Genet. Res., Camb. (2012), **94**, *pp.* 49–55.
doi:10.1017/S0016672312000055

Abstracts of papers presented at the 22nd Genetics Society's Mammalian Genetics and Development Workshop held at the Institute of Child Health, University College London on 17 November 2011

Edited by: NICHOLAS D.E. GREENE[1], ANDREW J. COPP[1] AND ANDREW WARD[2]
[1] *UCL Institute of Child Health, 30 Guilford Street, London WC1N 1EH*
[2] *Department of Biology & Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY*

Towards a cell therapy for retinal disease

J. LAKOWSKI[1], Y. T. HAN[1], R. A. PEARSON[2], A. GONZALEZ-CORDERO[2], E. L. WEST[2], S. GUALDONI[1], A. C. BARBER[2], M. HUBANK[1], R. R. ALI[2] and J. C. SOWDEN[1]
[1]*UCL Institute of Child Health, UCL Institute of Ophthalmology, University College London, London, UK;* [2]*Department of Genetics, UCL Institute of Ophthalmology, University College London, London, UK*

Retinal degenerative diseases are a major cause of untreatable blindness. Cell therapy to replace lost photoreceptors represents a promising future treatment. We previously demonstrated that transplanted postmitotic photoreceptor precursors, expressing the NrlGFP transgene, integrate into the diseased retina and restore light sensitivity.

Here, we performed microarray analysis of NrlGFP-expressing precursors to identify four candidate genes encoding cell surface antigens (Nt5e [CD73], Prom1, Podxl, and Cd24a). Cell selection strategies were developed using conjugated antibodies recognizing the cell surface antigens to isolate photoreceptor precursors by fluorescent activated cell (FAC)-sorting. To test the feasibility of using donor cells isolated via cell surface markers for retinal therapy, FAC-sorted cells from developing retinae were transplanted into the wild-type or degenerating mouse eye. Cells isolated by this method migrated into the outer nuclear layer and developed into mature photoreceptors. They showed an 18-fold higher integration efficiency than unsorted cells and 2·3-fold higher than cells sorted based on a single genetic marker, NrlGFP expression. These proof-of-principle studies show that transplantation competent photoreceptor precursor cells can be efficiently isolated from a heterogeneous mix of cells using cell surface antigens without loss of viability for the purpose of retinal stem cell therapy.

Regulation of cell protrusions during neural tube closure in mouse

ANA ROLO, MARK TURMAINE, CAROLINE HIRST, DAWN SAVERY, NICHOLAS D. E. GREENE and ANDREW COPP
Neural Development Unit, Institute of Child Health, University College London, 30 Guilford Street, London WC1N 1EH, UK

The final steps of neurulation involve fusion and remodelling of the neural folds at the dorsal midline. Using scanning electron microscopy, we imaged the site of fusion at the posterior neuropore (PNP), which is known to be rich in membrane protrusions. We describe the presence of distinct types of protrusions during different stages of PNP closure. Initially, the fusion site displays long, thin, finger-like protrusions that emanate from a lamelliform veil (filolamellipodia). Later, during final stages of PNP closure, these protrusions are replaced by elaborate membrane ruffles. To understand how these protrusions are regulated and whether they might play a role in neurulation, we genetically ablated Rac1 in the dorsal neural tube. Rac1 is a Rho-family GTPase known to regulate the formation of lamellipodia and membrane ruffles in many cell types. We observe that Rac1 conditional mutants lack the membrane ruffles characteristic of late PNP closure stages, but that at earlier stages they show filolamellipodia that are indistinguishable from controls. These observations are consistent with the finding that the Rac1 conditional mutants fail to close their PNP only at late stages, resulting in mild spina bifida or curled tail phenotypes, and indicate that the ruffles formed at the site of fold apposition might be required for correct fusion.

Cavitation of the middle ear and conductive hearing loss

HANNAH THOMPSON and ABIGAIL S. TUCKER
Dept of Craniofacial Development, Kings College London, London, UK

The middle ear is an air-filled cavity bridged by three auditory ossicles, which conduct sound from the ear-drum to the inner ear. The correct formation of the middle ear is essential for hearing, and problems can result in conductive hearing loss. In the early embryo, the middle ear is filled with neural crest cell mesenchyme; as development proceeds a process called cavitation occurs producing an air-filled space surrounded by an epithelial mucosa. How this process occurs is largely unknown. We hypothesize against the well-established "classic endoderm" model of middle ear cavitation, and propose an "endoderm rupture" model to explain how cavitation truly occurs. This model better explains the cases of retained mesenchyme (failed cavitation), which can lead to conductive hearing loss.

We have examined the origin of the middle ear mucosa in a number of transgenic reporter mice lines, to find it is not solely composed of endoderm, but is of mixed origin. Using immunohistochemistry, we have seen evidence of a mesenchmye-to-epithelial transition where neural crest cells produce epithelial cell proteins and redistribute around the middle ear cavity. These data support the "endoderm rupture" model of cavitation in the mouse.

The role of planar cell polarity signalling in mammalian spinal neurulation

SOPHIE E. PRYOR, VALENTINA MASSA, NICHOLAS D. E. GREENE and ANDREW J. COPP
Neural Development Unit, Institute of Child Health, University College London, London, UK

Among the many mouse models of NTDs, one group of genes critical for successful neural tube closure are members of the non-canonical Wnt/PCP signalling pathway. PCP signalling is required for the initiation of neural tube closure at the hindbrain-cervical boundary ('Closure 1'), via regulation of convergent extension (CE) cell movements in the midline tissues during post-gastrulation shaping. Components of the pathway are also emerging as candidates for causation of NTDs in humans, with putative PCP gene mutations identified in craniorachischisis and spina bifida patients.

In addition to the function of mouse PCP genes during Closure 1, our data suggest a key genetic modifying role for the *loop-tail* (*Lp*; *Vangl2*) mutation in predisposing to failure of low spinal neural tube closure. Mutations in the core PCP gene *Vangl2* increase the frequency and severity of spina bifida in the *grainyhead-like-3* hypomorph *curly tail* (*ct*), a well-established model of partially penetrant NTDs. This raises the question of whether CE also plays a role in low spinal neurulation. We are investigating the developmental mechanism(s) by which heterozygosity for the *Lp* mutation exacerbates *ct*-related spina bifida, to examine the role of PCP signalling in spinal neural tube closure. Our data indicate that *Lp* does not worsen the known *ct* mutant mechanism or prevent the formation of dorso-lateral bending regions in the neuroepithelium. Instead, *Lp* may cause defective CE cell movements and perhaps abnormal tailbud development.

A search for imprinting-specific sequence motifs in maternal germline differentially methylated regions and maternal imprinting control regions

HEBA SAADEH[1], CHARLOTTE PROUDHON[2], RACHEL DUFFIÉ[2], GUILLERMO CARBAJOSA[3], MICHELLE HOLLAND[3], VARDHMAN RAKYAN[3], DEBORAH BOURC'HIS[2], THOMAS DOWN[4] and REINER SCHULZ[1]
[1]*King's College London, Medical and Molecular Genetics, London, UK;* [2]*Institut Curie, UMR3215/Inserm U934, Paris, France;* [3]*Queen Mary University, Blizard Institute, London, UK;* [4]*Gurdon Institute, Cambridge, UK*

Genomic imprinting refers to the differential epigenetic marking of the parental genomes in mammals and flowering plants. In the germline, both parental genomes are being epigenetically re-programmed causing DNA methylation differences at distinct loci called germline differentially methylated regions (gDMRs). During the early stages of embryonic development, the parental genomes undergo a second wave of epigenetic re-programming during which differential methylation is lost at most but not all gDMRs. The few gDMRs that endure are termed imprinting control regions (ICRs) since they ultimately cause the parent-of-origin-specific expression of the imprinted genes in the mammalian genome. There are seventeen ICRs known to originate in the maternal germline, that is, they become methylated during oogenesis, and this process requires *DNA methyltransferase 3-like* (*Dnmt3l*). However, the signals that are necessary to target the *de novo* DNA methylation mechanism to gDMRs in the oocyte and the signals that protect specifically ICRs from *post* fertilization re-programming are incompletely characterized. One such necessary signal may be specific sequence motifs

https://doi.org/10.1017/S0016672312000055 Published online by Cambridge University Press

at gDMRs and ICRs. We precisely determined the genome coordinates of all known and some novel maternal ICRs in mouse using MeDIP-seq to generate and then compare the DNA methylation profiles of *Dnmt3l*−/+ embryos, wildtype embryos, sperm and C57/Bl6J × PWD/PhJ adult liver. As the coordinates of maternal gDMRs, we used the 1062 oocyte-specifically methylated CpG islands (>=75% methylation) that were identified by Smallwood *et al.* (*Nat Genet*, 2011) using RRBS-seq. Using motif discovery tools like MEME and NestedMICA we were able to identify a set of motifs that were significantly over-represented either in maternal gDMRs or in ICRs relative to a large set of non-imprinted CpG islands, the latter otherwise being similar to maternal gDMRs and ICRs (e.g. promoter-associated). Some of the over-represented motifs occur in all ICRs/gDMRs, and some are only shared between subsets of ICRs/gDMRs. We are in the process of annotating these identified motifs to evaluate their biological significance.

The ncRNAs in the *Dlk1-Dio3* domain function as tumor suppressors *in vivo*

NOZOMI TAKAHASHI, EITO YAMAGUCHI and KONO TOMOHIRO
Tokyo University of Agriculture

The *Dlk1-Dio3* domain on mouse distal chromosome 12 is an indispensable cluster of imprinted genes. This region contains the maternally expressed multiple non-coding RNAs (ncRNAs) (including *Gtl2*) and paternally expressed protein coding genes. Many miRNAs and snoRNAs are located at the downstream of *Gtl2*. The tumor-suppressive function of *Gtl2* has been reported in several types of human cancer cell line; however the function has not been well characterized in an *in vivo* experiment. Our previous studies showed that deletion of *Gtl2* on the maternal allele (*Gtl2* (−/+)) resulted in a loss of *Gtl2* expression and decreased expression of ncRNAs including many miRNAs located at the downstream of *Gtl2*. Therefore, to investigate the potential tumor suppressing activity of the ncRNAs, we conducted experimental teratomas induced by grafting E6.5 embryos under the kidney capsule of scid mice, and compared for weight and histopathology. The weight of teratomas derived from *Gtl2* (−/+) embryos increased significantly compared with WT, suggesting that the ncRNAs including *Gtl2* act as tumor suppressor genes *in vivo*. Subsequently, to investigate if expression levels of miRNAs in the domain are related to the tumor growth, we performed miRNA array analysis in the *Gtl2* (−/+) and WT teratomas.

Early development mechanics of expression and imprinting of the transcripts of the *Gnas* cluster

STUTI MEHTA[1,2], CHRISTINE WILLIAMSON[1], MARTIN FRAY[1], SIMON BALL[1] and JO PETERS[1]
[1]*Medical Research Council, Harwell, Didcot OX11 0RD, UK;* [2]*Biochemistry Department, University of Oxford, Oxford OX1 3QU, UK*

Genomic imprinting is an epigenetic phenomenon ensuring parent of origin specific expression of genes. Imprinted genes lie in clusters across the genome, their expression being regulated by control regions called Imprinting Control Elements (ICE). The *Gnas* cluster of imprinted genes lies on distal chromosome 2. A long non-coding RNA *Nespas*, originating from the ICE of the *Gnas* cluster is a regulatory RNA. A *Nespas* null mutant upsets imprinting of all transcripts in the cluster.

We find that *Nespas* controls imprinting of the other transcripts in the cluster via *Nesp*, its sense counterpart. The main function of *Nespas* is to repress *Nesp* on the paternal allele. De-repression of *Nesp* on the paternal allele, i.e. full length transcription of *Nesp* through the downstream *Exon1A* DMR leads to *de novo* methylation of the normally unmethylated paternal *Exon1A* DMR. This 'ectopic' methylation of the paternally inherited *Exon1A* DMR disturbs imprinting of downstream *Gnas*, leading to phenotypic abnormalities.

To probe into possible mechanisms that *Nespas* employs to silence *Nesp* on the paternal allele, a study of the mechanics of transcript expression and imprinting during early development was carried out. The results of this inquiry will be shared.

Loss of the imprinted signalling protein XLαs in mice results in increased sympathetic stimulation of the cardiovascular system

NICOLAS NUNN, RICHARD BARRETT-JOLLEY and ANTONIUS PLAGGE
Department of Cellular and Molecular Physiology, Institute of Translational Medicine, University of Liverpool, Liverpool, UK

XLαs is a signalling protein transcribed from the paternal allele of the imprinted *Gnas* locus. It is expressed only in certain tissues, including specific neurone populations, and mice lacking XLαs are lean and hypermetabolic. Using a paternal knock-out for XLαs, we are investigating whether the lean phenotype is associated with a general increase in sympathetic nervous system (SNS) activity. Firstly, we have shown that anaesthetized XLαs knock-out mice have

https://doi.org/10.1017/S0016672312000055 Published online by Cambridge University Press

increased blood pressure and body temperature compared with wild-types, indicating increased SNS stimulation of the cardiovascular system and brown adipose tissue. Using implantable radiotelemetry to record ECG in conscious freely moving animals, we have shown that XLαs knock-out mice have increased heart rate. Additionally, the sympatholytic drug reserpine causes a greater drop in heart rate in knock-outs compared with wild-types, reducing both to comparable levels. This confirms that the abnormally high heart rate in knock-outs is caused by increased SNS activity. Finally, following peripheral injection with Exendin-4, an agonist for the GLP-1 receptor, XLαs knock-outs showed a greater heart rate response. Together, these data suggest that XLαs inhibits the SNS, and that deficiency in mice results in increased SNS activity, possibly caused by increased sensitivity to SNS-stimulating neuropeptides.

In silico **analyses implicate** ***FoxA2*** **in** ***Pkd1l1*** **regulation**

D. V. LEORDEAN, D. T. GRIMES and D. P. NORRIS
MRC Harwell, Harwell Science and Innovation Campus, Oxfordshire, OX11 0RD, UK

Pkd1l1 (polycystic kidney disease 1-like 1) plays a crucial role in establishing left-right (L-R) asymmetry and interacts with the Ca^{2+} channel Pkd2. While *Pkd2* is broadly expressed, *Pkd1l1* is expressed specifically in the notochord and node. Characterizing the mechanisms controlling tissue-specific *Pkd1l1* expression will further our understanding of L-R determination.

Previous studies have shown *Pkd1l1* and *FoxA2* (a forkhead box transcription factor) to be spatiotemporally co-expressed during early development. Intriguingly, ChIP-Seq studies have revealed sequences 5′ to mouse and human *Pkd1l1* to bind FoxA2. *In silico*, we have identified FoxA2-specific binding sites within these regions, leading us to hypothesize that FoxA2 regulates *Pkd1l1*. Supporting this, certain FoxA2 binding sites are evolutionarily conserved. Moreover, the two regulatory sequences are non-homologous and we argue that both were active before the splitting of primates from rodents and lagomorphs, an event which led to increased activity in one regulatory sequence at the cost of the other. The evidence presented supports the hypothesis that FoxA2 regulates *Pkd1l1* expression. Furthermore, this study also indicates that the regulatory relationship between FoxA2 and *Pkd1l1* is conserved throughout the mammal lineage and possibly beyond.

Actomyosin cytoskeleton, Rho GTPase signalling and spinal neurulation

SARAH ESCUIN, NICHOLAS D. E. GREENE and ANDREW COPP
Neural Development Unit, Institute of Child Health, University College London, London, UK

Although there is evidence for a requirement of the actin cytoskeleton in neural tube closure, most mice with knock-out mutations in cytoskeleton-related genes exhibit only cranial neural tube defects (NTDs) and not spina bifida. Use of cytochalasin D (cytD), a drug that prevents the polymerization of actin monomers, blocks cranial neurulation and also inhibits the initial event of spinal neurulation (Closure 1). However, if treatment is begun after this stage, the spinal neural tube can close successfully in the presence of cytD. Median and dorsolateral hinge point formation both occur in cytD-treated embryos revealing that bending in the spinal region is independent of actin microfilament polymerization (Ybot-Gonzalez & Copp, 1999; *Dev Dyn* 215, 273).

On the other hand, a small percentage of mice with knock-out mutations in genes encoding the F-actin-associated protein Shroom3, or Marcks-related protein (Mrp), have spina bifida in addition to cranial NTDs. This indicates that neural tube closure in the spinal region may be regulated by partially redundant mechanisms involving, for example, cell adhesion events that mediate neural plate bending and actomyosin cytoskeletal events that regulate apical constriction.

To further investigate the role of actomyosin cytoskeleton-dependent apical constriction in mouse spinal neural tube closure, we have performed whole embryo culture in the presence of inhibitors including cytD, blebbistatin and Y27632 (specific for Rho kinase). Embryos were explanted at varying times after Closure 1, and cultured either for 5 hours or overnight in the presence of inhibitor. Following culture, the progression of spinal neurulation was assessed by measuring the length of the posterior neuropore, and by performing morphological and immunohistochemical analysis of transverse sections through the closing neural tube.

These studies confirm that cytD does not prevent spinal neurulation, and use of blebbistatin gives a similar result. In contrast, inhibition of RhoA signalling disrupts the progression of spinal closure. Immunohistochemical analysis indicates that the requirement for RhoA signalling may operate via regulation of the actomyosin cytoskeleton and the apical junctional complex in neuroepithelial cells.

https://doi.org/10.1017/S0016672312000055 Published online by Cambridge University Press

Eya1 mice as models for understanding middle ear developmental defects

LEENA JOSHI and ABIGAIL S. TUCKER
Department of Craniofacial Development, King's College London, Guy's Hospital, Great Maze Pond, London, UK

The mammalian middle ear is composed of three bony ossicles, the malleus, incus and stapes which function to conduct sound from the external ear, via the tympanic membrane, to the inner ear, through the oval window. Normal development of these three ossicles and the formation of a tissue- and liquid-free middle ear space are integral for this transduction of sound, with defects resulting in conductive deafness.

Branchio-oto-renal syndrome is a disorder of craniofacial development which causes defects in both the middle and inner ear structures and accounts for 2% of profoundly deaf children worldwide. Sufferers of this syndrome possess mutations in the Eya1 gene which is one of several genetic factors influencing the development of this syndrome.

Eya1 mice possess several middle ear defects which result in conductive deafness, including abnormal ossicular structures, middle ear cavitation defects and middle ear infections (otitis media). In humans any of these anomalies alone can contribute to varying degrees of conductive deafness. These mice can therefore be used as tools to investigate normal middle ear development of the ossicles and cavity alongside the disease mechanisms involved in formation of otitis media and middle ear defects associated with syndromes of craniofacial development.

***Grainyhead-like 2* and development of neural tube defects**

CAROLINE S. HIRST, MADELEINE R. BROUNS, SANDRA C. P. DE CASTRO, ANDREW J. COPP and NICHOLAS D. E. GREENE
Neural Development Unit, University College London Institute of Child Health, London WC1N 1EH, UK

Axial defects (Axd) mice are one of the few mutant models that closely resemble the genetic and environmental etiology of isolated spina bifida in humans. The *Axd* critical region has been mapped by linkage analysis and harbours the *grainyhead-like 2* gene. Although no coding mutation has been identified, qPCR analysis revealed a significant up-regulation of *Grhl2* expression, by approximately 4-fold, at the stage of spinal neurulation. These data suggest that the genetic defect in *Axd* mice could comprise a regulatory gain-of-function of *Grhl2*. In order to test this hypothesis, a genetic cross was performed to reduce *Grhl2* function in *Axd* heterozygotes. A *Grhl2* loss-of-function mouse mutant ($Grhl2^{GT}$) was generated using a gene-trap approach. $Grhl2^{GT}$ homozygous mutant embryos exhibited severe exencephaly with 'split face' and partially penetrant spina bifida. Importantly, normalization of spinal neural tube closure was seen in compound heterozygous embryos, carrying both the null ($Grhl2^{GT}$) and over-expressing (*Axd*) alleles, in comparison with *Axd* heterozygous littermates.

Since either loss- or gain-of-function of *Grhl2* can give rise to neural tube defects, it suggests that regulation of *Grhl2* expression is a key requirement for neurulation. *Axd* and $Grhl2^{GT}$ models have been utilized to further examine the developmental pathogenesis and molecular mechanism underlying *Grhl* related neural tube defects.

The zinc finger protein Hic2 is required for cardiac development and is implicated in a novel post-transcriptional control mechanism

IAIN M. DYKES, KELLY LAMMERTS VAN BUEREN and PETER J. SCAMBLER
Institute of Child Health, University College London, 30 Guilford St, London WC1N 1EH, UK

HIC2 is located on human chromosome 22q11 and its loss is associated with DiGeorge syndrome-like phenotypes. It is expressed in the developing heart, retina, DRG and spinal cord. Mice heterozygous for Hic2 have an increased risk of perinatal lethality possibly due to cardiac ventricular defects, and exhibit a high incidence of cataract.

Hic2 is a zinc finger protein and thus a putative transcription factor, yet our data suggest it may have a role in post-transcriptional regulation. Hic2 co-localizes in aggregates with components of Processing Bodies, protein complexes that mediate the degradation or transient silencing of mRNAs marked by micro RNAs. We used RNA-Immunoprecipitation to identify a set of mRNAs bound by Hic2-containing complexes in the embryo. These mRNAs encode many developmentally important genes including some with known roles in heart and neural development as well as Hic2 itself. Hic2 protein can in addition inhibit canonical Wnt signalling most likely by sequestering TCF4/beta-catenin, an "off DNA" mechanism demonstrated for its paralogue Hic1. Our data suggest that the mRNA binding capacity of Hic2 is reduced on Wnt pathway activation. This raises the possibility that Hic2 might serve to integrate paracrine signals with the post-transcriptional regulation of gene expression during development.

3D expression domains of activator genes of the cell cycle processes, during early stages of organogenesis in chick embryos

GORDANA PAVLOVSKA[1,2], DRAGOSLAV KOCEVSKI[2], MATTHEW TOWERS[1], MONIQUE WELTEN[1] and CHERYLL TICKLE[1]
[1]*Biology and Biochemistry Department, University of Bath, Bath, BA2 7AY, UK;* [2]*Faculty of Agricultural Sciences and Food, University "St. Cyril and Methodius" – Skopje, Macedonia*

Rapid proliferation of cells (governed by activation of transcription factors), spatio-temporal organization of progenitor cells (governed by morphogenic factors), and sculpting of the tissues (induction of apoptosis in transient cells), are major contributors to the normal development of the embryo. By combining wholemount in-situ hybridization method, Optical Projection Tomography (OPT) and Amira© software for digital 3D reconstruction, we were able to visually analyse the spatio-temporal interaction of multiple activator genes of the cell cycle processes in whole embryos and in developing tissues and organs.

The eight members of the E2F family of transcription factors with their complex activation and mutual interaction determine the faith of the cells in which they are expressed. We analysed in 3D the expression domains of this important family of transcription factors in the axial tissues and the developing hearts, in conjunction with Myc, D cyclins and other cell cycle genes at developmental stages HH21 and HH24, using normal chick embryos as model system. By analysing the 3D spatio-temporal co-expression domains of these genes we identified potential interactions. This methodology is widely applicable to the analysis of multiple expression patterns in other embryos. Data generated in this study contribute towards the '3D Chick Atlas Project'.

Mutations in the dynein assembly factor PF22 (DNAAF3) cause primary ciliary dyskinesia with absent dynein arms

M. SCHMIDTS[1], N. T. LOGES[2], J. FRESHOUR[3], A. DRITSOULA[1], D. ANTHONY[1], R. A. HIRST[4], C. O'CALLAGHAN[4], H. BLAU[5], H. OLBRICH[2], T. YAGI[6], H. MUSSAFFI[5], E. M. K. CHUNG[7], H. OMRAN[2], D. R. MITCHELL[3] and H. M. MITCHISON[1]
[1]*Molecular Medicine Unit, University College London (UCL) Institute of Child Health, London, UK;* [2] *Department of Pediatrics and Adolescent Medicine, University Hospital Freiburg and University Hospital Muenster, Germany;* [3]*Cell and Developmental Biology, SUNY Upstate Medical Univ, Syracuse, NY 13210, USA;* [4]*Department of Infection Immunity and Inflammation, University of Leicester, Leicester Royal Infirmary, Leicester, UK;* [5]*Pulmonary Unit, Schneider Children's Medical Center of Israel, Petah-Tikva, Sackler School of Medicine, Tel-Aviv University, Israel;* [6]*Department of Cell Biology and Anatomy, Graduate School of Medicine, University of Tokyo, Tokyo, Japan;* [7]*General and Adolescent Paediatric Unit, University College London (UCL) Institute of Child Health, London, UK*

The genetic disorder primary ciliary dyskinesia (PCD) arises from dysmotility of cilia in the respiratory tract, brain ventricles, oviduct and the embryonic node. Patients have chronic obstructive pulmonary disease, reduced fertility and situs abnormalities. PCD is genetically heterogeneous with 12 genes causing ~40% of all cases, two encoding proteins (KTU, LRRC50) involved in cytosolic axonemal dynein coassembly. We have identified mutations in the *C19ORF51* gene located within a previously mapped PCD locus. *C19ORF51* encodes a protein orthologous to PF22, a *Chlamydomonas* protein involved in the cytoplasmic assembly of the outer dynein arms preceding their import into the axoneme. *pf22* mutant flagella display a disturbance in their cytoplasm of dynein heavy chain stability and the co-assembly of heavy with intermediate chains, both essential for dynein arm assembly. PF22 appears to act downstream of KTU and LRRC50 in the dynein preassembly pathway. *PF22* knockdown in zebrafish causes a loss of the dynein arms, cilia dysmotility, and a typical ciliopathy phenotype with axis curvature, pronephric cysts, hydrocephalus and situs inversus. We propose the existance of a conserved multi-step pathway for formation of assembly-competent dynein complexes, and that PF22 (now renamed DNAAF3, 'dynein axonemal assembly factor 3') mutations causes PCD with situs inversus due to deficient cytoplasmic dynein assembly.

https://doi.org/10.1017/S0016672312000055 Published online by Cambridge University Press

Inversus viscerum (iv) – a robust and viable mouse model of PCD

SALONI H. PATEL[1], DANIEL T. GRIMES[1], ELIZABETH C. ADAM[2], PATRICIA M. GOGGIN[2], MARTIN D. FRAY[1], JANE S. LUCAS[2], MICHAEL T. CHEESEMAN[1], PETER M. LACKIE[2] and DOMINIC P. NORRIS[1]

[1] *MRC Harwell, Harwell Science and Innovation Campus, Oxfordshire OX11 0RD, UK;* [2]*Primary Ciliary Dyskinesia Group, Division of Infection, Inflammation and Immunity, University of Southampton Faculty of Medicine, Southampton NIHR Respiratory Biomedical Research Unit, Southampton General Hospital, Southampton SO16 6YD, UK*

The inversus viscerum (iv) mouse is a long established model for situs inversus, resulting from a spontaneous mutation in the axonemal dynein heavy chain 11 gene (Dnahc11). This laterality phenotype is due to immotile cilia in the embryonic node. Mutations in the human homologue DNAH11 have been identified in patients with primary ciliary dyskinesia (PCD). Here we show that iv mutants exhibit immotile cilia in the trachea. Mutants demonstrate otitis media, gross rhinitis and sinusitis; these phenotypes are remarkably reminiscent of PCD pathologies. A common problem in modelling PCD is the reduced viability of mutant mice due to the presence of complex heart defects, hydrocephalus and male infertility. iv mice do show a reduction in sperm count, sperm motility and progressivity. However, analysis of early post implantation embryos reveals no significant change in embryonic numbers. The birth rate in our iv colony is reduced compared with wild-type, indicating incidence of mid-gestation embryonic loss. In spite of this, these data reveal the iv mouse to be a robust model of human PCD both genetically and pathologically.

https://doi.org/10.1017/S0016672312000055 Published online by Cambridge University Press