CILIA2012 Conference Summary

A Personal View by Kheng Ng, UCL Student 2009-2012

Introduction

From 16 to 18 of May 2012, about 300 delegates including scientists and clinicians from over 19 countries congregated at the UCL Institute of Child Health, London for the CILIA2012 conference. This was the first international scientific conference organised by the Ciliopathy Alliance with support and sponsorships from various organisations such as Syscilia, Roche, Perkin Elmer, the Genetics Society, the Company of Biologists, EURO-WABB project, Deafness Research UK, Kidney Research UK, Cilia Journal, and Mammalian Genome Journal of Springer.

The theme of the conference, “Cilia in Development and Disease” ran through a total of 5 sessions on 17 and 18 May. These included: Clinical and novel aspects of ciliopathies, structure and function of cilia, cilia and development, cilia and disease as well as translational therapy and ciliotherapeutics.

The conference aimed to provide a forum to present the latest advances in scientific, clinical and translational research into cilia; promote opportunities for more junior investigators to present their research; encourage interactions between members of the cilia research community; and to bring researchers and clinicians together with the charitable patient organisations to foster communication and involvement between each group.

With the last aim in mind, an **evening reception** was held on 16 May, at the British Medical Association, that brought together research scientists, paediatricians, medical trainees, geneticists, educational/lobbying alliances, parent support groups, and family members. The keynote speaker was Joe Gleeson from University of California San Diego School of Medicine “Translating gene discoveries for patient benefit”.

On 17 May, the 2-day scientific programme was kicked off with a greeting from Professor Phillip Beales from the UCL Institute of Child Health.

Session 1: Clinical and Novel Aspects of Ciliopathies

Co-chaired by Professor Friedhelm Hildebrandt (University of Michigan) and Professor Heymut Omran (University of Münster).

Professor Omran introduced cilia structure and showed that DNAH5 loss of function mutation could result in non-motile cilia, and in mutant mice defective ependymal cilia causing failure of the cerebral aqueduct to close properly, leading to perinatal death. Primary Cilia Dyskinesia (PCD), hydrocephalus and the randomization of left-right axis specification were observed upon DNAH5 deficiency. Defects in Hydin caused caused a novel PCD variant without randomization of left-right asymmetry. There was intact cilia structure with lower beating amplitude, and as there were no obvious structural defects in the mutation, accurate diagnosis required TEM tomography which was able to show the central pair C2b projection was missing. Since it was hard to diagnose PCD by electron microscopy, Dr Jean-Francois Papon (INSERM, Creteil) suggested a recently developed high speed video microscopy to determine the quantitative parameters involved such as the length, beating angle, frequency and pauses of the cilia movement.

Dr Eva Lenassi (UCL) focussed on the causes of retinitis pigmentosa (RP), showing at least 3 Usher Syndrome 2A (USH2A) mutated variants appear to be RP-specific. Dr Valerie Cormiere-Daire (INSERM, Hôpital Necker Enfants Malades) presented a series of studies on Short Rib Polydactyly syndrome (SRPS). These patients often developed asphyxiating thoracic dysplasia (ATD) characterised by respiratory distress, development of kidney cysts, and disorders in liver metabolism such as cholestasis and liver fibrosis, with consequent retarded growth and neuromuscular development.

Charlie de Melo (AVENIR-Inserm) investigated the water reabsorption ability of primary cilia in kidneys of 33 *Bardet–Biedl syndrome* (BBS) patients with a mean age of 26 years. Although TEM showed no obvious malformation of the cilia structure, the water reabsorption ability was impaired in the subjects. It was found out that the loss of BBS proteins results in the failure of arginine vasopressin (AVP) to trigger water reabsorption in renal cells.

Professor Gillian Griffiths (University of Cambridge) showed striking similarities between synapses and cilia. The Hedgehog (Hh) signalling pathway found in cilia was also required for T-cell development, and centrosome docking in the plasma membrane of T-cells provided a docking point for endocytosis to deliver cytoxic molecules into the target cells. Hh signalling inhibitors such as cyclopamine prevent centrosomal localisation of the ring of actin formed during the delivery process of cytotoxins to target cells.

Professor Friedhelm Hildebrant (University of Michigan) reported a strategy to identify recessive mutations in patients who developed Nephronophthisis (NPHP) using homozygosity mapping, based on the identification of identical-by-descent markers located next to the disease genes. Exome capture was also employed, but was limited by the presence of hundreds of mutated variants and difficulty in filtering down the data obtained to explain the single recessive disease mutation. Mutation of FAN1, a nuclease involved in the DNA repairing mechanism, causes NPHP-like karyomegalic interstitial nephritis (KIN). Impaired DNA damage response (DDR) signalling was responsible for causing ciliopathy phenotypes such as the formation of kidney cysts. MRE11, CEP164 and FAN1 were both involved in DDR pathways, as well as ciliopathies.

Session 2: Structure and function of cilia

Co-chaired by Associate Professor Greg Pazour (University of Massachusetts) and Professor Bradley Yoder (University of Alabama).

Dr Duarte Barral (Lisbon University) showed that silencing of the Arl13b GTPase gene decreased cilia-dependent antigen presentation and inhibited membrane recycling due to impaired the early membrane trafficking. Arl13b mutation causes Joubert Syndrome, a ciliopathy characterized by neurological symptoms. Dr Oliver Blacque (Dublin University) focussed on the role of Arl13b in Joubert Syndrome. He studied the worm *C. elegans* showing Arl13b was highly motile at the ciliary membrane but the exchange with the dendritic component in the neurons was slow, indicating Arl13b is important at ciliary membranes, stabilizing protein transport.

The keynote speaker, Dr Greg Pazour (University of Massachusetts) showed the intraflagellar transport (IFT) IFT27 and IFT25 module is highly conserved in ciliated organisms except in *C. elegans and Drosophilla.* Silencing these genes is lethal to the organism due to defects in lungs and heart. Cilia along trachea were normal, so in contrast to most other IFT particles, IFT25/27 complex was not required for cilia assembly. However IFT25 mutants displayed Hh signalling defects with a 4-fold attenuation of pathway activation compared to controls; IFT25 was required to transport Smoothened (Smo) from cilium and Gli2 to ciliary tips.

Associate Professor Sudipto Roy (IMCB, Singapore) examined FoxJ1, the master regulator of cilia biogenesis, using zebrafish and a series of genetic screenings for FoxJ1 target validation. Subsequent functional validation of the target genes products which were fused with green fluorescent protein (GFP) was carried out using morpholinos knockdown studies, showing 64% of the studied gene products localised to the ciliary apparatus. These proteins were relevant for ciliogenesis and possible targets of ciliopathies.

Dr Jarema Malicki (University of Sheffield) suggested a model with B9d2 and Inversin required for transport of cargo in cilia, showing that transport of opsin is slower in Inversin, B9d2 and Nphp5 zebrafish morphants; B9d2 and Inversin are defective in the human cilia-related disorders Meckel Syndrome and Nephronophthisis. Dr Gert Jansen (Erasmus University) spoke on regulation of cilium length and IFT, by MAP kinase signalling. In mammals, 3 types of such kinases exist: MAK or RCK, MRK or ICK and MOK (MAPK/MAK) or RAGE1. Increased MRK expression decreases cilium length, while gene knockout increases length as well as IFT speed. MOK which was the most divergent of the three mentioned types of kinase had less significant effect in the prospective mutants, but knockdown increased cilia length with no effect on IFT speed.

Professor Bradley Yoder (University of Alabama) reported that single mutations in Nphp or MKS proteins cause functional defects but not severe structural defects in cilia morphology. OSM mutations were able to slow down the speed of IFT particles. Defects in transition zone which was located at the upper part of basal body, could result in the detachment of membrane from microtubules proving that the localisation of cilia membrane proteins was altered in NPHP and MKS mutants.

Professor Michel Leroux (Simon Fraser University) investigated the role of CHE-10 localized to the proximal end of the basal body and transition zone. Mutations cause abnormal IFT and the ciliary transition zone and basal body were lost. IFT assembly/function was impaired, which was suggested to be necessary for structural maintenance of the basal body and also required for the proper assembly of IFT particles.

Professor Andrew Jarman (Edinburgh University) showed the Fd3F transcription factor, likely a diverged FoxJ family member, regulates the function of cilia rather than their structure, as cilia were present in Fd3F mutants. Fd3F target genes were required for the formation and function of the motile zone of *Drosophila* chordotonal neuron cilia: loss of Rfx gene affects all the sensory neurons, but silencing Fd3F only affects chordotonal neurons.

Dr Chris Westlake (NIH-NCI, USA) showed Rab11-Rab8 proteins played an important role in ciliogenesis. Rabin 8 controls preciliary vesicle trafficking, important in the formation of cilia. EHD1 associates with Rab11-Rab8 proteins and aids in shaping of membrane during ciliary assembly. TRAPP1/II bound to Rabin 8 can target preciliary vesicles to centrosome.

Session 3: Cilia and development

Co-chaired by Professor Kathryn Anderson (Sloan-Kettering Institute New York) and Associate Professor Jeremy Reiter (University of California, San Francisco).

Jeremy Reiter reported that Tectonic1 (Tctn1) is essential to left hand axis development, it was found to localize to the cilia transition zone and to be required for dorsoventral patterning of the neural tube. Tctn1 and Tctn2 mutations cause Joubert Syndrome. B9d1 was found to localize Tmem231 at transition zone. B9d1 was found at the transition zone, but interestingly was dispensable in normal ciliary formation despite ubiquitous expression. B9d1 mutants were MKS-like, thus dysfunction of the transition zone can cause Meckel Syndrome.

Assistant Professor Rebecca Burdine (Princeton University) studied about the role of Ccdc151 and Ccdc114 in zebrafish, showing they likely dimerize together to play a role in left-right patterning. Ccdc114 was found to be required for proper kidney development. Dr Sarah Goetz (Sloan-Kettering Institute) showed that bartleby (bby) mutant mouse embryos exhibited reduced Shh signalling, due to a premature stop codon in Tau Tubulin kinase 2 (Ttk2). The basal body was normal in Ttbk2^{bby} mutants, but there was no localisation of ciliary IFT140 and IFT88. Cilia were also found to be restored in Ttbk2^{bby} cells by stable expression of Ttbk2-GFP which localised to the transition zone. Thus, TTbk2 is required for the recruitment of IFT complex to basal body.

Dr Yoshimi Greer (NIH-NCI*)* reported that casein kinase (CK) 1δ co-localized with γ-tubulin and is required for primary ciliogenesis. CK1δ. CK1δ siRNA blocked primary ciliogenesis in hTERT-RPE cells. In addition to the typical centrosomal microtubule organising centre (MTOC), the Golgi apparatus could also act as a site for γ-tubulin assembly and microtubule nucleation. AKAP450 and GM130 were also required for Golgi-derived microtubule nucleation, and this form of microtubule nucleation was disrupted after CK1δ siRNA. CK1δ is likely required to phosphorylate substrates important for this process, as its inhibitor PF670462 blocked Golgi derived microtubule nucleation and downstream ciliogenesis.

The plenary speaker, Associate Professor *John Wallingford* (University of Texas, Austin) spoke about the newly emerging roles for planar cell polarity (PCP) proteins in ciliogenesis. He showed that disruption of Fuz led to ciliopathy phenotypes, trafficking defects in the axoneme and basal body, and IFT43 was found to be missing in the axoneme. Currently, the search continues for an interface between and ciliogenesis, in light of the newly discovered broad roles of PCP proteins in ciliogenesis. The keynote speaker of the session, Professor Kathryn Anderson (Sloan-Kettering Institute) investigated Kif7 motor proteins in cilia. She noted

Kif7 is enriched at cilia tips in response to the presence of Shh, and Kif^{maki} mutant mice showed less stable cilia compared wild type mice. Kif7 was identified to be a motor protein involved in Shh signalling and axonemal microtubule stability.

Session 4: Cilia and disease

Co-chaired by Associate Professor Enza Maria Valente (University of Messina) and Professor Nicholas Katsanis (Duke University).

Professor Nicholas Katsanis (Duke University) discussed mutational loads in ciliopathies, identifying 18,000 rare point mutations and 1,100 insertions/deletions in ciliopathy patients, in the attempt to find the total mutational load in ciliopathies. Dr Isabelle Perrault (INSERM, Paris, France) showed IFT140 mutation is capable of causing Mainzer Saldino Syndrome, and reported that mutations in all IFT-A components were responsible for skeletal ciliopathies.. Ciliogenesis is lower in the fibroblast of MSS patients; the localization of IFT140 was unaltered but there was a change in the localization of retrograde ciliary transport proteins. However, there was no obvious correlation found between IFT140 and the severity of the disease.

Dr Ruxandra Bachmann-Gagescu (Washington University) showed Cc2d2a is required for the localization of Rab8, but not required for ciliogenesis. Opsins are mislocalized in Cc2d2a -/- zebrafish mutants, however, not all trafficking is abnormal, since transport of transducin and RIBEYE are unaltered. Dr Daniela Iaconis (Telethon Institute of Genetics and Medicine) showed Ofd1 interacts with the eIF3 complex in HEK293 cells. Besides a role in protein synthesis, Ofd1 may have some specific roles in mTOR signaling.

Session 5: Translational therapy and ciliotherapeutics

Co-chaired by Dr Peter Jackson (Genentech) and Associate Professor Rachel Giles (University Medical Center Utrecht).

After the poster awards, Associate Professor Rachel Giles (University Medical Center Utrecht) provided a brief introduction of the potential treatment approaches such as “prosthetic cilia”, signalling replacement therapy, and tissue and gene replacement therapy in ciliopathies. However, more experimental evidence must be considered in these therapeutic approaches before relevant clinical utilization. Following that, PTC124 was a hot topic in a presentation by Dr Uwe Wolfram (Mainz University). This Translational Read Through Inducing drug (TRID) can direct the ribosome from “skipping” the premature stop codon found in some specific USH1C mutant isoforms in human retina. Subsequently, functional USH1C protein is produced from the mutated USH1C RNA transcripts which harboured premature stop codons. Recovery of harmonin b’s actin bundling activity was observed following the application of TRIDs. Similarly, second generation TRIDs such as NB54 could induce this recovery with no increase of apoptotic cells. Thus, these drugs could be a potential treatment for specific retinal ciliopathies caused by nonsense mutations.

Finally, Professor Peter Jackson (Genentech) mentioned a newly discovered G-protein coupled receptor (GPR161). GPR161 null mice were embryonic lethal at E10.5, failing to form a closure in the forebrain so that detrimental neural tube defects were observed. Homozygous mutation of GPR161 was consistent with a loss of Hh signalling in these mutants. His studies also showed that ciliary localisation of GPR161 required Tulp3/IFTA complex. These proteins are highly conserved among vertebrates and GPR161 is highly expressed in melanoma as well as other forms of tumours.

Conclusion

Before the end of the conference, 7 poster prizes were awarded to the delegates which was followed by a concluding speech from Professor Phil Beales (UCL Institute of Child Health). A total of about 9 different organisms, 6 different organelles and almost all organs in the body was mentioned and discussed by these remarkable speakers. As a whole, CILIA2012 was deemed a fruitful event with delegates willing to share unpublished results as well as insights through a series of poster and oral presentations. In the closing speech, Professor Phil Beales (UCL Institute of Child Health) expressed his appreciation to all the delegates and working committee in ensuring the smooth running of the event. For more information, please do visit http://www.ciliopathyalliance.org/.

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