164 suppressed centriolar recruitment of TTBK2. Thus, binding to Cep164 is required for centriolar recruitment of TTBK2. Knockdown/rescue experiments of TTBK2 revealed that wild-type TTBK2, but not Cep164-non-binding mutants, rescued CP110 removal and ciliogenesis in TTBK2-depleted cells. Thus, Cep164 binding of TTBK2 is critical for its function to promote CP110 removal and ciliogenesis. We also found that TTBK2 phosphorylates Cep164 and Cep97, and suppresses Cep164 binding to Dishevelled-3.

Conclusion: Cep164, but not to EB1, is essential for centriolar localization of TTBK2 and its function in promoting CP110 removal and ciliogenesis. TTBK2 effectively phosphorylates Cep164 and Cep97.

P67

Merkelin guides orientation of basal bodies along the striated rootlet

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Cilia 2015, 4(Suppl 1)P67

Objective: Merkelin (MK3) functions in ciliogenesis and ciliary gating. MK3 appears to have similar functions in Paramecium tetraurelia, i.e. FLAG-MK3 is found associated slightly above each basal body and RNAi for MK3 leads to loss of cilia. RNAi for MK3 also leads to the disorganization of rows of basal bodies that run from anterior to posterior. In areas of misalignments, basal bodies with their post ciliary and transverse rootlets are found out of their expected rows. However, the rootlets are attached to the basal bodies at the expected angles relative to each other. We propose that MK3 guides new basal bodies as they move toward the anterior of the cell along the striated rootlet (SR) of the parent basal body. Basal bodies without MK3 lose their interactions with the parent’s SR. Without this guide to maintain orientation, new basal bodies migrate off the expected line and, when they form their SRs, these too cannot project toward the anterior as expected.

Method: We tagged 13 potential SR components and examined their location. Nine were associated with the SR, often with non-uniform distributions.

Results: Those sequences with SF assemblin domains (similar to those in the Chlamydomonas rootlet proteins) coded for proteins that we found in the Paramecium SRs; those without this domain were not in the rootlets. MK3 interacted sufficiently with the epitope tagged striated rootlet proteins to be pulled down with a GST-fusion of the 252 C-terminal residues of the Paramecium MK3.

Conclusion: MK3 interacts with a subset of SR proteins.

P68

Paramecium tetraurelia basal body unit isolation for Cryo-electron tomography studies

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Cilia 2015, 4(Suppl 1)P68

Objective: The Transition Zone (TZ) is defined as the most proximal region of the cilium overlapping with the most distal region of the basal body. This zone has been shown to play a crucial role in cilia biology since it is considered as the site of sorting of proteins that transit to cilia. Protein complexes housed at this zone are found mutated in MKS/NPHP ciliopathies. Although its organization varies from organism to organism, the TZ molecular composition and function are highly conserved. In Paramecium, the TZ is well structured with three distinct plates defined as the terminal, the intermediate and the axosomal plates. In this model, structural and molecular changes of the TZ are observed as anchored basal bodies become ciliated. Therefore, Paramecium appears to be a pertinent model to study the TZ at an ultrastructural level in correlation with its functionality.

Methods: To reach this goal, we have developed a technique to isolate Paramecium basal body cortex units. These units fit the cryo-electron tomography requirements allowing us their visualisation in native conditions at nanometric resolution.

Results: First cryo-tomograms obtained on these Paramecium units allow the observation of well-preserved basal bodies revealing the TZ with its three recognisable plates and the Y-links as well as at the proximal part of the basal body, the cartwheel and the radial spokes.

Conclusion: Thus, studies of the consequences of TZ protein depletion at high resolution are now achievable by combining new isolation protocols and cryo-electron tomography.

P69

Recruitment of FOR20 and OFD1 onto pericentriolar satellites and centrosomes depends on the formation of a ternary complex with KIAA0753

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Cilia 2015, 4(Suppl 1)P69

Stable microtubules organized on the basis of a nine-fold rotational symmetry constitute the structural basis of centrioles and basal bodies. A number of proteins associate with these structures to drive the process of centrosomes and cilia formation and to participate in their function. Pericentriolar satellites are small round-shaped particles closely associated with microtubules with PCM1 as a major component. To date they have been described only in vertebrates where they participate in the recruitment of proteins to the centrosomes and cilia. We identified the poorly characterized KIAA0753 protein as a direct interactor of FOR20, a pericentriolar satellite protein required for basal body integrity and ciliogenesis. This binary association allows the recruitment of OFD1, a centriole distal-end protein and regulator of centriole length, to form a ternary complex associated with pericentriolar satellites. Inhibiting
expression of KIAA0753 by small inhibitory RNA limits the recruitment of FOR20 and OFD1 onto pericentriolar satellites and centrosomes, and decreases cilia length in serum-starved RPE1 cells. We also show that KIAA0753 has a microtubule-stabilizing activity. We propose that pericentriolar satellite and associated proteins such as PC1 and KIAA0753 appeared in the course of early metazoan evolution to regulate ancestral eukaryotic centriole/basal body proteins such as FOR20 and OFD1.

P70
The role of Rabconnectin3a in cilia length regulation
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Cilia 2015, 4(Suppl 1)P70

Background: Using the zebrafish mutant for the deltaD gene (dll<sup>−/−</sup>), it was shown the involvement of Notch signaling in the control of cilia length in the cells of the fish laterality organ (Kupffer's Vesicle, KV) [1]. Further research based on KV specific microarray screening allowed the discovery of several genes with differential expression. Of these, 23% were associated with ciliogenesis and upon analysis, many proved to be involved in cellular trafficking.

Rabconnectin3a or rbcn3a was strongly downregulated in dll<sup>−/−</sup> KV cells. Homologs of this gene have been associated with Notch signaling in Drosophila and mammalian cells through the regulation of the V-ATPase activity [2,3]. Rbcn3a had also been associated with vesicular acidification in zebrafish hair cells [4] and with vesicular endocytosis and maturation in zebrafish neural crest migration [5].

Objective: We investigated the role of Rbcn3a in cilia length regulation.

Methods: We used a Morpholino against rbcn3a and fluorescent confocal imaging to explore cilia length. Furthermore we observed the consequences of reduced Rbcn3a in organ <em>in situ</em> by ISH. We also performed rescue experiments by injecting Rbcn3a full length mRNA at 1-cell stage dll<sup>−/−</sup> KO mutants.

Results: We showed that the downregulation of rbcn3a negatively regulates cilia length and that this can be rescued by rbcn3a overexpression in dll<sup>−/−</sup> embryos.

Conclusion: The ciliary phenotype in dll<sup>−/−</sup> mutants is partially due to the downregulation of rbcn3a. Our hypothesis is that a generalized decrease in endocytic acidification, by deregulating the V-ATPase activity, results in cilia malformation.

References

P71
New LKB1 function in the primary cilium
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The serine threonine kinase LKB1 is conserved and ubiquitously expressed throughout evolution. In humans, LKB1 is causally linked to the Peutz-Jeghers syndrome (PJS), an autosomal dominant inherited disorder characterized by melanotic macules of the lips and multiple gastrointestinal hamartomatous polyps. PJS patients have a high risk of developing malignant tumours, including breast and gastrointestinal cancers. Moreover, LKB1 expression loss is frequently found in several cancer types such as cervix, pancreas, or lung carcinomas which have led to classified LKB1 as a tumour suppressor. Mechanism(s) through which LKB1 exerts this tumour suppressor property remains an issue.

We and others have published results suggesting that the LKB1 complex is constitutively active in cells and that its regulation is in fact the result of its intracellular localization, allowing a spatiotemporal proximity with a subset of specific substrates. Although, LKB1 has been described to locate in the nucleus under ectopic expression, endogenous LKB1 appears to be mainly in cytosol, adherent junctions and primary cilium in polarized epithelial cells. LKB1 function(s) in cilia are still poorly understood even though involvement in mTOR repression has been proposed. Indeed, like for all proteins found in several cellular compartments, results from LKB1 inactivation is a mix of its functions loss in all compartments where its activity takes place impeding clear results for specific compartment. Thus and through a new knock out mouse model which leads to specifically LKB1 activity and function loss in cilia, we our work defines a new LKB1 function in this organelle which might be responsible, in part, for its tumour suppressor property.

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P72
A systemic proximity map of the centriole-cilia interface
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Cilia 2015, 4(Suppl 1)P72

Objective: Eukaryotic cilia/flagella are dynamic microtubule (MT)-based organelles. The ciliary axoneme is built from a radial scaffold of 9 MT doublets, plus a central MT pair, in the case of motile cilia. The axoneme is templated by a basal body (BB)/centriole derived from the mother centriole, in the case of animal cells, which presents sub-distal and distal appendages critical for cilia formation. In vertebrates, different cilia types fulfil diverse functions, critical for embryonic development and homeostasis of adult tissues. Cilia malfunction causes ciliopathies, but despite their biomedical implications, their molecular composition and mechanisms underlying their biogenesis still remain poorly defined.

Methods: In order to dissect the mechanisms involved in primary cilia assembly and disassembly we decided to focus on the mother centriole appendages and the T2 and define the interaction networks of their known components (~60 proteins) using a new mass spectrometry approach called BioID based on the proximity-dependent biotinylation of proteins by a promiscuous biotin ligase fused to the protein of interest.

Results: With this approach we have identified near neighbors and potential new interactors of ~60 known components of the centriole-cilia interface and the centriole transition zone which have been tested for their involvement in centrosome and cilia biology using automated high-throughput/high-resolution screens and precise morphometric measurements.

Conclusion: We identified several new regulators of ciliogenesis a subset of which we have characterized. The potential role of the most promising candidates in the potential onset of clinical features of ciliopathies will also be discussed.

P73
Art13b interferes with α-tubulin acetylation
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The serine threonine kinase LKB1 is conserved and ubiquitously expressed throughout evolution. In humans, LKB1 is causally linked to the Peutz-Jeghers syndrome (PJS), an autosomal dominant inherited disorder characterized by melanotic macules of the lips and multiple gastrointestinal hamartomatous polyps. PJS patients have a high risk of developing malignant tumours, including breast and gastrointestinal cancers. Moreover, LKB1 expression loss is frequently found in several cancer types such as cervix, pancreas, or lung carcinomas which have led to classified LKB1 as a tumour suppressor. Mechanism(s) through which LKB1 exerts this tumour suppressor property remains an issue.

We and others have published results suggesting that the LKB1 complex is constitutively active in cells and that its regulation is in fact the result of its intracellular localization, allowing a spatiotemporal proximity with a subset of specific substrates. Although, LKB1 has been described to locate in the nucleus under ectopic expression, endogenous LKB1 appears to be mainly in cytosol, adherent junctions and primary cilium in polarized epithelial cells. LKB1 function(s) in cilia are still poorly understood even though involvement in mTOR repression has been proposed. Indeed, like for all proteins found in several cellular compartments, results from LKB1 inactivation is a mix of its functions loss in all compartments where its activity takes place impeding clear results for specific compartment. Thus and through a new knock out mouse model which leads to specifically LKB1 activity and function loss in cilia, we our work defines a new LKB1 function in this organelle which might be responsible, in part, for its tumour suppressor property.
Background: The loss of Arl13b has been associated with cilia defects since 2007 [1]. Arl13b is a small G protein that localizes along the ciliary membrane but there is still no current knowledge about Arl13b ciliary role or effectors. Nevertheless, many studies aiming to understand cilia signaling pathways make use of mild Arl13b overexpression fused with GFP as a ciliary marker.

Objective: Study the impact of overexpressing Arl13b-GFP in ciliary formation and structure.

Methods: We used the zebrafish Kupffer’s vesicle as a dynamic ciliary growth system and performed a seven hour time-course experiment comparing the length of cilia measured by Arl13b-GFP or by acetylated α-tubulin. In order to evaluate the specificity of the alterations in α-tubulin acetylation pattern, we overexpressed different ciliary proteins that were also reported to increase cilia length.

Results: Arl13b-GFP injection increases cilia length and causes a specific decrease in the α-tubulin acetylation of both motile and primary cilia. We noted that this reduction is more accentuated right before the maximum ciliary length is achieved. Moreover, by blocking deacetylation with tubacin we were able to rescue acetylation levels but cilia length is maintained.

Conclusions: We concluded that Arl13b overexpression causes a specific and significant reduction in α-tubulin acetylation. We are currently investigating if there is any synergy between the loss of Mec17, the acetylate, and the overexpression of Arl13b. We hypothesize that Arl13b actively blocks α-tubulin acetylation to render the cilium more dynamic and allow it to grow more in the same time window.

Reference

P74
Developing a live cell assay for the centriole-cilium transition in flies
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Cilia 2015, 4(Suppl 1):P74

Cilia are essential organelles for organism development and have been linked with several human diseases, so called ciliopathies. A cilium is formed from a mother centriole that extends into a separate membrane compartment at the cell surface. Despite the large number of proteins associated with cilia formation/development the interplay of proteins that allow a cilium to form a ciliare are largely unknown. Using the well characterised Drosophila sensory organ precursor (SOP) cells as a model, we propose to dissect the molecular pathway of cilia formation with live cell imaging and electron microscopy. SOPs divide in a stereotypical manner to produce four cells, only one of which will form a cilium. We have started by imaging ciliento dynamics during the SOP divisions to determine how centrioles behave prior to differentiation and cillum formation. These very early studies reveal that centrioles are highly motile, but are tightly apically constricted in the SOP cells and most of their progeny. Further advances in the methodology will be discussed.

P75
Biochemical characterization of transmembrane proteins (TMEMs) in the ciliary transition zone
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Objective: Ciliopathies are a group of heterogeneous disorders caused by mutations in proteins associated with primary cilia. Many proteins that are mutated in ciliopathies Joubert syndrome (JBT5), Meckel-Gruber syndrome (MKS) and nephropathophysis (NPHP) are localized to the transition zone (TZ), a compartment of the proximal region of the cilium. In particular, a protein complex known as the “MKS-JBTS module” contains many transmembrane proteins (TMEMs) that are mutated in these conditions. Here, we aim to understand the role of the ciliary proteins TMEM67, TMEM138, TMEM216, TMEM237, TMEM17 and TMEM221 by characterizing their biochemical functions and potential interactions. We hypothesize that pathogenic missense mutations disrupt the putative TMEM complex or localization to the TZ. Furthermore, we aim to identify new interacting proteins of TMEMs, including potential ligands of the orphan receptor TMEM67.

Methods: The “Gateway” cloning system tagged TMEM138, TMEM216, TMEM237, TMEM17, and TMEM231 at the N-termini with GFP, TAP (streptavidin/FLAG) or the FLAG epitope. Constructs were exogenously expressed in ciliated mIMCD3 cells and expression confirmed by western blotting or immunofluorescence confocal microscopy. A construct for the N-terminal extracellular domain of TMEM67 (pSecTagA2-TMEM67-Nt) was made for secreted protein expression.

Results: IF microscopy confirmed the sub-cellular localization of tagged TMEM proteins to the cell membrane and basal body, and co-immunoprecipitations show that TMEM237 interacts with TMEM138 or TMEM216. High levels of TMEM67-Nt protein have been purified for use in future biochemical experiments.

Conclusions: Biochemical methods for the functional characterization of ciliary TMEM proteins should provide insights into the molecular pathogenesis of ciliopathy mutations.

P76
CSPP-L and EB3 localize to centriolar satellites and are required for satellite-dependent recruitment of ciliopathy proteins to the centrosome
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Cilia 2015, 4(Suppl 1):P76

Objective: Centrosome/Spindle Pole associated Protein 1 (CSPP1, JBTS21) mutations cause Joubert syndrome (JBT5) and JBT5-related ciliopathies. The large protein isoform CSPP-L is a ciliary protein required for ciliogenesis and stabilization of the ciliary protein RPGRIP1L (NPHP8/JBTS7/MKSS/FTM) at the ciliary transition zone (TZ). However, RPGRIP1L is dispensable for ciliogenesis and the mechanism by which CSPP-L promotes ciliogenesis is unclear.

Methods: We applied immunogold electron, immunofluorescence and fluorescence live cell microscopy to determine localization of CSPP-L at high spatial and temporal resolution. We elucidated the functional interplay of CSPP-L with centriolar satellites in hTERT-RPE1 and HeLa cells using biochemical analysis of CSPP-L complexes, siRNA modulated gene expression and quantitative immunofluorescence microscopy.

Results: We show that CSPP-L localizes to centriolar satellites, in addition to axonemal microtubule (MT) plus ends and the TZ, and that the MT plus end-tracking protein EB3 also localizes to satellites. CSPP-L complexed with the known satellite component PCM1 and GFP-CSPP-L showed satellite-like dynamics. Importantly, CSPP-L depletion decreased formation of PCM1, CEP290 and EB3 comprising satellites, whereas depletion or inactivation of EB3 impaired centrosomal localization of CSPP-L.

Conclusion: Our results identify a new link between MT plus ends and centriolar satellites, and suggest that CSPP-L contributes to ciliogenesis by promoting EB3- and dynine-dependent recruitment of satellite components to the centrosome.

P77
Identification of C. elegans RAB-5-dependent downstream effectors as regulators of ciliogenesis and ciliary membrane homeostasis
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Cilia 2015, 4(Suppl 1):P77

Cilia are microtubule based organelles enveloped by a specialised extension of plasma membrane. Serving as cellular antennae, cilia are essential for receiving extracellular cues and mediating downstream signal transduction crucial for normal cellular physiology and development. These functions are mediated in large part by the specialised ciliary membrane, which differs in composition from the plasma membrane, housing many of the receptors,
channels and effectors that mediate cilia-based signalling. However, the molecular mechanisms underlying the establishment, maintenance and regulation of the ciliary membrane remain poorly understood.

In this project we are seeking to identify and functionally characterize genes with ciliogenic roles in ciliary membrane transport and organisation. Specifically, we are taking a candidate gene approach using the Caenorhabditis elegans ciliated sensory neuronal model, employing assays for ciliary integrity (dye filling assay) and functionality (osmotic avoidance assay; foraging).

Using predicted loss-of-function alleles, we screened 55 mutants of evolutionarily conserved membrane organising and trafficking genes and identified 4 rabs-5-related endocytic gene mutants with defects in ciliary integrity and function. One of these, rabs-5, encodes a RAB-5 effector, which we found is expressed mainly in ciliated cells. rabs-5 mutants possess short cilia and an expanded periciliary membrane compartment, indicative of defects in ciliary membrane homeostasis. Transgenic expression of a rabs-5(WT) construct rescues the ciliary integrity and foraging defects of rabs-5 mutants, confirming the association of rabs-5 with cilia-related functions.

By taking a reverse genetics candidate gene approach in C. elegans, a number of RAB-5-dependent downstream effectors have been identified as potential regulators of ciliogenesis and ciliary membrane homeostasis.

**P78**

Structure of outer arm dynein molecule in respiratory cilia suggests an alternative mechanism of force generation

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Cilia 2015, 4(Suppl 1):P78

**Background:** Cilia are microtubule (MT)-based organelles that extend from the surface of eukaryotic cells. The ciliary movement is generated by microtubule (MT) sliding with axo

**Methods:** Here, we analyzed the conformational change and its distribution in each dynein head of mouse respiratory cilia by cryo-electron tomography and image processing.

**Results:** Most of two heads were in the same form and tightly packed in the non-nucleotide condition, whereas they are dissociated and alternatively moves in the presence of nucleotide. The external side of outer dynein arm shifts toward the neighboring b-tubule and the proximal end of axoneme, while the internal side of head only shifts toward the proximal end. In a significant number of dyneins in the presence of ADP-Vi, two heads overlap each other in the proximal shifting form, indicating that ciliary heterodimeric dynein translocates a microtubule by moving with short steps.

**Conclusion:** This indicates that, in contrast to the hand-over-hand motion of cytoplasmic dynein, during ciliary bending axonemal dynein translocates microtubules by moving with short steps.

**P79**

Primary cilia in myoblasts: a role in quiescence

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Cilia 2015, 4(Suppl 1):P79

Quiescence and self-renewal are hallmarks of adult stem cells, which are essential for tissue homeostasis and regeneration. Earlier considered as a state of hibernation, G0 is now emerging as a balanced state where both the cell cycle and tissue-specific programs are held in check by active mechanisms. Quiescent cells are also marked by the presence of primary cilia, which are microtubule-based membrane-encased centrosome-derived structures that act as cellular antennae transducing chemical and mechanical signals. Here we investigate the role of the primary cilia as a determinant for cell cycle regulation and quiescence.

Using a mouse skeletal muscle myoblast culture system that can undergo either reversible or terminal arrest, we show that myoblasts show an increased propensity for ciliogenesis as they enter reversible arrest. By contrast when triggered to differentiate to multinucleated myotubes (irreversible arrest), cilia are lost after a transient phase of ciliation.

Blocking ciliogenesis by RNAi-mediated knockdown of ciliary transport protein IFT88 causes defects in cell cycle exit, with knockdown cells continuing to proliferate under conditions of quiescence. IFT88 knockdown also show a significantly lower clonogenic potential indicating that the self-renewal ability of cilia-ablated cells is compromised. Furthermore, transcriptional profiling by microarray revealed a strong signature of cell cycle related genes many of which are centrosome associated.

Our data suggests that primary cilia are important for the specific regulation of the program of reversible quiescence. We will present recent results that probe the function of primary cilia in quiescence.

**P80**

Characterisation of homologues of known and putative dynein assembly factors in a Drosophila model

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With only two types of ciliated cells, Drosophila is a useful organism in which to study conserved aspects of ciliogenesis. Cilia with motile characteristics in Drosophila are represented just by the sperm flagella and the sensory receivers of chordotonal neurons, which are proprioceptive and auditory sensory neurons.

We recently used Drosophila to identify two new putative dynein arm assembly factors, CG11253 (ZMYND10 homologue) and CG31320 (HEATR2 homologue): impairment of function of either gene results in flies with immotile sperm and defective sensory transduction due to lack of dynein arms in the cilia. We have sought to extend these observations to other known or putative dynein assembly factor homologues to determine how much of this pathway is conserved and set up Drosophila as a model for exploring the function of these proteins in further detail. This analysis has used transcriptomic data of developing chordotonal neurons, gene regulatory analysis (regulation by Rfx and Fox factor, Fd3F), genetic analysis of sensation and male fertility, and protein interaction analysis. Currently, we are also carrying out mass spectroscopy.

**P81**

Insights into the role of Notch signalling in cilia motility regulation

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**Background:** Our previous work has demonstrated that Notch signalling modulates cilia length in the zebrafish left-right organizer (LRO) [1]. However, we also found that the axonemal motor Dnah7, an inner dynein arm motor protein, is upregulated in Notch signalling mutants where motile cilia number is exacerbated related to immotile cilia [1]. Moreover, the knock-down of this motor protein drastically affected cilia motility in the LRO, blocking the nodal flow.

**Objective:** To link Notch signalling to the regulation of cilia motility genes that may be upstream of dnah7.

**Methods:** Gene expression of potential motility-mediator targets were validated by qPCR in deltaD mutants. Whole mount in situ hybridization performed to assess dnah7 gene expression in zebrafish embryos at various stages of development. We used high-speed videomicroscopy to study olfactory pit and LRO cilia motility in dnah7 zebrafish morphants.

The ultrastructure of static cilia was analysed from embryos injected with dnah7-MO.

**Results:** Dnah7 is upregulated in deltaD mutants. Dnah7 is expressed in other organs with motile cilia, such as the olfactory pits, brain ventricles
and pronephros. Down-regulation of dnah7 showed also to affect motility in olfactory pits and pronephros. We are validating microarray data by qPCR and will present a model on how Notch signalling may affect the expression of dnah7.

Conclusion: Dnah7 knock-down affects cilia motility in various ciliated organs during zebrafish development. We found specific target genes from the foxj1 and rfx families, which encode regulatory factors that may be involved in a Notch signalling transcription pathway linked to cilia motility.

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**P83**

Function of the Ciliopathy gene RPGRIP1L in cortical neurogenesis

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Objective: The aim of this work is to identify the functions of the ciliary gene *Rpgrip1l/Ftm* in neurogenesis in the cerebral cortex. *Rpgrip1l/Ftm* encodes a protein enriched at the ciliary transition zone and is involved in ciliopathies with associated brain abnormalities, Meckel and Joubert syndromes. We have previously shown that *Rpgrip1l* is required for telencephalic morphogenesis.

Methods: We use a mouse knock-out mutant line, *Ftm* conditional, to identify the role of *Rpgrip1l* in cortical neurogenesis. Two distinct pools of progenitors undergo extensive cell divisions to form cortical projection neurons. Radial Glial Cells (RGCs) divide either symmetrically to expand the progenitor pool or asymmetrically to self-renew and produce an Intermediate Progenitor Cell (IPC), which divides once to form two neurons. The balance between differentiation and proliferation is coordinated by multiple signalling pathways, among which several depend on the primary cilium.

Results: In *Ftm* mutant embryos, the cortex is thinner with a reduction in the number of neurons and of IPCs. In contrast, the RGCs are present in normal numbers and they proliferate normally. This suggests a defect in the balance between symmetric (proliferative) and asymmetric (neurogenic) divisions, a hypothesis we are currently confirming. We showed that the reorganization of a short, repressor form of the Gli3 transcription factor partially rescues cortical neurogenesis in *Rpgrip1l* mutant background. We are currently investigating the molecular mechanisms of this defect downstream of Rpgrip1l and Gli3R.

Conclusion: Our results show that Rpgrip1l controls neurogenesis in the cerebral cortex, via the formation of the Gli3 repressor.

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**P84**

Pkd2 affects the architecture of zebrafish left-right organizer

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Background: Dorsal anterior clustering (DAC) of motile cilia in the left-right organizer (LRO) is crucial for normal fluid dynamics and correct laterality in zebrafish [1]. We directly demonstrated that *charon/dandS* transcription is negatively regulated by strong flow in zebrafish LRO [1] which suggests that LRO cells have the ability to sense fluid flow and influence gene expression patterns, but how? Pkd2 ion channel is a good candidate because it participates in a mechanosensory complex that senses fluid flow and induces a calcium inward flux in kidney cells [2] and in nodal cells [3]. In agreement, mouse and zebrafish mutants for Pkd2 have LR defects [4,5]. However, Pkd2 is also involved in cell polarity during migration [6] and in extracellular matrix deposition [7] implying a role for Pkd2 in cell morphogenesis.

Objective: Determine whether Pkd2 knockdown affects DAC of ciliated LRO cells.

Methods: dnah7 morpholino [1] was injected to generate static cilia without affecting DAC [1]. Each embryo was screened for static cilia by high-speed videomicroscopy. In parallel, Pkd2 knockdowns were imaged for flow dynamics followed by quantification of anterior / posterior cilia number through two-photon microscopy.

Results: Pkd2 knockdown, contrary to Dnah7 knockdown, caused a remodelling in the LRO architecture by disrupting DAC. Moreover, Pkd2 knockdown resulted in abnormal fluid flow as a consequence of defective DAC.

Conclusion: Comparing Dnah7 and Pkd2 knockdowns we concluded that Pkd2 mediated pathway affects LRO morphogenesis by a mechanism that seems to be independent of its role in fluid flow mechanosensation. Meaning that Pkd2 triggers additional responses from those caused by LRO flow.

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References

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**P85**

Automatic detection of beating cilia with frequencies estimations

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Objectives: Muco-ciliary clearance is the airway first mechanism of defence against environmental attacks such as micro-organisms or pollution. Cilia motility impairment can be either of genetic (primary ciliary dyskinesia) or acquired origin (environmental attacks), entailing chronic diseases. It is of interest for practitioners to evaluate cilia beating frequency easily, robustly and reliably. As yet, no fully automated method is available.

Methods: Ciliated cells were sampled in patients by brushing nasal mucosa and cilia beating was recorded using high speed video microscopy. We first estimated and removed the sensor pattern. We then stabilized the sequence assuming rigid transforms. We retained only the moving parts of the sequence and, after deblurring, characterized and segmented the moving parts in several regions of interest. The frequency was estimated for each region.

Results: We output the processed sequence, a labeled mask of the various beating zones and a chart of the frequency observed in each region. Hence we obtained synchronizations information between the different parts of the observed ciliated cells. An estimation of frequencies for each beating part is the final result.

Conclusion: With this new method, we propose a fully automatic estimation of cilia beating frequencies, which is able to deal with acquisition artifacts, such as sensor patterns, vibrations and noise, but also with the variety of frequencies we can observe on a single sample. We believe this may be a useful method for practitioners.
Conclusions: CCC1 is a publicly available tool, which will allow to clarify the roles of previously unknown cilary functions and to elucidate the molecular mechanisms underlying cilary-associated phenotypes.

P88
A systems biology approach towards the prediction of ciliopathy mechanisms
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Objective: Understanding the molecular and cellular mechanisms of ciliopathies is vital for dissecting their pathogenesis, identifying appropriate therapeutic targets and designing effective treatments. Recent advances in DNA sequencing technology have provided a torrent of genetic data that can now be used to elucidate the genetic basis of human diseases. A consensus has emerged among biologists that to fully exploit the available data, they have to be correlated with additional research. This is especially important for rare-disease genetics, because of the small number of available patients.

Methods: Computational methods could be employed to tackle this problem. Integration of diverse biological data can provide additional evidence to support experimental observations. We present a statistical method that predicts disease causing genes by integrating protein interaction data and clinical phenotype annotations. For a given protein in the network the method predicts the clinical phenotypes that may appear upon protein alteration based on the phenotypes associated with the neighbours of the protein.

Results: Application to protein interaction data from the SYSCILIA project (http://syscilia.org) and phenotype annotations from the HPD project (http://www.human-phenotype-ontology.org) successfully predicts candidate Nephronophthisis genes. The results also imply that abnormal Hh signaling may be the cause of Nephronophthisis and GPRC mislocalization a possible way by which cilia defects affect Hh signaling.

Conclusion: The results indicate that the developed method can be useful for the dissection of disease pathogenesis by predicting disease genes and drawing new hypotheses on the underlying mechanisms. Such hypotheses can assist in the design of new targeted experiments.

P89
Systematic exploration of the cilary protein landscape by large-scale affinity proteomics
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Objective: Mutations in different ciliopathy-associated genes often result in overlapping clinical phenotypes, which can in part be explained by disruption of overlapping functional protein modules. In this study we conducted large-scale affinity proteomics in a systems biology-based approach to boost insights into the assembly of these ciliary modules, and their connectivity in larger functional protein networks: the ciliary protein interaction landscape. This provides an important framework to deconvolute the pathways and processes that drive ciliopathies, and to understand the general importance of ciliary function for cellular homeostasis.

Methods: Using more than 220 known and potential ciliary proteins as baits, fused to the Strep/FLAG-tandem affinity purification tag (SF-TAP), we purified protein complexes from human embryonic kidney cells (HEK293T), which were analysed by mass spectrometry. In parallel, specific modules were scrutinized for binary interactions by yeast two-hybrid analyses. Existing and newly developed bioinformatic algorithms were employed to validate the confidence of the identified interactions and to define functional modules.

Results: We obtained low, medium and high confidence sets of protein interactions and modules. From this data we could assign novel components to known ciliary modules such as the anterograde and retrograde intraflagellar transport modules and the dynein-2 module. Due to the strong focus on ciliary proteins as baits and the integration of data from various sources, we could also identify several new modules, potentially with cilia-associated functions in health and disease.

Conclusion: Our systems oriented approach, employing affinity proteomics to define the ciliary network has resulted in a comprehensive description of known and candidate ciliary protein networks and modules, which can serve as a resource for candidate ciliopathy proteins and our understanding of pathogenic mechanisms underlying ciliopathies.

Cite abstracts in this supplement using the relevant abstract number, e.g.: Lu et al. From proteomic data to networks: statistics and methods reveal ciliary protein interaction landscape. Cilia 2015, 4(Suppl 1) P90