Genet. Res., Camb. (2009), **91**, *pp.* 133–141. © Cambridge University Press 2009
doi:10.1017/S0016672309000044 Printed in the United Kingdom

Abstracts of papers presented at the nineteenth Genetics Society's Mammalian Genetics and Development Workshop held at the Institute of Child Health, University College London on 20 and 21 November 2008

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Sponsored by: The Genetics Society and *Mammalian Genome*

Loss of Tbx22 causes submucous cleft palate and ankyloglossia

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Cleft palate is a common birth defect, with an incidence of approximately 1 : 1500. Mutations in *TBX22*, which encodes a T-box transcription factor, result in X-linked cleft palate and ankyloglossia. This is known to account for approximately 5% of all cleft palate patients.

Here, we show that disruption of *Tbx22* in mouse results in submucous cleft palate, partial ankyloglossia and choanal truncation. Although a small minority of mutants have overt clefts, all other male hemizygous and female homozygous mutant embryos show submucous cleft palate, where the palatal shelves have closed but where bone formation in the posterior hard palate is impaired. In the choanae, we observe that the oro-nasal membrane persists or is only partially ruptured. Each of these defects can cause severe breathing and/or feeding difficulties in the newborn pups, which results in about 50% postnatal lethality.

Our results suggest that Tbx22 plays an important role in the osteogenic patterning of the posterior hard palate, as well as regression of the tongue frenulum and oro-nasal membrane. These findings could have important implications for the molecular diagnosis in patients with submucous or soft palate clefts or in patients with unexplained choanal atresia.

Cellular and molecular characterization of abnormal rear leg (Arl) – a new mouse strain with a point mutation in cytoplasmic dynein

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Cytoplasmic dynein is a retrograde motor protein responsible for the movement and localization of cellular components such as mitochondria, Golgi apparatus and endoplasmic reticulum. Mutations in this protein have previously been shown to cause neurodegenerative disease-like symptoms in mice, indicating its importance in the normal development and function of neurons. Abnormal rear leg (*Arl*) is a mouse point mutation in the heavy chain of cytoplasmic dynein that is homozygous lethal at embryonic day 10 and causes abnormal gait and hindlimb clasping during tail suspension in heterozygotes, typical of neuronal dysfunction. In collaboration we have shown that the soma of sciatic nerve motor neurons are 20% smaller in *Arl* compared with wild type and in spinal cord dorsal and ventral roots, the number of myelinated sensory fibres is also decreased in *Arl* compared with wild type. In the present work, we have found that the reassembly of the Golgi complex, after disruption with the microtubule depolymerizing agent nocodazole, is significantly impaired

in *Arl* heterozygote mouse embryonic fibroblasts (MEFs) compared with wild-type littermate MEFs. We have also found that trafficking of endosomes is also significantly reduced in these Arl heterozygote MEFs. These data suggest that defective cargo transport may result in neuronal death and dysfunction.

Defects in branchial arch derivatives in Barx1 mutant mice

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The homeobox gene *Barx1* is a transcription factor important in proximal–distal patterning of the first branchial arch, fate determination in stomach development and critical for normal spleen development. Here, we show that loss of *Barx1* in the mouse causes various structures in the head and neck to be either hypoplastic, misshapened or fail to form completely. This includes a cleft secondary palate and missing cheekbones derived from the maxillary process. The mandible is consistently slightly shorter and the three processes for mandibular articulation are hypoplastic and the hyoid bone and cartilages derived from the 3rd to 6th branchial arch are also malformed. In the mid-ear, the malleus of the Barx1 mutant is hyperplastic, but with a vestigial tympanic ring, no gonial bone is found and there is a broken stapes cartilage. This suggests that Barx1 mice may have conductive hearing defects. Interestingly, in some cases in Barx1 mutants, the trabecular basal plate of the cranial base where Barx1 is not expressed has a significant bend near the preshenoid region instead of a normal straight rod shape, which may be the cause for the shortened length of the skull. Branchial arch patterning genes such as *Dlx*, *Gsc*, *Hoxa2*, *Lhx7* and *Pax9* showed no changes in their expression in Barx1 mutant. However, Msx1 expression in the proximal mandibule and the 2nd branchial arch is down regulated in the Barx1 mutant.

We studied the timing and formation of each skeletal element defective in the Barx1 mutant and concluded that Barx1 is essential for forming many of the branchial arch structures, probably by supporting the outgrowing of these tissues during development. Barx1 may be a candidate gene for human syndromes such as Pierre Robin's Sequence and Eagle's syndrome that exhibits a cleft palate and/or chewing and hearing problems.

Work supported by the Wellcome Trust.

Proteomic analysis of neural tube defects in the *curly tail* mouse

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Curly tail (*ct*) mice develop spinal neural tube defects (NTDs) that closely resemble the corresponding birth defects in humans, with multifactorial aetiology 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. *Grainyhead-like-3* (*Grhl3*) is a candidate gene for *ct*, since knockout mice for *Grhl3* exhibit NTDs and its expression correlates spatio-temporally with the causative cellular defect in the hindgut. We identified a putative regulatory mutation in the *Grhl3* gene, and showed by transgenic Bacterial artificial chromosome (BAC) rescue that increased expression of *Grhl3* prevents NTDs in *ct* embryos. This suggests spina bifida in *ct* embryos results from reduced expression of *Grhl3*. In order to further investigate the pathogenesis of NTDs in *ct*, we have applied two-dimensional gel-based proteomic approaches. These studies have revealed differences in the proteome of *ct* and wild-type embryos involving several proteins, which are being analysed for their possible contribution to NTDs susceptibility. We identified a polymorphism in *Lmnb1* that differs between *curly* and wild-type genetic backgrounds. This polymorphism is being investigated as a potential modifier of NTD risk in *ct*.

Characterizing *rks*: a mutant generated in a recessive ENU screen

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Specification of the mammalian left–right axis occurs early in embryogenesis. The first molecular asymmetries are observed at late headfold stage (~7·5 dpc in mice) in the embryonic node, a ciliated pit-like structure located at the distal tip of the embryo. Nodal cilia have been shown to rotate, causing

a leftward flow of fluid that is generally accepted to specify the left side of the embryo. The mechanism by which this flow is detected and converted into a robust asymmetry in gene expression is the subject of debate.

rks is a point mutant displaying striking right pulmonary isomerism, heart patterning defects and consequent oedema. *rks* individuals have immotile cilia but the phenotype is not consistent with the effect of immotile cilia alone. The well-characterized mutant *iv* has immotile cilia and randomly assigns the left side, whereas *rks* has a phenotype consistent with a lack of specification of leftness.

I have identified a single base substitution in a functional domain of a candidate gene for *rks*. It is a member of a gene family implicated in the generation of calcium ion fluxes in response to flow in the kidney. I propose that this gene acts to form a calcium ion channel integral to ciliary motion and the downstream specification of leftness in development.

FGF expression in the developing cerebellum suggests roles in cerebellar morphogenesis and development

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Earlier studies have defined important roles for FGF8 signalling during development of the midbrain and cerebellum. The major classes of cerebellar neurons are only born and differentiate after embryonic day (E) 12.5, when the expression of *Fgf8* has been reported to cease. A key process required for normal cerebellar development is the extensive proliferation of granule cell precursors in the external granule cell layer during the early post-natal period. To investigate whether Fgf signalling is required during these later stages of cerebellar morphogenesis, we determined the expression of all *Fgf* genes at key stages of cerebellar development after E12.5. Several *Fgf* genes are expressed in cell-type- and region-specific patterns, suggesting multiple functions during the development of different cell types and specialized regions within the cerebellum. Among them, *Fgf3* is strongly expressed in parasagittal domains of Purkinje cells from E16.5 to P7. We investigated the role of *Fgf3* during cerebellum development by analysing *Fgf3* null mutants, and provide evidence that *Fgf3* might regulate granule cell proliferation.

The Medical Research Council at Harwell: providing the research community with tools and services for mouse functional genomics

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The Medical Research Council (MRC) at Harwell offers a range of tools and services to the scientific community for the creation, maintenance, study and archiving of mouse models for human diseases:

- A state of the art specific pathogen-free mouse holding and breeding facility.
- The Frozen Sperm and Embryo Archive, which acts as the UK node for European Mouse Mutant Archive consortium.
- Mutagenesis service for the production of chemically induced mutations in the mouse genome.
- Transgenics service for mouse genome engineering through pronuclear injection and gene targeting.
- Advanced phenotyping platforms including metabolic, sensory and behavioural testing.

The MRC at Harwell is a member of both the EUCOMM and EUMODIC consortia.

We present in further details some of the tools and services available at the MRC Harwell.

We also introduce our R&D programme, aimed and developing and enhancing methods and tools for transgenesis.

Milking *Grb10* for novel roles

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Imprinted genes are those that are expressed from only one allele in a parent-of-origin-specific manner. The *Grb10* gene is imprinted in both mouse and human but is unique among imprinted genes for demonstrating tissue-specific, monoallelic expression from each of the parental alleles. We have demonstrated that *Grb10* expression in the central nervous system is exclusively from the paternally derived allele, while the maternally derived allele is the source of *Grb10* transcripts in most other tissues. Further, we have demonstrated that maternally and paternally expressed *Grb10* influence distinct physiological processes – growth/metabolism and behaviour, respectively.

Recently, our work has revealed that the transcription factor Stat5 binds to a conserved enhancer element at the *Grb10* locus and is required for *Grb10* expression in three specific tissues: brain, placental labyrinth and mammary epithelium. This observation is suggestive of a novel role for Grb10. Consistent with the characterized function of the Grb10 protein as a cytoplasmic adaptor molecule, our current model proposes that Grb10, through Stat5, is involved in prolactin signalling in these three tissues. This novel role would be independent of the imprinting status of *Grb10*, unlike the previously described roles specific to the maternally and paternally derived alleles.

Assessment of candidate imprinted genes in the human term placenta

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Imprinted genes demonstrate expression from one parental allele only. They have been shown to be important in fetal and placental development, behaviour, cancer and several well-characterized dysmorphic syndromes. Imprinting is a rare and extreme mode of allelic imbalance in expression, a more subtle variation we call 'differential allelic expression' might be more common and could account for milder phenotypic differences. Using two high-throughput technologies alongside bioinformatics predictions, normal-term human placental tissue was screened to find new imprinted genes and to ascertain the extent of differential allelic expression in this tissue.

Twenty-three family trios were analysed, each consisting of placental cDNA, placental genomic DNA (gDNA) and gDNA from both parents. First the Sequenom mass spectrometry was used to test 104 single nucleotide polymorphisms (SNPs) in 104 candidate genes as a pilot project. Second, the Illumina Allele-Specific Expression (ASE) BeadArray™ platform was used to test 1536 SNPs in 932 genes in the cohort of tissues. Differential allelic expression was detected in at least one (more than one) informative individual for 19·8% (7·3%) of the SNPs tested. No new imprinted genes were found. In conclusion, differential allelic expression is common in the human term placenta and true imprinted expression appears to be rare.

This work is supported by the Wellcome Trust and the Wellbeing of Women/RCOG Charity.

Tissue- and transcript-specific genomic imprinting of the tumour suppressor gene *Blcap*

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The bladder cancer-associated protein gene (*BLCAP*; previously *BC10*) is a tumour suppressor that limits cell proliferation and stimulates apoptosis. *BLCAP* protein or message are downregulated or absent in a variety of human cancers. In mouse and human, the first intron of *Blcap*/*BLCAP* contains the distinct *Neuronatin* (*Nnat*/*NNAT*) gene. *Nnat* is an imprinted gene that is exclusively expressed from the paternally inherited allele. Previous studies found no evidence for imprinting of *Blcap* in mouse or human. Here, we show that *Blcap* is imprinted in mouse and human brain, but not in other mouse tissues. Moreover, *Blcap* produces multiple distinct transcripts that exhibit reciprocal allele-specific expression in both mouse and human. We propose that the tissue-specific imprinting of *Blcap* is due to the particularly high transcriptional activity of Nnat in brain, as has been suggested previously for the similarly organized and imprinted murine *Commd1*/*U2af1-rs1* locus. For *Commd1*/*U2af1-rs1*, we show that it too produces distinct transcript variants with reciprocal allele-specific expression. The imprinted expression of *BLCAP* and its interplay with *NNAT* at the transcriptional level may be relevant to human carcinogenesis.

Characterization of *Nespas*, a non-coding imprinted RNA

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Non-coding transcripts are thought to be involved in gene regulation within their imprinted clusters. However, the mechanism(s) of regulation have not

yet been established, although several models have been proposed.

Nespas is the non-coding antisense transcript of the imprinted *Gnas* cluster; it is maternally imprinted and located on mouse distal chromosome 2. Our work aims to characterize *Nespas* and study its function, in order to help identify which of the gene regulation models applies to the *Gnas* cluster.

We have used northern analysis to show *Nespas* is expressed during the second half of gestation and peaks at 13·5 dpc. We have identified two transcripts one of >10 kb in length and a previously unknown one of 0.8 kb. The latter is localized to the nucleus and we have used 5′- and 3′-RACE techniques to investigate its sequence and composition.

Little is known about the transcripts of the *Gnas* cluster in the placenta. We have shown *Nespas* is expressed in the placenta and are looking for imprinted expression of the cluster's main transcripts, including *Nespas*, at three gestational time points.

For the functional studies, we are doing an experiment to ascertain if transcription of *Nespas* across its cognate sense gene *Nesp* is required for silencing *Nesp*, by truncating the *Nespas* transcript 10 kb from its start site.

Wt1 antisense in the mouse

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WT1 proteins regulate target genes via transcriptional transactivation and repression and post-transcriptional mechanisms and are associated with mesenchyme to epithelial transitions during development, particularly in nephrogenesis. *Wt1* is essential for kidney development in the mouse, and mutations cause Wilms' tumour of the kidney in man.

The *WT1* antisense transcript (*WT1-AS*) is a candidate regulator of WT1 in the kidney. During nephrogenesis, human *WT1-AS* is expressed coordinately with *WT1*. In transcription assays, *WT1-AS* can upregulate WT1 protein. *WT1-AS* is regulated by genomic imprinting, but imprinted expression is absent from some Wilms' tumours. These observations suggest that *WT1-AS* is a regulator of WT1 during nephrogenesis.

We have developed a mouse model in which *wt1-as* expression is disrupted. The human *WT1-AS* promoter is not phylogenetically conserved, so our approach was to target a conserved region (mcr2) upstream of *wt1* that we propose may act both as a DNA element and as a transcribed RNA. The model consists of two mouse lines: a knock-in line in which the *wt1-as* transcript should be prematurely terminated (T; for truncation) and a knock-out line, in which mcr2 is also deleted (D; for deletion).

The mouse models will be described and our endeavours to characterize them.

The role of Eda signalling in salivary gland development

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Hypohidrotic ectodermal dysplasia (HED) is a syndrome characterized by defective ectodermal organ development. This includes the salivary glands (SG), which have an important role in providing lubrication for digestion and protection of the oral cavity. In humans and mice, HED is caused by mutations in *Eda* pathway genes. Defects in *Eda* (the ligand) or *Edar* (the receptor) lead to SG dysplasia in adult mice. However, the role of Eda signalling in embryonic SG development has not been thoroughly investigated. Therefore, this work aims to examine the expression pattern of *Eda* pathway genes in developing SGs and characterize the embryonic SG phenotype of mice deficient in Eda pathway components.

We show that *Edaradd* (the intracellular component of the pathway) is expressed in the SG epithelium and mirrors the expression of *Edar*, suggesting that *Edaradd* interacts with *Edar* in the SGs as in other ectodermal organs. Furthermore, we show that the SGs of mice deficient in *Eda* pathway genes are hypoplastic with reduced epithelial branching. This is accompanied by changes in apoptosis patterns and gene expression. Specifically, *Shh* is downregulated in the mutant SGs, suggesting that the *Eda* pathway acts through *Shh* to regulate SG development.

This work is supported by NFED and MRC.

Novel phenotyping methods using microMRI

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Transgenic mice are an integral part in the study of gene function in development and disease. Determining phenotype is crucial. μMRI is an emerging technique for high-throughput imaging of mouse embryos: capable of imaging up to 32 in a single scan and producing high-resolution three-dimensional (3D) data (as low as $\sim$15 μm). Any organ system can be assessed for phenotype by inspection of each embryo by a trained observer. However, this is a time-consuming and labour-intensive process. Semi-automated, morphometric methods of analysis, where large numbers of embryos may be compared at once, show promise in combining high-throughput imaging with high-throughput analysis. Voxel and deformation-based morphometry are techniques that can detect subtle differences in anatomy between two populations. They combine a number of subjects into a population atlas and two such groups may be compared statistically to look for differences. We present our work on optimization to phenotype the embryo cardiovascular system, using defects seen in *Chd7*$^{+/-}$ mice (a model of human CHARGE syndrome) as an example. We present an initial step in applying statistical morphometry methods to phenotyping the mouse embryo, creating an embryo atlas by co-registering wild-type embryos. With further development, this may enable subtle anatomical differences between transgenic populations to be detected.

The role of fibroblast growth factor (Fgf) signalling in thymus and parathyroid organogenesis

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The thymus and parathyroid glands develop from the 3rd pharyngeal pouch endoderm in the mouse embryo. Previous research has shown that *Fgf8* is necessary for the formation of the 3rd pouch by E9.5 and that signalling through FGFR2(IIIb) is required for proliferation of the thymic primordium at E12.5. It is not known whether Fgf signalling plays a role between E10.5 and E12.5, stages when subdivision of the 3rd pouch into prospective thymus and parathyroid domains becomes apparent.

Sprouty genes encode intracellular feedback antagonists of Fgf signalling. Simultaneous deletion of both *Spry1* and *Spry2* results in thymus and parathyroid hypoplasia, and thymic migration defects. The 3rd pouch forms in these embryos, allowing us to investigate the role of Fgf signalling between E10.5 and E12.5. *Spry1* and *Spry2* are expressed in all tissues of the 3rd pharyngeal arch at E10.5. Gene expression analysis in the 3rd arch suggests that Fgf signalling is involved in patterning of the 3rd pouch endoderm into thymus and parathyroid domains. We further show that growth of the organ primordia, but not their separation from the pharynx, is sensitive to alterations in Fgf signalling in neural crest cells.

Anti-apoptotic agents can reduce deleterious levels of cell death causing ocular coloboma in the *lamb1* zebrafish model leading to milder ocular phenotypes

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Ocular coloboma is a congenital eye anomaly arising from incomplete fusion of the optic fissure between 5 and 7 weeks post-conception in humans and 36–48 h post-fertilization (hpf) in zebrafish. Apoptosis is known to play a transient role in the formation and closure of the optic fissure. Using the *lamb1*$^{-/-}$ zebrafish model of ocular coloboma, deleterious levels of cell death were detected at the site of the unfused optic fissure, whereas wild-type controls showed negligible apoptosis. Translational bypass therapy with aminoglycoside drugs resulted in complete closure of

the optic fissure in *lamb1*$^{-/-}$ mutants by 6 dpf with a corresponding reduction of cell death. To test the hypothesis that optic fissure closure was apoptosis-dependent, the anti-apoptotic agents, curcumin and zFAD-fmk, were tested in *lamb1*$^{-/-}$ embryos. Both drugs greatly reduced the size of the coloboma and the level of apoptosis, providing further molecular evidence that cell death is required for optic fissure remodelling. The use of relatively safe drugs, such as curcumin, as a prenatal treatment may limit the severity of colobomatous defects, or be used as an adjunctive treatment to minimize the long-term toxic effects of more definitive pharmacological therapies.

This work is supported by St. Mary's Development Trust.

Effects of age and *Pax6*-deficiency on mouse limbal stem cell function

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Previous analysis of corneal epithelial stripe patterns in mouse chimeras and X-inactivation mosaics in our lab has provided indirect evidence that limbal stem cell function declines with age and is reduced in *Pax6*$^{+/-}$ mice. We are investigating these two predictions by comparing active limbal stem cell numbers in two ways. K5-*LacZ* transgenic mice produce rare β-galactosidase-positive clones in the corneal epithelium, marking limbal stem cell lineages. As predicted, the frequencies of marked corneal epithelial clones were lower at 30 weeks than at 15 weeks and lower in *Pax6*$^{+/-}$ K5-*LacZ* than in wild-type K5-*LacZ* corneas. The second approach involved comparing the frequency of BrdU label-retaining cells, which will include putative stem cells. Mice were exposed to BrdU for 1 week and chased for 10 weeks. Putative stem cells divide less frequently than other cells and so retain the BrdU during the chase period. Results failed to show the predicted depletion of label-retaining cells in older mice or in *Pax6*$^{+/-}$ mice. It is unclear why analysis of label-retaining cells failed to support the mosaic studies. One possible explanation is that cell-turnover differences may affect label-retaining cell numbers.

This work is supported by Fight for Sight.

Investigating the role of Dicer1 in male and female reproductive tract development

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Since the discovery of microRNAs (miRNAs) in *Caenorhabditis elegans*, a whole number of distinct classes of small RNAs have been identified, with many implicated to play varied and essential roles in mammalian development. Numerous studies that investigate miRNA function focus on the processing enzyme Dicer1. This RNAseIII enzyme cleaves the precursor miRNA to produce the active form and is essential for development. Deletion of Dicer1 is embryonic lethal at 7·5 dpc. Despite the current interest in miRNAs as genetic regulators, it is still largely unclear what role they play in the development of the male and female reproductive tract. This study uses a conditional allele of the Dicer1 gene and two *Cre* expressing lines, driven by *Amhr2* and *HoxB7*, to investigate the effect of Dicer1 deletion on male and female reproductive tract development. The data presented here highlight an essential role for Dicer1 in the correct morphology and function of the female reproductive tract and confirms recent findings that suggest Dicer1 is required for female fertility. Our findings also demonstrate a previously undefined role for Dicer1 and miRNAs in the kidney. Male reproductive tract development, however, remains largely unaffected in both cases.

A novel 'Synodiporic' mechanism to explain the ocular phenotype in the *Mp* mouse

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The *Mp* mouse has severe limb and ocular malformations and is a model for human disease. We have mapped the causative mutation to an inversion on chromosome 18, resulting in a C-terminal truncation of the Fbn2 protein (Fbn2^{Mp}). Other loss-of-function Fbn2 alleles in the mouse have identical limb defects no eye phenotypes. Therefore, we investigated the cause of the 'worse than null' *Fbn2* mutation in *Mp*. The ocular malformations were characterized by a failure of correct ciliary body (CB) development, loss of vitreous and retinal lamination defects. Intracellular aggregates of Fbn2^{Mp} were identified in cells at the developing peripheral retina. These cells have been previously identified as the source of

Wnt2b essential for CB development and retinal lamination. Markers of the unfolded protein response (UPR) were activated in *Mp* eyes in regions overlapping with $Fbn2^{Mp}$ localization, and the distribution of Wnt responsive factors and ciliary markers were disrupted. We propose a novel 'synodiporic' mechanism of pathology, where specific mutations that trigger activation of the UPR affect the endoplasmic reticulum (ER) processing of other factors coexpressed within the same cells. Such effects may have significant implications for human genetic disease analysis, and may provide explanation for other 'worse than null' mutations.

Wnt-Ca^{2+} signalling in kidney development

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A number of *Wnt* genes are expressed during, and are known to essential for, early kidney development. It is typically assumed that the products of these genes will exert their effects through the β-catenin Wnt signalling pathway; however, we have found expressional and functional evidence that the non-canonical Ca^{2+}/NFAT (nuclear factor of activated T cells) signalling pathway may also be important downstream of *Wnt4*. To facilitate further identification of NFAT activity, we have generated reporters driven by NFAT-responsive elements. Transgenic mice are now being generated to examine activity *in vivo*.

The second, complementary approach we are taking is to manipulate the pathway in cells, embryos and components of the embryonic kidney. Cre-regulated dominant-negative and constitutively active forms of NFAT have been generated and will be employed on both the wild-type and *Wnt4*-deficient background. In addition to these genetic manipulations, drugs have been used to alter the pathway in cultured kidney rudiments. Treatment with cyclosporin A (CSA), an inhibitor of calcineurin-NFAT Ca^{2+} signalling, specifically decreases nephron formation – a phenotype similar to that in $Wnt4^{-/-}$ embryos. We are now attempting to rescue the $Wnt4^{-/-}$ phenotype using Ionomycin, an activator of the Ca^{2+}/NFAT pathway.

By combining the described reporters, lines and reagents – along with similar tools for the canonical pathway to investigate potential cross-talk – it is hoped that the functions of Wnt-Ca^{2+} signalling in kidney development can be dissected.

Role of Zic2 in mammalian neural tube closure

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Zinc finger proteins belonging to the Zic family play critical roles in animal development. Five mouse *Zic* genes have been identified, *Zic1–Zic5*, which encode a family of 2C2H-like zinc finger transcription factors of which three members, *Zic2*, *Zic3* and *Zic5*, have been implicated in the causation of neural tube defect (NTD).

The ENU-generated mouse model of spina bifida, the Kumba mouse ($Zic2^{Ku}$), carries a mutation in the zinc finger domain region of *Zic2*. Morphological analysis of neurulation in $Zic2^{Ku/Ku}$ embryos shows that Zic2 is required for normal bending of the neural plate. Absence of dorsolateral bending during neural tube closure can explain the subsequent development of spina bifida in $Zic2^{Ku/Ku}$ embryos. In order to better understand the Zic2 involvement in neurulation, we have performed a microarray analysis of the posterior region of somite-matched wild-type and mutant embryos. Some of the differentially expressed genes will be discussed.

Recently, the double mutant for *Zic2* and *Zic3* was found to develop craniorachischisis, the most severe form of NTD, usually associated with the planar cell polarity (PCP) pathway. We therefore generated and analysed compound mutants for *Zic2* and PCP mutant genes. The genetic interaction revealed by this study will be discussed.

Evidence for generation of human β-defensin copy number variation by germline crossover

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Copy number variation in human β-defensin genes involves a cluster of at least seven β-defensins on human chromosome 8p23.1. These genes encode proteins known to have antimicrobial properties. In this study, we have measured and validated diploid copy number variation at *DEFB4* (one of the seven loci) in reference (CEPH) pedigrees by using *HSPD*-paralogue ratio test (PRT) and two multiallelic loci. We found *DEFB4* copy numbers to be variable in CEPH family members with between 2 and 7 copies per diploid genome in these three assays, consistent with previous studies. Additionally, we also

demonstrated that recombination between homologous chromosomes in the interval between two distinct defensin loci is the simple but unexpected explanation for the origin of new variation in copy number, which arises at the very high rate of 0.7% per gamete. Our segregation data also allowed us to map the loci containing variable numbers of β-defensin repeat units by linkage. One of these sites is consistent with the location in distal 8p23.1 indicated on the genome assembly, but there is also a separate location about 5 Mb proximally. The existence of two distinct loci for variation in β-defensins is unusual, and may be the only such example in the genome, but it nevertheless illustrates that mechanisms of copy number variation may be more varied and complex than hitherto suspected.

https://doi.org/10.1017/S0016672309000044 Published online by Cambridge University Press