Genet. Res., Camb. (2017), vol. 99, e5. © Cambridge University Press 2017
doi:10.1017/S0016672317000039

Abstracts of papers presented at the 27th Genetics Society's Mammalian Genetics and Development Workshop held at the UCL Great Ormond Street Institute of Child Health, University College London on 18th November 2016

Edited by: NICHOLAS D. E. GREENE[1], ANDREW J. COPP[1] AND CYNTHIA L. ANDONIADOU[2]
[1]*UCL Great Ormond Street Institute of Child Health, 30 Guilford Street, London, WC1N 1EH*
[2]*KCL Craniofacial Development and Stem Cell Biology, Floor 27 Tower Wing, Guy's Campus SE1 9RT*

The role of *Hesx1* in mouse embryonic stem cells and pre-implantation development

SARA POZZI AND JUAN PEDRO MARTINEZ-BARBERA
UCL Great Ormond Street Institute of Child Health, Developmental Biology and Cancer Programme

Hesx1 is a homeobox transcriptional repressor well known to be involved in the correct specification and development of the forebrain and pituitary gland. Moreover, inactivating mutation in this gene have been previously linked with Septo Optic Dysplasia (SOD), a congenital condition causative of hypopituitarism and optic-nerve hypoplasia. Although *Hesx1* has been isolated for the first time from embryonic stem cells (ESC) almost two decades ago, its role in stemness is still partially unknown. In this study I aim to elucidate the role of *Hesx1* in the maintenance of ESC fate. Specifically, I show how the core pluripotency network composed by OCT4, SOX2 and NANOG directly interact with *Hesx1,* and how *Hesx1* is regulated by extracellular signalling previously descried as pivotal for the maintenance of ESC self-renewal and pluripotency. Molecular studies here presented show that *Hesx1* is a target of the JAK/STAT signalling and that its lack of expression influences the behaviour of ESC and their ability to maintain a pluripotent state. Importantly, I demonstrate how *Hesx1* is expressed in the pre implantation embryo, and I discuss a possible conserved role for this transcription factor *in vivo.*

Nanog, pluripotency and the germline revisited

HARRY G. LEITCH[1,2,3], MAN ZHANG[1,4], WALFRED W.C. TANG[6], NICOLA FESTUCCIA[4], JENNIFER NICHOLS[5], M. AZIM SURANI[6], AUSTIN SMITH[5] AND IAN CHAMBERS[4]
[1]*Joint first authors*
[2]*MRC Clinical Sciences Centre, London, England*
[3]*Institute of Clinical Sciences, Faculty of Medicine, Imperial College London, London, England*
[4]*MRC Centre for Regenerative Medicine, Institute for Stem Cell Research, School of Biological Sciences, University of University of Edinburgh, Scotland*
[5]*Wellcome Trust-Medical Research Centre Stem Cell Institute, University of Cambridge*
[6]*Gurdon Institute, University of Cambridge.*

Primordial germ cells (PGCs) are the embryonic precursors of the gametes. As they only give rise to either sperm or eggs, depending on the sex of the embryo, they are traditionally considered a unipotent lineage. However, upon fertilization they form the totipotent zygote, a single cell with the potential to generate an entire organism. PGCs are the cells of origin of testicular teratocarcinoma and can acquire pluripotency spontaneously when grafted to ectopic sites. Furthermore when placed in culture, in the presence of certain cytokines, they can efficiently undergo conversion to naive pluripotent stem cells called embryonic germ (EG) cells. Thus, we have recently hypothesized that PGCs exhibit a latent form of pluripotency that persists to a lesser or greater degree throughout germline development (Leitch and Smith, 2013). Indeed, during their development PGCs express the entire network of transcription factors known to function in maintaining pluripotency in embryonic stem (ES) cells. In particular, the pluripotency gene Nanog is highly expressed in PGCs and has been implicated as an essential factor in germline development (Chambers et al., 2007; Yamaguchi et al., 2009). However, a recent study presented some evidence that Nanog null iPS cells may be capable of germline transmission (Carter et al., 2014). Here we formally assess the requirement for Nanog in PGC development using both knockout ES cells and conditional knockout approaches. Furthermore,

we perform genetic rescue experiments using an *in vivo* knock-in approach, and an established *in vitro* model for PGC induction. These unpublished findings have implications for current models of germline development and, in particular, the role played by pluripotency factors.

WDR11-mediated Hedgehog signaling links congenital hypogonadotropic hypogonadism, Kallmann syndrome and ciliopathy

YEON JOO KIM[1], JI YOUNG LEE[1], MASATAKE ARAKI[2], KIMI ARAKI[2], TIMOTHY MOHUN[3], PARIS ATALIOTIS[1], NIGEL BROWN[1], HYUNG-GOO KIM[4], JUAN PEDRO MARTINEZ-BARBERA[5], DANIEL OSBORN[1], LAWRENCE LAYMAN[4] AND SOO-HYUN KIM[1]
[1]*St. George's Medical School, University of London, London, UK*
[2]*Institute of Resource Development and Analysis, Kumamoto University, Japan*
[3]*Francis Crick Institute Mill Hill Laboratory, London, UK*
[4]*Medical College of Georgia, Augusta University, Augusta, USA*
[5]*Birth Defects Research Centre, UCL Great Ormond Street Institute of Child Health, London, UK*

The Hedgehog (Hh) signaling pathway plays a fundamental role in normal development and homeostasis. The primary cilium, a microtubule-based organelle present in most vertebrate cells, is an organizing center for extracellular signals including Hh. *WDR11* is known to be mutated in congenital hypogonadotropic hypogonadism (CHH) and Kallmann syndrome (KS). However, the biological activities of WDR11 are poorly understood. Here we report that WDR11 is a downstream component of the Hh signaling pathway and required for normal ciliogenesis. Disruption of *WDR11* expression in mouse and zebrafish models results in dysgenesis of multiple organs that are affected in CHH/KS and other human ciliopathies, as well as mid-line disorders such as holoprosencephaly, indicating a potential genetic overlap among these disorders. Using mouse and human cell lines, we demonstrate that WDR11 translocates from the nucleus to the cytosol and cilia in response to Hh signaling, and modulates processing of the Hh effector protein GLI3. Loss of WDR11 results in nuclear accumulation of GLI3 repressor, accompanied by attenuation of Hh target gene response. Our study reveals a novel disease mechanism mediated by WDR11 and the Hh signaling pathway, suggesting that CHH/KS could be a part of the spectrum of human ciliopathies.

The importance of the SHH pathway in hypothalamic-pituitary development

GABRIELA CARRENO AND JUAN PEDRO MARTINEZ-BARBERA
UCL Great Ormond Street Institute of Child Health, Developmental Biology and Cancer Programme

Mouse studies have demonstrated the necessity of sonic hedgehog (SHH) for normal proliferation of Rathke's pouch (RP) precursors. However, the possible function of SHH in pituitary cell specification remains to be assessed. In this study we aim to determine the function of SHH during normal pituitary development, specifically, we aim to test whether SHH is relevant for cell specification during pituitary development and before its effect on RP progenitor proliferation. We firstly show that conditional over activation of the SHH pathway in the *Hesx1*-cell lineage leads to over-proliferation of the embryonic pituitary but normal pituitary specification and differentiation. Secondly, we show that deletion of *Shh* in the *Hesx1*-cell lineage arrests RP development with complete loss of pituitary tissue by E12·5. Molecular analyses indicate that *Shh* is not only required for proliferation, but also, for normal specification of RP precursors. Without hypothalamic SHH signalling, the critical RP progenitor markers *Lhx3* and *Lhx4* are not expressed, leading to a possible change in cell fate into the surrounding oral ectoderm.

Cranial neural crest cells are the major driver underlying the facial phenotypes in orofacial digital syndrome 1

HADEEL ADEL A-LAMI, WILLIAM BARRELL AND KAREN LIU
Craniofacial Development and Stem Cell Biology, King's College London, UK

Ciliopathy patients frequently have characteristic facial features such as cleft lip, wide nose with a broad, flat nasal bridge and hypertelorism, or widely spaced eyes. It has been difficult to pinpoint the underlying cellular causes of these phenotypes. Using a genetic model in mouse, we found that mutation of the causative gene, *Ofd1*, could lead to facial widening and micrognathia. We determine the critical interactions by using tissue-specific drivers to delete *Ofd1* in mice. We find that *Ofd1* is required in the neural crest cell lineage, which contributes to the majority of the facial skeleton. Loss of *Ofd1* in this lineage leads to prolonged and aberrant migration of the neural crest cells toward the facial prominences. The consequence of this increased population of neural crest cells is the loss of the mesodermally derived

https://doi.org/10.1017/S0016672317000039 Published online by Cambridge University Press

muscle lineages and premature condensation of the skeletal precursors. Our studies highlight a key role for cilia in the neural crest lineage and uncover the steps leading to facial changes in ciliopathy patients.

High Throughput Imaging and Phenotyping of Homozygous Lethal Mouse Lines at MRC Harwell

JAMES CLEAK, SARA JOHNSON, ZSOMBOR SZOKE-KOVACS, MRC HUSBANDRY TEAM, NEIL HORNER, JAMES BROWN, HENRIK WESTERBERG AND LYDIA TEBOUL
Mary Lyon Centre, MRC Harwell Institute, Harwell Science and Innovation Campus, Oxfordshire, OX11 0RD

The twenty institution International Mouse Phenotyping Consortium has come to the end of their five-year project to generate and characterise 5000 conditional knockout mouse lines. They have made freely available to the research community a broad phenotypic characterisation and expression profile of each targeted locus. MRC Harwell has been responsible for the production, distribution and analysis of over 500 of these lines.

We will describe the pipeline of work focusing on gene expression at E12·5, identification of the window of lethality and the acquisition of morphological data by optical projection tomography (OPT) and micro computed tomography (microCT) of embryonic lethal lines. We will present some of these lines along with the software used to reconstruct and analyse this data.

With the successful re-funding of the consortium for a further five years and 7000 new knockout mouse lines to be generated and characterised we touch on the challenges ahead and the role that MRC Harwell will take.

Role of sonic hedgehog during early development of the lingual epithelium

ANAHID A. BIRJANDI, KAREN J. LIU AND MARTYN T. COBOURNE
Craniofacial development and Stem cell biology, Kings College London

The mammalian tongue dorsum is characterized by multiple rows of fungiform papillae, which contain differentiated taste buds and contribute to taste sensation. Sonic hedgehog (Shh) is a secreted signaling molecule, which progressively localizes to the fungiform papillae during specification and subsequent morphogenesis of these repetitive structures. It has been demonstrated using inhibitor experiments in explant culture that Shh has multiple roles during the development of fungiform papillae such as regulating local induction and patterning of these structures, whilst constitutive activation in transgenic mice alters lingual epithelial cell fate. We have investigated the role of Shh signaling during tongue development using tamoxifen-inducible Cre-mediated ablation immediately following the initiation of tongue development and prior to specification of the papillary placodes. We find that early loss of *Shh* results in the formation of ectopic epithelial thickenings in the anterior tongue that initially resemble disorganized placodes. These thickenings later develop into bulb–like structures with disrupted morphology, innervation and delayed growth. Our results demonstrate a role for Shh in cell fate determination in undifferentiated tongue epithelium. Specifically, Shh has a dual role both before and after formation of the fungiform papilla. Interestingly, the ectopic bulb-like structures that appear in the anterior tongue in the absence of Shh signaling resemble tongue nodules. These nodules are often found on the tongue dorsum of patients affected by ciliopathies, which suggests a potential mechanism underlying these defects associated with disrupted Hedgehog signaling.

Deleterious growth restricting effects of human SAMD9 mutations are rescued by dynamic genomic changes

FEDERICA BUONOCORE[1], PETER KÜHNEN[2], JENIFER P. SUNTHARALINGHAM[1], IGNACIO DEL VALLE[1], MARTIN DIGWEED[3], HARALD STACHELSCHEID[4], NOUSHAFARIN KHAJAVI[2], MOHAMMED DIDI[5], ANGELA F. BRADY[6], OLIVER BLANKENSTEIN[2], ANNIE M. PROCTER[7], PAUL DIMITRI[8], JERRY K. H. WALES[9], PAOLO GHIRRI[10], DIETER KNÖBL[11], BRIGITTE STRAHM[12], MIRIAM ERLACHER[12], WEI CHEN[13], GLENN ANDERSON[14], DEBORAH MORROGH[15], DALE A. MOULDING[16], SHANE A. MCKEE[17], CHARLOTTE M. NIEMEYER[12], ANNETTE GRÜTERS[2] AND JOHN C ACHERMANN[1]
[1]*Genetics and Genomic Medicine, UCL Great Ormond Street Institute for Child Health, London, UK*
[2]*Institute for Experimental Pediatric Endocrinology and Department for Pediatric Endocrinology, Charité, Berlin, Germany*
[3]*Department for Human and Medical Genetics, Charité, Berlin, Germany*
[4]*Berlin Institute of Health, Berlin, Germany*
[5]*Department of Paediatric Endocrinology, Alder Hey Children's NHS Foundation Trust, Liverpool, United Kingdom*

https://doi.org/10.1017/S0016672317000039 Published online by Cambridge University Press

[6]North West Thames Regional Genetics Service, Northwick Park Hospital, Harrow, United Kingdom
[7]Institute of Medical Genetics, University Hospital of Wales, Cardiff, United Kingdom
[8]Academic Unit of Child Health, Sheffield Children's Hospital, Sheffield, United Kingdom
[9]Department of Paediatric Endocrinology and Diabetes, Lady Cilento Children's Hospital, South Brisbane, Australia
[10]Department of Neonatology, University of Pisa, Pisa, Italy
[11]Pediatric Endocrinology, Karlsruhe, Germany
[12]Department for Pediatrics and Adolescent Medicine, Division of Pediatric Hematology and Oncology, Medical Center University of Freiburg, Faculty of Medicine - University of Freiburg, Germany, and German Cancer Consortium (DKTK), and German Research Center (DKFZ), Heidelberg, Germany
[13]Max Delbrück Center for Molecular Medicine, Berlin
[14]Histopathology Department, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom
[15]NE Thames Regional Genetics Laboratory Service, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom
[16]Developmental Biology & Cancer, UCL Great Ormond Street Institute for Child Health, London, UK
[17]Department of Genetic Medicine, Belfast City Hospital, Belfast, UK

Intrauterine growth restriction (IUGR) is a common condition that is sometimes associated with additional features, and in some cases endocrine dysfunction. We investigated eight children who had IUGR together with a complex multisystem disorder involving gonadal, adrenal and bone marrow failure. Several children died in the first 2 years of life. Using NGS and targeted capture, heterozygous *de novo* missense mutations in *SAMD9* (chr 7q21·2) were identified in all eight children. SAMD9 was shown to be a growth repressor and these mutations result in gain-of-function, leading to reduced cell proliferation. Furthermore, progressive loss of the mutated gene through the development of monosomy 7 (-7), deletions of 7q (7q-) and secondary somatic loss-of-function (nonsense and frameshift) mutations in *SAMD9* rescued the growth restricting effects of the mutant proteins in bone marrow and was associated with increased length of survival. However, two patients with -7 /-7q developed myelodysplastic syndrome, most likely due to haploinsufficiency of related 7q21.1 genes. These findings provide strong evidence that dynamic somatic changes can occur in specific tissues. These changes can modify disease phenotype and influence survival. Tissue-specific adaptability such as this may be an under-recognised mechanism modifying the phenotype of human genetic.

A new model of *Zac1* loss-of-imprinting to investigate the pathophysiology of 6q42 TNDM

SALAH AZZI[1], FLORENCE NAILLAT[1], ANNE SEGONDS-PICHON[2], HEBA SAADEH[1,2], SIMON ANDREWS[2], SEBASTIAN SMALLWOOD[1] AND GAVIN KELSEY[1,3]
[1]Epigenetics Programme, Babraham Institute, Cambridge CB22 3AT, United Kingdom
[2]Bioinformatics Group, Babraham Institute, Cambridge CB22 3AT, United Kingdom
[3]Centre for Trophoblast Research, University of Cambridge CB2 3EG, Cambridge, United Kingdom

ZAC1/PLAGL1 is an imprinted gene whose loss-of-imprinting (LoI) causes 6q24 Transient Neonatal Diabetes Mellitus (TNDM). In order to gain better insights into the pathophysiology of TNDM, we generated a molecularly faithful model in mice ($Zac1^{LOM}$), in which LoI and biallelic expression of *Zac1* is caused by failure to establish a methylation imprint on the maternal allele of the *Zac1* promoter during oogenesis. At birth, $Zac1^{LOM}$ mice have increased weight, which resolves by weaning, and reduced serum insulin, despite normal blood glucose, and become glucose intolerant with age. In addition, $Zac1^{LOM}$ mice presented with cartilage abnormalities and macroglossia. Histological analysis revealed muscle hyperplasia in $Zac1^{LOM}$ tongues and RNA-seq showed deregulation of genes, such as *Scube3*, *Slc7a10* and *Pax3*, involved in muscle development. *Zac1*, which encodes a transcriptional co-regulator, is proposed to belong to an imprinted gene network (IGN) in which it has been characterised as a hub. However, analysis of the IGN in RNA-seq datasets from multiple tissues showed that biallelic expression of *Zac1* did not markedly alter expression of the IGN. Moreover, we found that imprinted genes are dynamically regulated during tongue development, indicating wide-ranging roles of imprinted genes in the development of this organ. In conclusion, the mouse $Zac1^{LOM}$ model will enable detailed molecular analysis of as yet poorly understood clinical phenotypes in 6q24 TNDM, such as macroglossia.

The 3D organisation of the Dlk1/Gtl2 imprinted domain in mammalian development

CAROL A. EDWARDS, DIONNE J. GRAY AND ANNE C. FERGUSON-SMITH
The Department of Genetics, University of Cambridge, Downing Street, Cambridge CB2 3EH

The Dlk1/Gtl2 domain is imprinted in eutherian mammals with the paternally inherited chromosome

https://doi.org/10.1017/S0016672317000039 Published online by Cambridge University Press

expressing the protein coding genes *Dlk1*, *Rtl1* and *Dio3* and the maternally inherited chromosome expressing the long non-coding RNA gene *Gtl2* and clusters of miRNAs and snoRNAs. Imprinted gene expression in the domain is controlled by an intergenic differentially methylated domain (IG-DMR), however, the mechanism by which it does this has yet to be elucidated. The chromosome conformation capture based technique 4Cseq allows you to interrogate all genomic interactions that occur between a viewpoint and the rest of the genome. We have used allele specific 4Cseq to investigate the 3D organisation of the Dlk1/Gtl2 region in mouse development. Data will be presented for allele and tissue specific topology within this imprinted domain.

Identification, characterization and inheritance of mammalian metastable epialleles

TESSA M. BERTOZZI[1], ANASTASIYA KAZACHENKA[1], MARCELA K. SJÖBERG[2], NIC WALKER[1], DAVID J. ADAMS[2] AND ANNE C. FERGUSON-SMITH[1]
[1]*University of Cambridge, Cambridge, United Kingdom*
[2]*Wellcome Trust Sanger Institute, Cambridge, United Kingdom*

Endogenous retroviruses (ERVs) are non-coding repetitive elements representing 10% of the murine genome. Active ERVs are capable of random genomic insertions via retrotransposition, potentially leading to damaging mutagenesis. They are therefore silenced via heavy DNA methylation. Metastable epialleles exhibit variable DNA methylation between genetically identical individuals and the few that have been robustly identified to date are all ERVs. They include the A^{vy} locus, where an ERV of the intracisternal A-particle (IAP) class was inserted spontaneously upstream of the agouti coat colour gene. The insertion is associated with variable DNA methylation at the IAP-LTR promoter, variable expressivity of coat colour phenotype, and transgenerational epigenetic inheritance.

We conducted a genome-wide screen identifying novel ERVs that possess features of metastable epialleles and find that most are IAPs. Less than 1% of IAPs are epigenetically metastable, and several show an inverse correlation between their LTR promoter methylation and expression of nearby genes. We studied the heritability of these loci and found that the vast majority exhibits metastability in the next generation, with two exhibiting parental origin effects. Germline methylation levels indicate that the variable methylation states observed in somatic tissue undergo reprogramming, suggesting a mechanism that reconstructs the methylation metastability transgenerationally.

Genetic insights on provisional extracellular matrix remodeling in birth defects

SUNEEL APTE
Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio, USA

Extracellular matrix (ECM) of the developing embryo increases in complexity and changes its character throughout gestation. Beginning with adhesive glycoproteins and basement membranes, which represent the needs of early cell aggregates, sheets and tubes, the growing embryo next acquires an interstitial ECM that grows in volume. This is a highly hydrated, malleable, carbohydrate-rich provisional ECM distinct from the mechanically robust collagen-rich interstitial ECM of adult tissues. The components of this ECM, such as hyaluronan and the proteoglycan versican, as well as the proteases that remodel it-the ADAMTS proteases- are crucial for development. Several ADAMTS proteases are specifically required for versican remodeling during morphogenesis as evidenced by diverse developmental anomalies seen in ADAMTS mutant mice. These include defects in craniofacial and neural development, eye, heart, vascular, limb and skeletal development. In my presentation, I will discuss how versican proteolysis is central to the process of converting provisional ECM to mature interstitial ECM and synchronized with concurrent cellular events.

Biomechanical coupling of the closing spinal neural tube facilitates neural fold apposition

GABRIEL L GALEA[1], YOUNG-JUN CHO[1], GAUDEN GALEA[2], MATTEO A. MOLÉ[1], ANA ROLO[1], DAWN SAVERY[1], DALE MOULDING[1], EVANTHIA NIKOLOPOULOU[1], NICHOLAS D. E. GREENE[1] AND ANDREW J. COPP[1]
[1]*Developmental Biology of Birth Defects, UCL Great Ormond Street Institute of Child Health, UCL, London WC1N 1EH, UK*
[2]*WHO Regional Office for Europe, UN City, Marmorvej 51 DK-2100, Copenhagen Ø, Denmark*

Mammalian neural tube closure initiates at multiple 'closure sites', where the neural folds first become apposed. A single closure site is believed to initiate spinal closure, extending through 'zippering' in a rostral-to-caudal direction. We observed that laser ablation of the recently-fused zippering point (ZP) causes rapid re-widening of the open neuropore, suggesting the ZP withstands tension. ZP tension, inferred from the re-widening magnitude following

https://doi.org/10.1017/S0016672317000039 Published online by Cambridge University Press

ZP ablation, increases with advancing somite stage until ~25 somites, after which it decreases. Concomitantly, neuropore structure changes from being spade-like to elliptical, with cellular zippering protrusions forming at the caudal as well as rostral canthus. Ablating either canthus causes neuropore re-widening, suggesting both facilitate neural fold apposition. To identify force-generating neurulation 'motor(s)', we produced strain maps from relative cell displacements following rostral ZP ablation before (15–20som) or after (25–30som) caudal ZP formation. In each case the distal neuropore, where neuromesodermal progenitors (NMP) reside, underwent constriction, indicating it is biomechanically coupled to the rostral ZP. Apical constriction of NMP region cells, drawing the neural folds medially, was confirmed in live-imaged embryos. Thus, NMP zone constriction narrows the midline gap across which zippering proceeds in rostral-to-caudal as well as, when completion of closure is imminent, caudal-to-rostral directions.

Vertical Telescoping bends the mammalian salivary gland epithelium

JINGJING LI, ANDREW ECONOMOU AND JEREMY GREEN
King's College London, London, UK

Ectodermal organs, such as teeth, salivary gland, mammary gland, and hair follicles, share similar morphogenetic processes in early stages of development. Primordia of these organs are initiated as local epithelial thickenings, which subsequently invaginate into the mesenchyme, forming organ placodes and buds. We previously reported that in teeth, FGF-driven cell division stratifies the early placode. Later, Shh-promoted converging cell intercalation in the suprabasal canopy of the stratified placode drives downward bending and constriction of the placode into a bud shape. Mammary gland and hair follicle invaginations involve similar suprabasal converging force. However, salivary gland invaginates as a hollow structure, ruling out suprabasal contraction as a mechanism. Our new analysis now also rules out some classic cellular mechanisms of epithelial invagination, including apical constriction, basal wedging, and basal relaxation. We observe, instead, that cells in an invaginating salivary gland exhibit a "vertical telescoping" behaviour in which there is vertical downward shear of more central cells relative to their more peripheral neighbours. This is a novel mechanism to bend an epithelium with potential widespread relevance to placodal and potentially non-placodal invaginations.

Neural crest progenitor pool at the frontal bones can repair with more efficiency than parietal mesoderm

DANIEL DORO,
KSHEMENDRA SENARATH-YAPA,
ANNIE LIU, INYOUNG CHO AND KAREN LIU
Craniofacial Development and Stem Cell Biology – King's College London, UK

The skull bones are derived from two different embryonic lineages: the neural crest and mesoderm. Intriguingly, we find that neural crest cells from both frontal bone or dura mater are more osteogenic *in vitro*, and can also confer increased ossification potential to parietal osteoblasts. Whether this distinctive potential correlates with the ability of these cells to repair bone injuries is not yet well understood. To test this, we have used *Wnt1cre/+; Rosa*^{mTmG} mice, which express membrane green fluorescent protein (mGFP) in frontal bone, dura mater and other neural crest derived tissues. At 40 days after birth, we performed 2 mm calvarial defects in the right frontal bone and left parietal bone of each mouse. We then, allowed 4 days of repair after surgery and harvested the heads for histological analysis. We find that even in the early stages of repair, wounds in frontal bones show increased infiltration of neural crest cells deriving from bone, periosteum and dura mater, whereas parietal bones show less contribution from surrounding bone and periosteum, with significant dura mater derived repair. Taken together, the data suggests that the defective bone healing previously reported in parietal bones may be result of a lack of neural crest derived progenitors and inability of mesodermal progenitors to repair the wound. Future plans include transplanting neural crest-derived bone into parietal defects to test whether this will improve healing at the parietal wound site.

Hidden asymmetries in tooth development

RUPALI LAV, JUAN FONS ROMERO AND ABIGAIL TUCKER
Department of Craniofacial Development and Stem Cell Biology, King's College, London

Tooth organogenesis relies on an elegant orchestration of signalling interactions specifying the fate of competent dental epithelial stem/progenitor cells. These dental precursor cells have been shown to express the transcription factor *Sox2*. In molars, this expression exhibits a variation across the different developmental stages of the tooth- starting with an initial global expression to a progressive restriction to the lingual

https://doi.org/10.1017/S0016672317000039 Published online by Cambridge University Press

aspect of the developing tooth germ. FGF signalling has been shown to influence *Sox2* expression in various tissues including the mouse incisor where FGF10 and FGF8 are associated with its maintenance and enhancement respectively. Unlike the incisor however, the molar does not possess a persistent cervical stem cell niche and therefore serves as a better model for comparison with human teeth. Our investigation is aimed at elucidating the role of FGF signalling in the modulation of *Sox2* expression during the development of the murine molar. We have utilized explant cultures of murine mandibles and molar tooth germs to perform a series of loss of function experiments targeting the FGF signalling pathway. Through these experiments, we have highlighted the role of FGF signalling in the regulation of *Sox2* expression during different stages of molar tooth development.

Role of WNT/β-catenin signaling in adult salivary gland maintenance and repair/regeneration

ARAZ AHMED AND ISABELLE MILETICH
King's college London, Craniofacial Development and Stem Cell Biology Department, Floor 27, Guy's Hospital, London, UK

Following injury, WNT signalling is activated and plays a crucial role in the repair of a number of adult organs. In the salivary glands, it has been shown that WNT signalling is activated in ductal epithelial cells following salivary gland hypofunction caused by ligation of the main excretory duct (Hai *et al.,* 2010). Furthermore, studies in irradiated mice have revealed that expression of WNT1 in the salivary gland epithelium at the time of irradiation partially rescues the saliva flow rate, suggesting WNT signalling can prevent radiation-induced salivary gland dysfunction (Hai *et al.,* 2012).

Using $Axin2^{CreERT2/+};R26^{mTmG/+}$ WNT reporter mice, we have found that during homeostasis few submandibular gland (SMG) cells have active WNT/β-catenin signalling, which are mostly located within the parenchyma of the gland, and more specifically within intercalated ducts -where salivary glands stem cells are believed to be located. After injury caused by ligation of the main excretory duct of the SMG, a significant increase in WNT/β-catenin signalling occurs within the sparse stroma of the gland -mainly in a population of elongated cells within the gland capsule, septa, and around the excretory ducts and blood vessels in contrast with data previously obtained by Hai *et al.* (2010). Further investigation revealed the majority of the stromal cells with active WNT/β-catenin signalling are CD45+ inflammatory cells including F4/80+ macrophages. We show that WNT signalling activation peaks 6 days after ligation and that the increase in WNT/β-catenin signalling after injury corresponds with the timing of infiltration of different inflammatory cells, including macrophages. We identified that a number of WNT ligands comprising Wnt2 2b, 5b, 7b, 9a, and 11 are upregulated 3 days after injury. Our results suggest that WNT signalling may have two separate roles in salivary gland maintenance and regeneration during homeostasis and after injury. Further work will investigate whether inflammatory cells and active WNT/β-catenin signalling are required for salivary gland repair.

LATS1/2 kinase in hippocampal development

NOREEN EDER AND SILA ULTANIR
Developmental Neurobiology, The Francis Crick Institute, 1 Midland Road, London, NW1 1AT, United Kingdom

The Hippo signalling pathway is well characterised in its involvement of controlling tissue growth and homeostasis. Dysregulation of this kinase pathway is associated with aberrant cell growth and neoplasia. Interestingly, it is also found that members of this highly conserved pathway, such as Warts/Lats1/2 exhibit additional roles in the dendrite morphogenesis in flies. Correct wiring of the neural circuitry is dependent on proper dendrite arborisation and synapse formation. Molecular mechanisms regulating these important processes are being investigated.

Our aims are 1) to test if Lats1/2 signalling regulates mammalian neuronal development and 2) to explore its downstream mechanism by identify its substrates. To address the first question we are using conditional knockout mice in which Lats1/2 are deleted from excitatory neurons in the cortex and hippocampus starting at committed neuronal progenitor stage via Nex-Cre. Preliminary data from Lats1/2 conditional knockout mouse, gives us a first indication that is necessary for maintaining brain structure via suppressing YAP. In order to address Lats1/2 downstream effectors more broadly, we are taking mass spectrometry approaches.

The protective function of the oxidation resistance 1 gene in ALS.

MATTHEW G. WILLIAMSON, KAY E. DAVIES AND PETER L. OLIVER
MRC Functional Genomics Unit, Department of Physiology, Anatomy and Genetics, University of Oxford, Parks Road, Oxford, OX13PT, UK

Mutations in the gene encoding the transactive response DNA binding protein (TDP-43) are known to cause

https://doi.org/10.1017/S0016672317000039 Published online by Cambridge University Press

~5% of familial ALS cases. Interestingly though, >90% of all ALS cases present TDP-43 pathology which is characterised by neuronal cytoplasmic inclusions of aggregated TDP-43. Oxidative stress (OS) is believed to be a key contributory factor early in ALS pathology and is known to promote TDP-43 aggregation. Oxidation resistance 1 (OXR1) is an antioxidant protein known to regulate neuronal survival in response to OS and was recently found to bind wild-type and mutant TDP-43^{M337V}. Furthermore, overexpression of Oxr1 in cells expressing specific TDP-43 mutants decreased TDP-43 mislocalisation and improved cellular pathology. We investigate the hypothesis that OXR1 is a key neuroprotective factor during ALS pathogenesis *in vivo* by crossing a new transgenic mouse line that overexpresses Oxr1 in neurons with a novel TDP-43^{M337V} mutant mouse line. We report that neuronal overexpression of Oxr1 significantly improves motor deficits observed in homozygous TDP-43$^{M337V/M337V}$ mice. Current investigations are underway to assess the pathological phenotype of these mice. Our findings suggest Oxr1 shows promise as a modulator of disease progression in TDP-43-related ALS and provides further evidence that OXR1 may have therapeutic benefits for the treatment of ALS.

Non-monotonic phenotypes and gene expression changes in an allelic series of *Chd8*-deficient mice

PHILIPP SUETTERLIN[1], KIMBERLEY L. H. RIEGMAN[1], SHAUN HURLEY[1], CONOR MOHAN[1], ANGELA CARUSO[2,3], JACOB ELLEGOOD[4], IVAN CRESPO-ENRIQUEZ[1], CATERINA MICHETTI[2,5], ROBERT ELLINGFORD[1], OLIVIER BROCK[6], ALESSIO DELOGU[6], PHILIPPA FRANCIS-WEST[1], JASON P. LERCH[4], MARIA LUISA SCATTONI[2], CATHY FERNANDES[7,8] AND M. ALBERT BASSON[1,8]

[1]*Department of Craniofacial Development and Stem Cell Biology, King's College London, Floor 27, Guy's Hospital Tower Wing, London, SE1 9RT, UK*
[2]*Neurotoxicology and Neuroendocrinology Section, Department of Cell Biology and Neuroscience, Istituto Superiore di Sanità, Viale Regina Elena 299,00161 Rome, Italy*
[3]*School of Behavioural Neuroscience, Department of Psychology, Sapienza University of Rome, via dei Marsi 78, 00185, Rome, Italy*
[4]*Department of Medical Biophysics, University of Toronto, Mouse Imaging Centre, Hospital for Sick Children, 25 Orde Street, Toronto, Ontario M5T 3H7, Canada*
[5]*Center for Synaptic Neuroscience and Technology, Istituto Italiano di Tecnologia, Genova, Italy*
[6]*Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology & Neuroscience, King's College London, UK*
[7]*MRC Social, Genetic & Developmental Psychiatry Centre, PO82, Institute of Psychiatry, Psychology & Neuroscience, King's College London, De Crespigny Park, London SE5 8AF, UK*
[8]*MRC Centre for Neurodevelopmental Disorders, King's College London, 4th floor, New Hunt's House, London SE1 1UL, UK*

Truncating CHD8 mutations are amongst the highest confidence autism risk factors identified to date. To investigate how reduced Chd8 gene dosage may predispose to autism, we constructed a mouse Chd8 allelic series. Whereas the pan-neuronal, homozygous deletion of Chd8 results in brain hypoplasia, we find that Chd8 heterozygous mice display subtle brain hyperplasia and only minor gene expression changes. A small additional decrease of Chd8 expression in Chd8 hypomorphs causes robust changes in the expression of 168 autism-associated genes and hyperplasia of several autism-associated brain areas. Unexpectedly, neither Chd8 heterozygous nor hypomorphic mice display autism-like behaviours. Together, these data show that gene expression and brain growth respond in a non-monotonic fashion to changes in Chd8 expression. We propose that CHD8 haploinsufficiency represents a sensitised genetic background that is not necessarily sufficient to cause autism, but may strongly predispose to autism by reducing the threshold for additional autism risk factors.

Identification of novel candidates for BOR syndrome

RAMYA RANGANATHAN, MONICA TAMBALO, JINGCHEN CHEN AND ANDREA STREIT

Dental Institute, Craniofacial Development and Stem Cell Biology, King's College London, London SE1 9RT, UK

In humans, mutations in the nuclear factors Six1 and Eya1 cause Branchio-Oto-Renal (BOR) syndrome, an autosomal-dominant disorder characterised by hearing loss, branchial fistulae and renal anomalies. However, only 50% of BOR patients harbour Six1 or Eya1 mutations suggesting that other causative mutations remain to be discovered. These may either affect the coding or the non-coding regulatory regions of new candidate BOR. In chick, activation of Six1 targets is important for normal development of the inner ear primordium, the otic placode. We therefore

https://doi.org/10.1017/S0016672317000039 Published online by Cambridge University Press

hypothesised that understanding the molecular mechanisms downstream Six1 will reveal new BOR candidates. Using a combination of RNAseq, ChIPseq and in vivo experiments we have identified many active enhancers that harbour Six1 binding sites and are associated to otic-enriched genes. Interestingly, of these potential Six1 targets more than half correspond to human deafness loci. Currently, we are investigating the functional relationship of Six1 and its targets, as well as their role in ear formation.

A new biological and clinical resource for research into pregnancy complications: The Baby Biobank

NITA SOLANKY[a], LYDIA LEON[a], C. MAURER[a], SAYEDA ABU-AMERO[a], LESLEY REGAN[b] AND GUDRUN E. MOORE[a]
[a]*UCL Institute of Child Health, 30 Guilford Street, London WC1N 1EH*
[b]*Obstetrics and Gynaecology Department, St Mary's Hospital, Imperial College, Praed Street, London W2 1NY*

About 20% of pregnancies are affected by some form of complication. Research has shown that anomalies in implantation, development, and growth of the fetus; placental dysfunction; and maternal problems such as hypertension or infection during pregnancy can all lead to adverse outcomes. However, the molecular aetiology of such events remains poorly understood, in part due to the lack of large sample and datasets.

This prompted us to set up the Baby Biobank which now contains maternal, paternal and baby biological samples from 2515 pregnancies. These pregnancies include 236 with Fetal Growth Restriction, 133 Preeclamptic, 373 Preterm Birth, and 232 with Recurrent Miscarriage. Over 1,500 'normal' pregnancies with none of these complications were also collected for use as control samples, and 636 of these are classified as 'perfect' controls with no recorded problems associated with the mothers' health, pregnancy, or delivery. The Baby Biobank also contains up to 200 fields of data for each pregnancy.

This presentation outlines the nature of these sample sets and their availability to academia and industry, with the intention that their widespread use in research will make significant contributions to the improvement of maternal and fetal health worldwide.

http://www.ucl.ac.uk/tapb/sample-and-data-collections-at-ucl/biobanks-ucl/baby-biobank

https://doi.org/10.1017/S0016672317000039 Published online by Cambridge University Press