Genet. Res., Camb. (2011), **93**, *pp.* 433–440. © Cambridge University Press 2011
doi:10.1017/S0016672311000383

Abstracts of papers presented at the 21st Genetics Society's Mammalian Genetics and Development Workshop held at the Institute of Child Health, University College London on 11 November 2010

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*

Mechanisms underlying spina bifida in the Zic2 loss-of-function mutant, *Kumba*

SABA RAZA-KNIGHT, VALENTINA MASSA, DAWN SAVERY, NICHOLAS D. E. GREENE and ANDREW J. COPP
Neural Development Unit, UCL Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK

Spina bifida aperta is a common and disabling neural tube defect in humans; it occurs when the caudal progression of neural tube closure from the hindbrain/cervical boundary is interrupted. Spinal neural tube closure requires the formation of 'hinge points', which are localized regions of bending in the neuroepithelium. Dorsolateral hinge points (DLHPs) are essential for lumbosacral neural tube closure between the 16- and 30- somite stages: mice homozygous for the *Zic2* loss-of-function allele, *Kumba* (*Zic2*$^{Ku/Ku}$) lack DLHPs and have a fully penetrant spina bifida. Both Bmp and Shh signalling can directly inhibit DLHP formation (Ybot-Gonzalez *et al.*, 2007). Although *Zic2*$^{Ku/Ku}$ mutants have a notochordal defect and diminished *Shh* expression, downstream effectors such as *Ptc* and *Gli2* are unaltered. A change in neural tube patterning might also affect neuroepithelial bending, but the expression domains of the dorsal marker, Pax3, and ventral marker, Nkx6.1, are normal. However, the effector of Bmp receptor-mediated signalling, phospho-Smad 1/5/8, is upregulated in the *Zic2*$^{Ku/Ku}$ neuroepithelium. In parallel with our pathway analysis, we have also identified an orphan nuclear receptor, GCNF/Nr6a1, that is downregulated in the *Zic2*$^{Ku/Ku}$ neuroepithelium, and is linked to Bmp pathway activation (Lan *et al.*, 2003). Our data so far suggest that Bmp receptor-mediated signalling, but not Shh signalling, contributes to the *Zic2*$^{Ku/Ku}$ spina bifida.

Molecular characterization of *hitchhiker*, a mouse mutant exhibiting spina bifida

VICTORIA L. PATTERSON[1], ANJU PAUDYAL[1] and JENNIFER N. MURDOCH[2]
[1]*Mammalian Genetics Unit, MRC Harwell;* [2]*School of Biological Sciences, Royal Holloway University of London, UK*

Hitchhiker (*hhkr*), a mutant exhibiting spina bifida, exencephaly and polydactyly, was identified through an ENU G3 recessive screen at Harwell. The mutation was mapped to *Tubby like protein 3* (*Tulp3*), and is predicted to cause loss of all functional domains. A yeast 2-hybrid screen using Tulp3 as bait identified multiple potential protein interactions, most robustly an interaction with Tripartite motif protein 71 (Trim71), a stem cell specific E3 ubiquitin ligase involved in the control of cell fate in *C. elegans*. This is particularly interesting as examination of cellular processes in the hindbrain of *hhkr* mutant embryos revealed a significant increase in proliferation in this region.

We are confirming this potential interaction using an immunoprecipitation approach, the preliminary results of which suggest the interaction can be verified. Examination of mRNA distribution confirms that *Trim71* is expressed throughout neurulation in tissues relevant to the phenotypes of *hhkr*, and this expression is not altered in *hhkr* mutant embryos. We are investigating the dorsal-ventral patterning of the neural tube of *Trim71* mutant embryos, and are crossing *hhkr* to *Trim71* in order to examine any resultant genetic interaction, and refine the role of Tulp3 in promoting proper neural tube closure.

A cell-based screen for genes involved in mammalian cilia formation and function

EMMA HALL, PLEASANTINE MILL and IAN JACKSON
Institute of Genetics and Molecular Medicine, MRC Human Genetics Unit, Edinburgh, UK

Primary cilia are essential for mouse development, where they have been shown to be important for key signalling events. They are necessary for Hedgehog (Hh) signalling and mouse mutants for cilia genes show defects reminiscent of perturbed Hh responses. We are developing a cell-based RNAi screen to identify new genes involved in cilia formation and/or function. We are screening a subset of candidate genes, identified by cross-species analysis plus proteomic and transciptomic studies. The screen provides two readouts, one image-based readout identifies genes required for cilia formation, assayed by high-throughput immunofluorescence microscopy. The second functional readout measures Hh responsiveness using a luciferase reporter, which is altered when cilia are perturbed. Preliminary results will be presented. Identification of novel ciliogenic genes could aid the search for the diverse functions of primary cilia in development and perhaps help explain the varied phenotypes seen in human ciliary diseases, known as ciliopathies.

Examining the role of MAPK signalling in mouse gonad development

RACHEL BRIXEY, NICK WARR, ASHA DOPPLAPUDI, PAM SIGGERS, DEBORA BOGANI, MANOLIS PASPARAKIS and ANDY GREENFIELD
Sexual Development Group, Mammalian Genetics Unit, MRC Harwell, Harwell Science & Innovation Campus, Oxfordshire OX11 0RD, UK

Sexual development begins with the process by which the bipotential gonads of the embryonic urogenital ridge develop into either testes or ovaries. Sex determination occurs at around 11·5 dpc in the mouse and depends on the presence or absence of the Y chromosome and the associated activity of the testis-determining gene, *Sry*, in supporting cell precursors.

The boygirl (*byg*) mutant was identified at Harwell during an ENU-based screen. On the C57BL/6J background, XY *byg*/*byg* homozygotes exhibit embryonic gonadal sex reversal, associated with reduced and delayed expression of *Sry*. The defective gene in *byg*, *Map3k4*, is a component of the mitogen-activated protein kinase (MAPK) signalling pathway, however the other components of this MAPK pathway in sex determination have not yet been determined. I am currently focusing on the relationship between p38 MAPK, one of the MAPKs known to be activated downstream of MAP3K4, with the transcriptional regulation of *Sry* and sexual development more broadly. To do this I have utilized a conditional knockout approach to delete the *p38β* isoform using *Sf1-Cre* and *Amhr2-Cre*. To address the possibility that there could be functional redundancy amongst the four different p38 isoforms, I am working on siRNA-mediated knockdown approaches in organ culture models.

Assisting research into human embryonic and fetal development

D. GERRELLI[1], S. SUREN[1], V. MORRISON[1], Y. CHENG[2], L. OVERMAN[2], M. CROSIER[2], S. LISGO[2], S. LINDSAY[2] and A. J. COPP[1]
[1]*UCL, Institute of Child Health, London;* [2]*Institute of Human Genetics, Newcastle University, Newcastle, UK*

The Human Developmental Biology Resource (HDBR) is a unique resource funded by the MRC and Wellcome Trust. It provides human embryonic and fetal tissue for gene expression studies related to congenital disease, including both birth defects and inherited metabolic disorders. Use of the material should particularly illuminate developmental gene expression underlying aspects of functioning that characterize humans as opposed to lower animals (e.g. higher brain function, language). This research is essential if we are to introduce new methods for prevention of congenital defects and develop an improved understanding of "what makes us human".

The HDBR has ethics approval for the collection, storage and distribution of material between 4 and 12 weeks of gestation. The material can be used to generate cell lines, stem cells, protein, RNA and DNA. In addition, paraffin wax and frozen sections of embryos and early fetuses are available for *in situ* hybridization and immunohistochemistry. For users who do not have experience in gene expression analysis the HDBR offers an in-house gene expression service using *in situ* hybridization and/or immunohistochemistry.

Further details can be found at www.HDBR.org.

https://doi.org/10.1017/S0016672311000383 Published online by Cambridge University Press

Development of a conditional knock-out for XLαs; A G-protein-like signalling molecule encoded by the imprinted *Gnas* locus

STEFAN KRECHOWEC* and ANTONIUS PLAGGE*
**Cellular and Molecular Physiology, Institute of Translational Medicine, University of Liverpool, Liverpool L69 3BX, UK*

XLαs (eXtra Large αs) is an NH2-extended variant of the 'α-stimulatory' subunit of the trimeric G-protein, Gαs. *Gnasxl*, the transcript encoding XLαs, is derived through the use of an alternate promoter and first exon and shows strict maternal imprinting. At birth, knock-out mice display poor feeding, increased neonatal mortality and very limited adipose development, while adult survivors go on to develop a healthy exceptionally lean, hypermetabolic phenotype, showing increased sympathetic tone and weighing ~45% lighter with less than half the body fat of wild-type controls. To investigate the possible tissue-specific functions of XLαs a conditional knock-out is required. Since the complexity of the imprinted Gnas locus does not allow a standard approach to be used we designed a conditional gene trap strategy for the *Gnasxl* transcript. Tissue-specific Cre expression causes an inversion/activation of a silent gene-trap cassette, resulting in truncation of XLαs and the formation of a lacZ fusion marker protein. A neural-specific knock-out has been undertaken using the well established Nestin-Cre strain. Results from this cross have revealed both novel and unexpected insight into the tissue distribution and expression of XLαs.

Inheritance of a copy number variant in the imprinted *PHLDA2* gene promoter significantly increases fetal birth weight

MIHO ISHIDA[1], DAVID MONK[2], ANDREW DUNCAN[1], SAYEDA ABU-AMERO[1], JIEHAN CHONG[1], SUE RING[3], MARCUS PEMBREY[3], PETER HINDMARSH[1], JOHN WHITTAKER[4], PHILIP STANIER[2] and GUDRUN E. MOORE[1]
[1]*Clinical and Molecular Genetics Unit, Institute of Child Health, University College London, UK;* [2]*Imprinting and Cancer Group, Bellvitge Institute for Biomedical Research, L'Hospitalet de Llobregat, Spain;* [3]*Department of Community Based Medicine, University of Bristol, UK;* [4]*Non-communicable Disease Epidemiology Unit, London School of Hygiene and Tropical Medicine, University of London, UK;* [5]*Developmental Biology Unit, Institute of Child Health, University College London, UK*

Intrauterine growth restriction (IUGR) is the second leading cause of perinatal mortality and morbidity, after prematurity. Imprinted genes play an important role in placental and fetal growth in eutherian mammals. Our previous study showed a significant correlation between lower birth weight and increased *PHLDA2* expression in term placentae without loss of imprinting. To investigate the increased expression of *PHLDA2* in smaller birth weight babies, sequence analysis of the promoter region was performed in the Moore cohort (n = 285). A tandem 15 bp duplication was found in most individuals (86%), while a rarer, single copy number variant (CNV) in the remainder was found to reduce *PHLDA2* promoter efficiency *in vitro*. Meta-analysis of the polymorphism frequency and birth weight data from the Moore (n = 263), Rayne (n = 339) and ALSPAC (n = 9,327) cohorts showed that a maternally inherited CNV in the baby results in increased birth weight. Moreover, when the mother is homozygous for this polymorphism, the influence on the baby's birth weight is twice as high. This suggests a cumulative effect of the CNV through the mother's mother. To our knowledge, this is the first evidence of genetically inherited regulation of imprinted gene expression, which has a direct and significant effect on fetal birth weight.

https://doi.org/10.1017/S0016672311000383 Published online by Cambridge University Press

Elucidating the mechanism of imprinting of *Ddc*

ADAM PRICKETT, RUTH B. MCCOLE, WILL PUSZYK, REINER SCHULZ and REBECCA J. OAKEY
Department of Medical & Molecular Genetics, King's College London, 8th Floor Tower Wing, Guys Hospital, London SE1 9RT, UK

Dopa Decarboxylase (Ddc) is an enzyme that plays a fundamental role in the biosynthesis of catecholamine neurotransmitters and serotonin. A short form transcriptional variant of *Ddc* called *Ddc_exon1a*, which originates from an alternative promoter at exon 1a, is highly expressed in the trabecular cardiomyocytes during development of pre-natal heart and is progressively silenced during postnatal development. *Ddc_exon1a* has been shown to be epigenetically regulated via genomic imprinting in mouse heart in a tissue-specific and transcriptional variant-specific manner.

Ddc imprinting is controlled via a differentially methylated region (DMR) located in a CpG Island (CGI2) within the adjacent imprinted gene *Grb10*. A bioinformatic screen using ChIP-seq data from an ES-cell line has identified a CTCF binding site at CGI2 which we have confirmed *in vivo* using ChIP-qPCR. Furthermore, we have preliminary data which show that binding of CTCF at CGI2 is allele-specific in somatic tissues. It has been proposed that a CG rich region (CGI3) adjacent to CGI2 may control the different imprinting patterns of *Grb10* found in different tissues. We are using ChIP, bisulphite sequencing and bioinformatic analysis in order to assess the role of CGI2 and CGI3 in imprinting of *Ddc*.

Manual curation of mammalian proteins in UniProtKB

MICHAEL GARDNER and THE UNIPROT CONSORTIUM[1,2,3]
[1]*European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SD, UK;* [2]*Swiss Institute of Bioinformatics, Centre Medical Universitaire, 1 rue Michel Servet, 1211 Geneva 4, Switzerland;* [3]*Protein Information Resource, Georgetown University, 3900 Reservoir Road, Washington, DC 20057-1414, USA*

The UniProt Knowledgebase (UniProtKB) aims to provide the scientific community with a comprehensive and authoritative resource for protein sequence and functional information. Given the importance of mammalian model data in biomedical research, a major focus is the high-quality curation of human proteins and their orthologs in other mammals. Manual curation involves (1) the extraction of experimental results from scientific literature to enrich protein records with a wide range of functional information, (2) the manual verification of each sequence and clarification of any discrepancies, and (3) the assessment of the output of a range of analysis programmes to ensure that sequence features are correctly reported. The process also facilitates the standardization of experimental data – a step necessary for development of methods that enable the semi-automated transfer of annotation to uncharacterised or related proteins. UniProtKB/Swiss-Prot currently contains the complete manually reviewed human proteome, comprising approximately 20,300 canonical sequences and 14,700 isoforms, and an additional 45,000 reviewed entries from other mammals such as mouse, rat, apes, cow, sheep and dog. It offers a number of features that are specifically useful in the context of developmental research and is a valuable resource for those working in the field. Ongoing efforts continue to improve the quality of mammalian sequences in collaboration with HAVANA, Ensembl, HGNC and RefSeq, and to extend the coverage of curated proteins in mammalian species. All data are freely available from www.uniprot.org.

Epigenetic signatures at imprinted loci in the mouse

KIRSTEN R. MCEWEN and ANNE C. FERGUSON-SMITH
Department of Physiology, Development and Neuroscience, University of Cambridge, Downing Street, Cambridge CB2 3EG, UK

Genomic imprinting provides a useful paradigm to study epigenetic mechanisms. Imprinting refers to the expression of a gene from one chromosome homologue in a parental-origin-specific manner, which is dependent on epigenetic control by a *cis*-acting imprinting control region (ICR). Using high-throughput data extraction with subsequent analysis, we have found that particular histone modifications at imprinted genes are more likely to associate with either imprinting repression or more canonical 'developmental' repression (McEwen & Ferguson-Smith, 2010). Furthermore, we have identified a specific tri-mark signature comprising H3K4me3, H3K9me3 and H4K20me3 at all ICRs in mouse ESCs. We have further examined this epigenetic signature as a predictor of novel ICRs. Non-imprinted loci enriched with the tri-mark are identified, suggesting an intriguing function for this rare epigenetic profile. We have shown that publicly available whole genome data can be mined and analysed in order to generate novel findings for functional categories. Our results identify epigenetic profiles at imprinted loci that are likely to

associate with distinct mechanisms of gene repression. This form of analysis is therefore a useful tool to elucidate the complex epigenetic code associated with genome function and determine underlying features conferring epigenetic states.

McEwen, K. & Ferguson-Smith, A. (2010). Distinguishing epigenetic marks of developmental and imprinting regulation. *Epigenetics & Chromatin* 3:2.

The role of epialleles in growth disturbance syndromes

JENNIFER FROST[1,2], REINER SCHULZ[2], WILLIAM PUSZYK[2], SAYEDA ABU-AMERO[1], KHALID HUSSAIN[1], RAOUL HENNEKAM[1], REBECCA OAKEY[2] and GUDRUN E. MOORE[1]
[1]*Clinical and Molecular Genetics, The Institute of Child Health, University College London, UK;* [2]*Medical and Molecular Genetics, Guy's Hospital, King's College London, UK*

The role of epigenetics in human disease is not fully understood. Epialleles may act independently of DNA sequence to cause disease, known as pure epialleles. Alternatively, they may act to modify the effect of sequence variation such that disease manifests, known as facilitated epialleles. Or they may be obligatory epialleles, themselves determined by the DNA sequence. Delineating each type is vital for understanding disease pathogenesis.

We have collected a unique group of patients with diseases and syndromes of growth disturbance of unknown genetic cause, whose inheritance patterns and phenotypes point to the involvement of epialleles in their pathogenesis, i.e. asymmetric growth restriction or overgrowth, mosaicism and cases of discordant inheritance in monozygotic twins. Within our group we include patients with Silver Russell Syndrome, a potential genomic-imprinting related growth disorder, whose aetiology has been correlated with hypomethylated DNA epialleles on chromosome 11.

One of the most important and well studied epigenetic modifications is DNA methylation. To establish the role of DNA methylation in the disease phenotypes of our patients we carried out genome-wide methylome analyses, using methylated DNA immunoprecipitation followed by Next Generation Sequencing (MeDIP-seq). We present preliminary bioinformatic analyses of MeDIP-Seq data, showing candidate differentially methylated regions identified by comparing patients with healthy controls.

A new mutation in the imprinted *Gnas* cluster gives rise to a hyperactivity phenotype

SALLY EATON, SIMON BALL, CHARLOTTE TIBBIT, CHRISTINE WILLIAMSON and JO PETERS
MRC Harwell, Mammalian Genetics Unit, Harwell Science & Innovation Campus, Oxfordshire, OX11 0RD, UK

The imprinted *Gnas* cluster contains a complex group of paternally, maternally or biallelically expressed genes. Within this cluster are *Gnasxl*, which is exclusively paternally expressed and *Gnas*, which is maternally expressed in specific tissues. Both *Gnasxl* and *Gnas* determine the alpha subunit of the Gs protein. Deficiency of either *Gnasxl* or *Gnas* is associated with severe phenotypes shortly after birth.

We describe here a new mutation in the *Gnas* cluster designated *Caspa*. Upon maternal transmission of the mutated allele we observe a reduction in expression of *Gnas* in imprinted tissues, and global up-regulation of *Gnasxl* transcript levels. These mice present with a marked hyperactive phenotype that is observed from the day after birth. This phenotype is generally lethal; most *Caspa*/+ mice die before weaning. This lethality appears to be associated with a failure to gain weight. The *Caspa*/+ phenotype appears similar to that observed in mice with paternal uniparental partial disomy for distal chromosome 2 (PatDp.dist2). We hypothesize that the phenotype observed in the *Caspa*/+ mice is due to over-expression of *Gnasxl*. Rescue experiments in which the *Caspa*/+ mutation is crossed with either a mutation that corrects for *Gnas* expression or a mutation that corrects for *Gnasxl* expression supports this hypothesis.

Investigating genetic factors underlying hypopituitarism and septo-optic dysplasia in humans

SUJATHA A. JAYAKODY[1], CYNTHIA ANDONIADOU[1], MASSIMO SIGNORE[1], CARLES GASTON-MASSUET[1], LARYSA H. PEVNY[2], THIERRY BRUE[3] and JUAN-PEDRO MARTINEZ-BARBERA[1]
[1]*Neural Development Unit, Institute of Child Health, UCL, UK;* [2]*Department of Genetics and Neuroscience Centre, University of North Carolina, USA;* [3]*Service d'Endocrinologie, diabète et maladies métaboliques, Hôpital de la Timone, France*

Significant advances in the understanding of pituitary gland development in mouse have lead to the identification of a number of genes implicated in the pathology of hypopituitarism, with or without syndromic features such as Septo-Optic Dysplasia (SOD). To date, a total

of 15 mutations in the *paired*-liked homeodomain transcriptional repressor *Hesx1* have been documented. Here, we characterize three novel HESX1 mutations identified in patients with hypopituitarism. We demonstrate that mutations occurring within the homeodomain impede DNA binding of mutant protein however repressor activity is maintained.

Hypopituitarism has also been associated with mutations in human *Sox2*, a SOXB1-HMG box transcription factor. The molecular mechanisms responsible for this remain poorly understood. To study the role of *Sox2* in the pituitary, we utilized the Hesx1-cre knock-in mouse to genetically ablate *Sox2* from the developing pituitary. Our preliminary data demonstrate that these mutants display profound anterior pituitary hypoplasia in addition to a defect in the specification of the Pit1$^+$ lineage. Taken together, we highlight that it is essential to investigate factors required for normal development of the pituitary. This will ultimately lead to a better understanding of hypopituitarism and SOD in humans. This work is supported by a CHRAT studentship.

Comparing the incidence of mosaicism detected by FISH in murine blastocyst cultured *in vitro* and *in vivo*

AISHA ELAIMI[1], TANYA V. SABHNANI[2], HANAN SULTAN[1], ADEL ALDURAIHEM[1], KATAYOON GARDNER[3] and JOYCE C. HARPER[1]

[1]*University College London Centre for PG&D, Institute of Women's Health, UCL, 86-96 Chenies Mews, London, WC1E 6HX, UK;* [2]*Centre for Reproductive and Genetic Health, University College London Hospital, London;* [3]*Institute of Child Health, UCL, London, UK*

The majority of *in-vitro* derived human preimplantation embryos are chromosomally abnormal/mosaic but whether the same pattern exists in-vivo is unknown. This would be impossible to demonstrate in humans. Hence we chose murine embryos to study this difference owing to their ease of manipulation and compared the incidence of mosaicism between *in-vivo* and *in-vitro* cultured embryos. Fluorescent In situ hybridization (FISH) was developed using probe for chromosomes 2 and 11 on two groups of mouse embryos. In vitro group were obtained 48 hours following superovulation and mating of the female mice and cultured in vitro until the blastocyst stage. In vivo group were obtained by sacrificing female mice on day 5. FISH was performed on all blastocysts obtained. The same experiment was repeated in a different lab using exactly the same mouse species and the same protocol to validate the obtained results. The comparison between the incidence of mosaicism of the in vivo group and in vitro group indicated a significant difference with a p value $<0{\cdot}05$ (8% vs. 31%). These results were repeated in the second lab with a p value $<0{\cdot}05$ (12% vs. 46%). Our data show that murine embryos are an efficient model to study the effect of culture conditions on aneuploidy. Moreover, in vitro cultured embryos showed a significant increase in mosaicism in comparison to the in-vivo group.

Functional analysis of a prion disease modifier linked to incubation time

RABIA BEGUM[1], SARAH LLOYD[2] and JOHN COLLINGE[1,2]

[1]*MRC Prion Unit, UCL Institute of Neurology, London, UK;* [2]*Department of Neurodegenerative Diseases, UCL Institute of Neurology, London, UK*

Prion diseases are transmissible spongiform encephalopathies caused by the aberrant conformational conversion of PrP^c to PrP^{Sc}. These disorders are characterized by prolonged, clinically silent incubation periods that are influenced by a number of factors including host genetic background. The major genetic determinant of incubation time is variation within the prion gene, *Prnp*, however, quantitative trait analyses have identified other modifiers, including *HECTD2*, that contribute to this effect. *HECTD2* is thought to be an E3 ubiquitin ligase with no known substrates. To investigate the role of HECTD2 in prion disease it is important to confirm its effect on incubation time using mouse models and to define its function by identifying its substrates.

To assess the effect of HECTD2 on incubation time, a knockout mouse model is being characterized and challenged with a range of prion strains. To identify the substrates, the yeast-two-hybrid system was employed. Preliminary studies indicate that the screen identified a range of different interactors including those expressed in the central nervous system. This includes *Stmn2*, a gene previously implicated in vCJD susceptibility.

The characterization of HECTD2 will allow us to understand the molecular basis of incubation time with the ultimate goal of providing new therapeutic targets.

https://doi.org/10.1017/S0016672311000383 Published online by Cambridge University Press

Lamin B1, a modifier gene of neural tube defects in the *curly tail* mouse

SANDRA C. P. DE CASTRO[1], KIT-YI LEUNG[1], PETER GUSTAVSSON[2], DAWN SAVERY[1], ANDREW J. COPP[1] and NICHOLAS D. E. GREENE[1]
[1]*Neural Development Unit, Institute of Child Health, University College London, Guilford Street, London, WC1N 1EH, UK;* [2]*Karolinska University Hospital, Stockholm, Sweden*

Curly tail (*ct*) is the best characterized mouse model of spinal NTDs, which closely resemble the corresponding birth defects in humans, with multifactorial etiology influenced by environmental and genetic factors. Spina bifida in *ct*/*ct* embryos results from reduced proliferation in the hindgut endoderm, which causes excessive curvature of the caudal region of the embryo, and inhibits closure of the posterior neuropore. The *ct* mutation is a hypomorphic allele of *Grainyhead-like-3* (*Grhl3*) with reduced expression due to a putative regulatory mutation. This is confirmed by the *Grhl3*-transgenic *curly tail* strain which reinstatement of *Grhl3* levels results in normal closure of the posterior neuropore. A two-dimensional protein electrophoresis analysis was used to compare *ct*/*ct* and matched wild-type embryos at the time of posterior neuropore closure in order to investigate the pathogenesis of NTDs in the *ct* strain. Migration differences have been found to result from a genomic polymorphism leading to a variation in lamin B1 protein sequence. *In vitro* analysis were suggestive lamin B1 variant could affect the dynamics of the nuclear lamina. Analysis of sub-strains of mice carrying different combinations of the *Lmnb1* polymorphism and the *Grhl3* mutation suggest that *Lmnb1* could potentially act as a modifier of NTD risk in *curly tail* mice. This work is supported by the MRC.

Investigating molecular interactions between Tulp3 and Rgnef

ANJU PAUDYAL[1], VICTORIA L. PATTERSON[1] and JENNIFER N. MURDOCH[2]
[1]*Mammalian Genetics Unit, MRC Harwell;* [2]*School of Biological Sciences, Royal Holloway University of London, UK*

Carrying a mutation in *Tubby like protein 3* (*Tulp3*) gene, *Hitchhiker* (*hhkr*) displays multiple developmental defects including spina bifida, exencephaly and polydactyly. We and others have recently reported that Tulp3 functions as a negative regulator of the Sonic hedgehog signalling.

To investigate the molecular function of Tulp3 by identifying its interacting partners, we carried out a yeast 2-hybrid screen using Tulp3 as bait. This screen identified 11 potential protein interactions, all of which were expressed at the developmental stage that is important for neural tube closure. Rho-guanine nucleotide exchange factor (Rgnef, also called p190RhoGEF), is one of the potential interacting partners of Tulp3 identified in this screen.

Analysing the expression domain of *Rgnef* mRNA, revealed that it is an excellent candidate gene for regulating Shh signalling with *Tulp3* during patterning of the neural tube. It is most highly expressed in cells where a high level of Shh is present i.e. the floor plate. This expression domain of *Rgnef* is not affected in *hhkr*. To confirm this potential interaction we performed co-immunoprecipitation study and our preliminary results suggest that Tulp3 and Rgnef may form a protein complex.

Haploinsufficiency for *Map3k4* and *T*-associated sex reversal (*Tas*)

NICK WARR, DEBORA BOGANI, PAM SIGGERS, RACHEL BRIXEY, ASHA DOPPLAPUDI, LYDIA TEBOUL, SARA WELLS, MICHAEL CHEESEMAN & ANDY GREENFIELD
Mammalian Genetics Unit & Mary Lyon Centre, MRC Harwell, Harwell Science & Innovation Campus, Oxfordshire OX11 0RD, UK

T-associated sex reversal (*Tas*) describes the formation of ovotestes or ovaries in XY mice harbouring the hairpin tail deletion (T^{hp}) when on the C57BL/6J-Y^{AKR} genetic background. One explanation of this phenomenon invokes haploinsufficiency of an autosomal testis-determining gene in the T^{hp} deletion that is postulated to reduce expression of the Y-linked testis-determining gene, *Sry*. *Tas* mimics certain human conditions, in which loss of one allele of a sex-determining gene is sufficient to cause XY sex reversal. We recently reported that mice heterozygous for a null allele of *Map3k4* on C57BL/6J-Y^{AKR} exhibit a *Tas*-like phenotype: XY ovotestis development, testicular hypoplasia and occasional gonadal sex reversal. Haploinsufficiency of *Map3k4* is therefore sufficient to phenocopy $T^{hp}/+$ XY sex reversal. However, these experiments did not exclude the possibility that loss of other loci in the deletion or indeed that the deletion itself might also contribute to sex reversal, i.e. they did not demonstrate that *Map3k4* haploinsufficiency is necessary for *Tas*. Here, we report the generation of a novel mouse line carrying a functional *Map3k4* bacterial artificial chromosome (BAC) transgene. We are determining whether

this transgene rescues abnormalities of C57BL/6J-Y^{AKR} $T^{hp}/+$ gonad development which would suggest that only *Map3k4* haploinsufficiency can cause *T*-associated sex reversal.

Mouse phenotyping using high resolution 3D microscopic magnetic resonance imaging

FRANCESCA C. NORRIS[1,2], JON O. CLEARY[1,3], MARC MODAT[4], BENJAMIN SINCLAIR[1,3], KAREN MCCUE[5], JACK A. WELLS[1], JUAN PEDRO MARTINEZ-BARBERA[6], SEBASTIEN BRANDER[7], ELIZABETH FISHER[7], PETER J. SCAMBLER[5], SEBASTIEN OURSELIN[3,8] and MARK F. LYTHGOE[1]

[1]*Centre for Advanced Biomedical Imaging, Department of Medicine and UCL Institute of Child Health, University College London, UK;* [2]*Centre for Mathematics and Physics in the Life Sciences and EXperimental Biology (CoMPLEX), University College London, UK;* [3]*Centre for Medical Image Computing, Departments of Medical Physics and Bioengineering and Computer Science, University College London, UK;* [4]*Department of Medical Physics and Bioengineering, University College London, UK;* [5]*Molecular Medicine Unit, UCL Institute of Child Health, University College London, UK;* [6]*Neural Development Unit, UCL Institute of Child Health, University College London, UK;* [7]*Institute of Neurology, University College London, UK;* [8]*Dementia Research Centre, National Hospital for Neurology and Neurosurgery, London, UK*

Effective methods for high-throughput screening and analysis are crucial for phenotyping the increasing number of mouse mutants that are being generated to investigate the role of genes in human diseases and development. Magnetic resonance imaging is an ideal phenotyping platform due to its inherent non-invasive and three-dimensional nature. We can image up to 40 embryos at E15.5 in a single overnight scan, generating an MRI dataset of all the embryos, which may be viewed on slice-by-slice basis. Furthermore, we have developed automated techniques for morphometric assessment of these large MRI datasets. *Segmentation propagation* enables rapid and non-invasive calculation of tissue volumes in a population using an average, composite atlas. *Tensor-based morphometry* is a fully automated technique that enables unsupervised and unbiased detection of local, volumetric changes in a population on a voxel-wise basis, which are not visible to the human eye. In addition, we are investigating active staining techniques for MR histology, which may allow specific cellular structures to be targeted using MRI, and developing diffusion tensor imaging techniques in the mouse embryo to investigate local microstructure, which may not be visible using histological and standard MRI approaches. This work is supported by the ESPRC and BHF.