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Is neural crest cell delamination necessary for normal cranial neural tube closure?

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Prior to cranial neural tube closure, the neural folds adopt a biconvex morphology which is thought to be due to expansion of the underlying mesenchyme. Dorso-lateral hinge points (DLHPs) then form, which allow the dorsal tips of the neural folds to 'flip around' resulting in apposition of the tips and facilitating subsequent fusion. Cranial closure is particularly prone to perturbation, leading to exencephaly in many mouse mutants and as a result of a variety of teratogenic influences. This may reflect mechanical tensions affecting the closing cranial neural folds. For example, the presence of ventral flexures of the body axis at the mid- and forebrain levels mechanically opposes the formation of DLHPs. Several processes have been implicated as important in overcoming these mechanical tensions, thereby assisting in cranial neural tube closure. These include contraction of actin microfilaments at the luminal surface of the neuroepithelium and apoptosis in the dorsal and dorsolateral neuroepithelium. The latter may act to increase flexibility in the dorsal neural folds, enhancing DLHP formation. Neural crest cells (NCC) originate in the dorsal tips of the neuroepithelium and undergo an epithelial-to-mesenchymal transition, allowing them to delaminate, exit the neuroepithelium and migrate extensively throughout the embryo to form numerous derivatives. We hypothesized that delamination of the NCC from the neuroepithelium may enhance the mechanical flexibility of the dorsal tips of the neural folds, allowing the 'flip around' event to occur. The spatial and temporal correlation of NCC delamination and apposition of the neural folds supports this idea, as does a plethora of mouse mutant models, such as Splotch, Cited2, Zic5 and Msx2 mutant mice, which demonstrate a co-occurrence of exencephaly and neurocristopathies. Further experimental evidence will be presented in support of this hypothesis.

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Computerized image analysis of striping patterns in the corneal epithelia of X-inactivation mosaic, PAX77 transgenic mice

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The dosage of the transcription factor Pax6 is crucial for normal eye development. Mice hemizygous for the PAX77 transgene overexpress human *PAX6*. These mice have small eyes and a variety of ocular defects. PAX77 transgenic mice were crossed with male mice harbouring a *nLacZ* transgene on their X chromosome. As the X chromosome is inactivated randomly during development, the resulting hemizygous $X^{LacZ/-}$ females show mosaic *LacZ* expression. In these X-inactivation mosaics, random clumps of *LacZ*-positive cells are seen in the cornea of young animals. This pattern resolves at between 8 and 10 weeks forming radial stripes that represent chords of clonally related, inwardly migrating cells. By measuring the number and width of stripes around the epithelium's circumference and correcting for the effects of different proportions of *LacZ*-positive cells, an estimate of the number of functional coherent stem

cell clones maintaining the tissue can be derived. An automated method was developed using image analysis software to analyse these striping patterns. This produced results that did not differ significantly from previous work using a laborious manual approach. Wild-type animals demonstrated a decline in the estimated number of functional coherent clones with age. In PAX77 animals the clone number was initially reduced but did not decline with age.

Effect of palatal shelf fusion on bone formation

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In mammals, the secondary palate originates from paired palatal shelves that extend from the maxillary processes of the first branchial arches. Following shelf fusion at the midline, the palatal mesenchymal cells condense and undergo intramembranous ossification to form the palatal bones and sutures. Growth factors, such as TGFβs and FGFs, which initiate palatogenesis, are known to be involved also in osteogenesis. The relationships between these developmental processes have not been fully investigated. We used palate organ cultures as a model to study the effects of impaired shelf fusion on bone formation. Fusion was prevented either by physically separating the shelves or by using tgfβ 3 antisense, and the expression of markers of bone differentiation was analysed by RT-PCR and immunohistochemistry. Early in palatal bone formation, expression of *tgfβ 1*, *tgfβ 3*, *fgfr2IIIc*, *snail*, *twist*, *runx2*, *collagen I* and *osteopontin* transcripts was affected when fusion was prevented, but some differences were observed depending on the method used to induce 'clefting'. These results suggest that lack of palatal fusion affects the onset of osteoblast differentiation and that differences in the aetiology of cleft palate might result in different bone formation defects.

Making a left–right axis in culture

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Vertebrates have three major axes: anterior–posterior, dorsal–ventral and left–right (L/R). The research of our group is concerned with understanding how the L/R axis is established. The L/R axis in mammals is believed to be established at 7·5 days of development when nodal cilia produce a leftward flow across the embryonic node. This results in left-sided expression of *Nodal*, *Lefty2* and *Pitx2* in the lateral plate and asymmetry of *Nodal*, *Cerl-2* and *LPlunc1* at the node. Previous work has shown that embryo culture can perturb the establishment of L/R asymmetry: rat embryos cultured from before the early neural plate stage show heart situs and embryonic turning defects; mouse embryos cultured from a similar stage of development show perturbed *Pitx2* expression. We are culturing mouse embryos from 7·0–8·0 d.p.c. (days post-coitum; Theiler stages 10c–12a) through to 8·5 d.p.c. to examine the effect on asymmetric marker expression, and are using this approach to examine expression of other asymmetric markers. Initial data using a Nodal-lacZ reporter is consistent with previous work, demonstrating that culture interferes with asymmetric *Nodal* expression.

Identification of a novel left–right asymmetrically expressed gene in mouse

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Internally, all vertebrates show left–right (L–R) asymmetry in organ positioning and morphology. While much is now understood about the establishment of asymmetry in mammals, it is clear from L–R mutant phenotypes that gaps in our understanding exist. In a microarray-based screen, designed to identify asymmetrically expressed genes, we have identified a gene novel to the L–R pathway that shows asymmetric expression. This gene is strongly expressed in the left lateral plate mesoderm at E8.5 and shows a highly dynamic and asymmetric expression pattern at the node between E7.5 and E8.5. As both aspects of this expression pattern develop, expression is seen simultaneously in the *left* lateral plate mesoderm and to the *right* of the node – a truly novel expression pattern. We present a preliminary analysis of the expression pattern and of its control by known members of the L–R pathway.

Understanding how muscle pattern is determined in the developing limb bud

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The morphogenetic events orchestrating the development of the definitive muscle pattern in vertebrates are poorly understood. Using the developing limb as our model system, we want to gain insight into the molecular and cellular events that regulate the coordinated morphogenesis of muscles and tendons/connective tissues in the limb bud. In particular, we are studying the involvement of *Meox2*, a homeobox gene, and its interaction with other genes implicated in these processes. *Meox2* is a homeodomain-containing transcription factor with a dynamic expression in somites, migrating limb myoblasts and premuscle masses; our data also suggest that *Meox2* regulates specific molecular pathways in the limb-mesoderm-derived connective tissues/tendons as in muscles. Genetic removal of *Meox2* leads to a unique and stereotypical patterning defect of the limb musculature, as well as in tendon formation and maturation. A primary focus of investigation is to characterize the molecular changes in *Meox2* mutant limbs further to include epistatic analysis of genes expressed in tendon precursors and in limb mesoderm. Further analysis of the role of *Meox2* in regulating muscle and tendon development will be instructive in understanding the mechanisms involved in their coordinated morphogenesis.

A mechanistic and comparative analysis of the imprinted *Inpp5f_v2* domain

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Inpp5f_v2 is a transcript variant of the inositol polyphosphate phosphatase gene *Inpp5f*, and is expressed exclusively from the paternally derived allele in the developing murine nervous system. The promoter region on the maternally derived allele is hypermethylated relative to the paternal allele in somatic tissues. This differential methylation is established in gametes and is dependent on *Dnmt3l* expression in the oocyte. Using E8.5 embryos derived from *Dnmt3l* −/− mothers, we show that loss of maternally derived methylation at this locus results in biallelic expression. Comparative sequence analysis indicates that the unique first exon of *Inpp5f_v2* originated at some point shortly after the divergence of the eutherian and marsupial lineages, and acquired the ability to splice onto exons 16–20 of the more ancient *Inpp5f* gene, which is biallelically expressed in mouse. In summary, we have demonstrated the mechanism of imprint control at a fifth imprinted domain on mouse chromosome 7 and provided insights into the evolutionary origins of imprinting at this locus.

A pharmacological induction strategy reveals a role for KIT in embryonic stem cell differentiation

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The stem cell factor (SCF)/KIT signal transduction pathway is known to be an important regulator for proliferation, differentiation, migration, adhesion and survival in a multitude of cell types but little is known about its role in the survival and/or differentiation of embryonic stem cells. We analysed the role of the SCF/KIT signal transduction pathway in murine embryonic stem (ES) cells homozygous for $Kit^{W\text{-}lacZ}$, a targeted deletion at the Kit locus. Kit null ES cells ($Kit^{W\text{-}lacZ/W\text{-}lacZ}$) do not survive when induced to differentiate upon leukaemia inhibitory factor (LIF) withdrawal and this phenotype is mimicked in wild-type cells ($Kit^{+/+}$) when grown in the presence of a KIT-neutralizing antibody, ACK2. We engineered ES cells that carry a knock-in allele, designated $Kit^{W\text{-}FKB}$, encoding an inducible form of KIT in which the extracellular domain is replaced with the FKB domain. This allows for activation of the receptor in the presence of the pharmacological agent, AP20187. The phenotype of Kit null cells was reversed when $Kit^{W\text{-}\ lacZ/W\text{-}FKB}$ cells were grown in the presence of AP20187, and western blotting using KIT phosphotyrosine 730 specific antibody directly associated this rescue with activation of KIT by AP20187. Our data strongly support a role for KIT in the survival of differentiating ES cells *in vitro* by suppressing apoptosis.

Screening mouse chromosome 18 for novel imprinted genes

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To date, only one imprinted gene (*Impact*) has been identified on mouse chromosome 18. However, mice with Uniparental Disomy (UPD) for chromosome 18 die in gestation, suggesting other imprinted genes map to chromosome 18. Mice with UPD contain chromosomes that have originated from just one parent of origin. RNAs from these mice can be used to assay genes that are expressed in a parent-of-origin-dependent manner. A comparison was carried out of Affymetrix GeneChip expression patterns in whole 8·5 d.p.c. embryos with a maternal duplication versus those with a paternal duplication of chromosome 18 to identify candidate imprinted genes. The imprinting status of candidates was confirmed using allele-specific assays. Further analysis of parent-of-origin imbalance of chromosome 18 is being studied using mice with trisomy for chromosome 18. Comparisons are made between trisomy mice with two maternally derived chromosomes and trisomy mice with two paternally derived chromosomes. This should reveal the effect of copy number on gene expression. To date, we have found one gene that is monoallelically expressed from the maternal allele. However, it maps in the centre of a 1 Mb domain rich in maternal and paternally expressed candidate genes. Features of this cluster may determine why mice with UPD 18 die early, and identify developmentally significant genes.

Dynamics of placental imprinting: a comparison between mouse and humans

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Genes that are imprinted in both the embryo and extra-embryonic tissues show extensive conservation between mouse and human. Here we examine the human orthologues of mouse genes imprinted only in the placenta, assaying allele-specific expression and epigenetic modifications. In contrast to their imprinted expression in mouse, these genes are expressed biallelically in human from early trophoblast through to term. This lack of imprinting correlates with absence of allelic histone modifications (H3K27me3;H3K9me2;H3K9ac;H3K4me2) that are thought to contribute to regulation of imprinting in the mouse. These data indicate that, like imprinted X-inactivation, autosomal imprinting specific for placenta is not conserved in the human.

Using comparative genomics to investigate the molecular mechanism underlying FSH muscular dystrophy (FSHD)

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FSHD is an autosomal dominantly inherited human neuromuscular disorder, caused by deletions within a tandem array of a 3·3 kb repeats (D4Z4) located at 4qter. Unaffected individuals have 12–100 repeats at D4Z4, while FSHD patients have <11. Although this mutation was identified in 1992, the disease mechanism remains obscure. Each 3·3 kb repeat has an open reading frame (ORF) containing two homeoboxes; however, no transcript has been identified and it is unclear whether D4Z4 contains a functional gene. One model proposes that D4Z4 deletions alter local chromatin structure, perturbing expression of nearby genes. Here we show that within D4Z4 homologues in apes, Old and New World monkeys, only the ORF is conserved. Furthermore, we have identified a mouse homologue (mD4Z4) in which 5 kb repeats (each containing two homeoboxes) are arranged in a large tandem array. Phylogenetic analysis shows that the mD4Z4 homeodomains are most closely related to those in human and primate D4Z4. By RT-PCR and *in situ* hybridization, mD4Z4 is transcribed in a variety of tissues. Epitope-tagged mD4Z4 protein localizes to the nucleus, consistent with its predicted function as a transcription factor. We suggest that the hypothesis that human D4Z4 encodes a protein should be revisited.

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Comparative sequence analysis of imprinting evolution in the Dlk1/Dio3 domain in extant mammals

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Genomic imprinting is a process by which some mammalian genes are expressed from only one

parentally inherited allele with the other allele being silenced. We are using a comparative sequence approach to investigate the evolution of genomic imprinting function and mechanism in the Dlk1/Dio3 orthologous regions of two mammals. Bacterial clone maps of this region in a marsupial, *Macropus eugenii* (tammar wallaby), and a monotreme, *Ornithorhynchus anatinus* (platypus), are being constructed and sequenced. Dlk1/Dio3 is an ideal region in which to study imprinting evolution as it contains three paternally expressed genes – *Dlk1*, *Dio3* and *Rtl1* – and is also rich in genomic features such as differentially methylated regions (DMRs), large non-coding RNAs, small functional non-coding RNAs and antisense transcripts. Analysis of the orthologous region in chicken identified *Dlk1* and *Dio3* but none of the non-coding transcripts or *Rtl1*, suggesting that these features may have been co-opted into the region to help regulate imprinting. This analysis will allow us not only to ascertain the imprinting status of the genes in this region in these mammals, but also to identify genomic features which might correlate with imprinting control and contribute to our understanding of the epigenetic control of genome function. Furthermore, identification of evolutionarily conserved regions from chicken, platypus, marsupial, mouse and man should provide insight into regulatory elements involved in the control of these important developmentally regulated genes regardless of their imprinting status.

Generation of hypomorphic *Pax9* mouse mutants as a model for oligodontia in humans

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Heterozygous mutations of the *PAX9* gene have been shown to cause oligodontia (lack of more than six teeth) in humans. Haploinsufficiency of *PAX9* has been suggested as the underlying genetic mechanism, but it is not known how this gene dosage reduction affects tooth development. We have generated a hypomorphic *Pax9* mutant allele (*Pax9-neo*) in mice which produces decreased levels of *Pax9* wild-type mRNA by alternative splicing. Homozygous *Pax9-neo* mutants exhibit hypoplastic or missing lower incisors and third molars, and when combined with the *Pax9* null allele (*Pax9-lacZ*), the compound mutants consistently develop severe forms of oligodontia. Furthermore, these mutants show defects in enamel formation of the continuously growing incisors, whereas molars exhibit increased attrition and reparative dentin formation. Missing molars are arrested at different developmental stages and posterior molars are consistently arrested at an earlier stage, suggesting that decreased Pax9 protein levels affect the dental field as a whole. We conclude that *Pax9* is required at multiple stages of tooth development and suggest that variations in *Pax9* expression levels were involved in modulating dental patterns during mammalian evolution.

This work was funded by the Wellcome Trust.

The morphology and genetics of the developing mammalian jaw joint: the role of the Tgf-beta superfamily of signalling molecules in patterning the mammalian jaw articulation

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The shift of the primary jaw joint in mammals from the quadro-articular to the squamosal-dentary has led to a change in the role of the dentary, which now forms the mandible completely, from a simple tooth-bearing membranous bone to an element of increased complexity and patterning. This has included the development of one articular process, the condylar, flanked by two non-articular processes, the angular and coronoid, each important as muscle attachment sites. In addition, secondary cartilages cap the condylar processes and, depending on the species, one or both of the non-articular processes. Mouse knockout studies indicate that the transforming growth factor beta (Tgf-beta) superfamily of secreted proteins is important in the patterning of the mandible. Tgf-beta2 knockouts and Tgfbr2 wnt1/cre knockouts have diminished proximal mandibles, including a loss of the angular process, whilst preserving the secondary cartilage. We show that Tgf-beta2 is expressed in the mesenchyme around the developing articular region of the mandible, and especially in the mesenchyme of the future angular process. A culture system has been developed to investigate the development of the dentary, enabling physical manipulation, addition of growth factors in heparin beads and the addition of inhibitors to the medium or in beads.

Identification of *chuzhoi*, a new mutant with severe neural tube defects, from a recessive mutagenesis screen

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Defects in neural tube closure constitute one of the most common causes of human congenital malformation. However, the genetic causes underlying these defects remain largely undetermined. During ongoing mouse ENU mutagenesis programmes with screening for recessive mutations, we have observed several new mutants with neural tube defects. One mutant currently under analysis is *chuzhoi*. This mutant exhibits craniorachischisis, the most severe form of neural tube defect characterized by an open neural tube from the midbrain/hindbrain boundary throughout the spine. Using a positional cloning strategy we have identified the causative mutation in *chuzhoi* as a splicing defect in the *Ptk7* gene. We are currently performing intercrosses with other mutants with craniorachischisis, including *circletail* and *crash*, in order to characterize genetic interactions with these mutants.

This work is funded by the Medical Research Council.

Zic2-associated holoprosencephaly is due to a defect in prechordal plate development

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Holoprosencephaly (HPE) is the most common defect of forebrain development and in extreme cases results in cyclopia with a proboscis. Nine genes are linked to HPE in humans and five are members of the Sonic hedgehog (SHH) pathway. In addition, mutations in *ZIC2* also cause HPE. The Zic genes are members of the Gli superfamily of transcription factors and the Gli genes are transcriptional mediators of hedgehog signals. On the basis of homology to, and interactions with, the Gli genes, it has been proposed that *Zic2* may act downstream of *Shh* during patterning of the mammalian forebrain. We have isolated a point mutation in the fourth zinc finger domain of mouse *Zic2* which renders the protein unable to bind DNA and ablates the *trans*-activation ability of *Zic2* in cell-based assays. Embryos homozygous for this mutation develop HPE and die at 13·5 d.p.c. with HPE. The assumption that *Zic2* functions downstream of *Shh*, predicts that the forebrain expression of *Shh* in a structure called the prechordal plate (PCP) should be intact in mutant embryos. Unexpectedly, we find that in mutant embryos *Shh* expression in the 9·5 d.p.c. PCP is downregulated or absent. Moreover at earlier stages of development several markers of the PCP are severely depleted, indicating that PCP development fails in these mutants. We show that *Zic2*-associated HPE occurs due to aberrant node function during gastrulation.

Role of the homeobox gene Hesx1/HESX1 in forebrain and pituitary formation in mouse and humans

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Central to this project is the study of the homeobox gene Hesx1/HESX1 (mouse/humans). This gene is a transcriptional repressor expressed in the rostral region of the developing vertebrate embryo. $Hesx1^{-/-}$ mice show variable defects affecting the forebrain and the pituitary gland. In humans a comparable phenotype exists called septo-optic dysplasia (SOD). Various mutations in HESX1 have been associated with SOD and other forms of hypopituitarism. Two recessive mutations of interest are R160C and I26T, located in two different but equally important regions of the protein. HESX1-R160C is associated with a severe phenotype affecting the forebrain and the pituitary gland. This mutation abrogates the DNA binding ability of HESX1. HESX1-I26T, on the other hand, gives rise to a severe pituitary phenotype but with no forebrain abnormalities. HESX1-I26T binds DNA but the mutation diminishes the repressor activity of the protein very significantly. Reasons underlying these differences in the phenotype are not fully understood. To test whether there is a genotype–phenotype correlation, we have introduced these two mutations in the mouse Hesx1 locus by homologous recombination in embryonic stem (ES) cells. The resulting $Hesx1^{I26T/I26T}$ mice show variable eye defects ranging from normal eyes to bilateral anophthalmia. Although mice carrying the R160C mutation have not been generated yet, the two mutant proteins produce different phenotypes in ES cells. As the differences in phenotypes might be due to the disruption of specific protein–protein interactions brought about by the HESX1 mutations, we have carried out a yeast

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two-hybrid screen, and five Hesx1 partners have been identified and partially characterized. Three are nuclear proteins that can repress transcription whilst the other two are proteins of unknown function. Functional analysis of these interactions is under way.

The role of the actin-binding protein Thymosin $\beta 4$ in Hand1-mediated cardiac morphogenesis

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The bHLH transcription factor *Hand1* plays a fundamental role in cardiac looping morphogenesis, an essential process for correct chamber orientation and alignment of the major vessels of the heart. The molecular mechanisms of looping morphogenesis remain undefined. *Thymosin β4* (*Tβ4*) was identified as a potential downstream target of *Hand1* by representational difference analysis. The function of *Tβ4* in remodelling the cytoskeleton is consistent with a role in cell migration that may mediate cardiac looping. We have shown by electrophoretic mobility shift assay (EMSA) that *Hand1* can bind to consensus E-box and Thing1 box binding sites within the *Tβ4* promoter and results in a 3- to 4-fold increase in luciferase reporter activity. Furthermore, *Tβ4* is temporally and spatially co-expressed with *Hand1* in the left ventricle and outflow tract of the developing heart (E10.5). In order to elucidate the *in vivo* role of *Tβ4* in cardiogenesis, we have generated a cre-lox conditional model of RNAi-mediated *Tβ4* knockdown, driven in the heart by crosses with Nkx2-5CreKI mice. Embryos at E14.5 displayed a thin non-compacted myocardium with abnormal blood-filled epicardial nodules, impaired angiogenesis and failure in branching of the aorta. The observed defects are consistent with a failure of migration of epicardium-derived cells into the myocardium and analyses to confirm this are under way.

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Identification of potential Tbx1 targets in a mouse model of DiGeorge syndrome

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DiGeorge syndrome is characterized by craniofacial, cardiovascular, thymic and parathyroid defects most often resulting from a heterozygous 3 Mb deletion of chromosome 22q11. Haploinsufficiency of the *TBX1* transcription factor is considered to be the major underlying cause of this syndrome. Heterozygous mouse models in which a region of MMU16 homologous to HSA22q11 is deleted (*Df1*) and $Tbx1^{+/-}$ mice exhibit aortic arch, thymic and parathyroid defects; $Tbx1^{-/-}$ mice display more severe defects of pharyngeal development. Our microarray experiments have identified a number of candidate *Tbx1* targets by comparing E9.5 $Df1/Tbx1^{lacZ}$compound heterozygotes with wild-type embryos. In order to more clearly define cell autonomous effects of *Tbx1* and circumvent problems associated with tissue loss, we have developed a novel method of comparing *Tbx1*-expressing cells. The *Tbx1* null allele was generated by knocking a lacZ reporter gene into exon 5. By using a fluorescent lacZ substrate, we have isolated specific *Tbx1*-expressing cells by FACS and compared the expression profile of $Df1/Tbx1^{lacZ}$cells with $Tbx1^{+/lacZ}$ cells. Analysis of the microarray data has identified a number of potential transcriptional targets of *Tbx1*. Generating a comprehensive list of *Tbx1* targets will be of vital importance in understanding the development of the pharyngeal system.

Analysis of folate metabolism in the prevention of neural tube defects

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Neural tube defects (NTD) arise when the process of neural tube closure fails during embryonic development, and comprise a group of common and severe birth defects in humans. Folate supplementation has been shown to effectively reduce the occurrence of neural tube defects, whereas suboptimal maternal folate is a known risk factor for human NTD. However, despite extensive research into folate metabolism during the embryonic period of neural tube closure (neurulation), the mechanism by which folate status affects the incidence of NTD remains unknown. Using a folate-sensitive NTD mouse model, we have shown that maternal folate deficiency resulting from a folate-deficient diet causes a significant increase in the incidence of NTD in mutant embryos. Further to this, intracellular and extracellular folate levels have been measured in neurulation-stage embryos to specifically examine how the maternal folate-deficient diet affects embryonic folate metabolism. A series of whole embryos from the

folate-deficient and the normal diet group have been analysed for comparison. Results suggest that intracellular folate levels are highly regulated in accordance with embryonic growth and development, whilst extracellular folate levels are dependent on gestational age.

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Neuroendocrine expression of mouse arylamine *N*-acetyltransferase 2

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Mouse arylamine *N*-acetyltransferase 2 (mNAT2), like its human orthologue hNAT1, is a polymorphic enzyme, with roles in xenobiotic and endobiotic metabolism. The role of mNAT2 in xenobiotic metabolism has been well characterized *in vitro* using medicinal and environmental arylamines as substrates and acetyl coenzyme A as acetyl donor. Extending previous work indicating endogenous roles in folate metabolism and in acetyl coenzyme A homeostasis, mNat2 gene activity is described here during embryogenesis, using a LacZ knock-in/Nat2 knockout mouse model. During the development of the peripheral nervous system, mNat2 is expressed within neurogenic placodes and Rathke's pouch as well as in folate-sensitive, neural-crest-derived sympathetic ganglia. In adult mice, adrenal glands of both sexes show high mNAT2 activity. These results indicate a role for mNAT2 in cells linking environmental input with neuroendocrine response. Many cell types expressing mNat2 are cholinergic. Although *in vitro* enzyme assays show choline is not a substrate, mNAT2 activity may determine the acetyl coenzyme A pool available for acetylcholine synthesis. The phenotypic effect of deleting the mNat2 gene on the sex ratio and mNat2 genotype distribution has been examined in rapid (C57Bl/6) and slow acetylator (A/J) strains, and shows that the effect of the mNat2 null allele is dependent on the genetic background.

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HUMOT: Human and Mouse Orthologous Gene Nomenclature

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The HUGO Gene Nomenclature Committee (HGNC) and the Mouse Genomic Nomenclature Committee (MGNC) have a long history of working together in assigning official nomenclature to orthologous human and mouse genes. Since 2004 we have strengthened this collaboration with the instigation of the HUMOT, Human and Mouse Orthologous Gene Nomenclature, project. We are utilizing all the currently available online comparative genomic data to rapidly identify and assign parallel nomenclature where possible to all human and mouse orthologous gene pairs. There is a need in both the human and mouse research communities to establish the relationships between genes in these genomes. Here we review many of the resources currently available for identifying orthologues and paralogues, and for viewing regions of synteny between genomes. We discuss how these can and are being used in the process of nomenclature assignment and also introduce the HGNC Comparison of Orthology Predictions search tool, HCOP (Wright *et al.*, 2005; http://www.gene.ucl.ac.uk/cgi-bin/nomenclature/hcop.pl). HCOP enables users to compare predicted human and mouse orthologues for a specified gene, or set of genes, from either species according to the orthologue assertions from the Ensembl, HGNC, Homologene, Inparanoid, MGI and PhIGs databases. This tool provides a useful one-stop resource to summarize, compare and access various sources of human and mouse orthology data. If you have any queries with regard to our work then please contact us at nome@galton.ucl.ac.uk.

Spina bifida: from chromosomal abnormality to candidate gene

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In Sweden, the prevalence of spina bifida in newborn children is approximately 2·5 in 10 000 births. We are collecting blood samples from Swedish patients with spina bifida for DNA extraction and EBV cell transformation. Cytogenetic analyses show that

two patients with spina bifida carry rare structural chromosomal abnormalities. The approach of using chromosomal rearrangements in key patients may be an important first step towards the identification of a disease gene since a chromosomal rearrangement may pinpoint the localization of a disease gene. We hypothesize that the chromosomal rearrangements in the two patients with spina bifida may result in a phenotype because of the disruption of a functional gene or genes. In order to positionally clone candidate genes for neural tube defects, a detailed mapping of the chromosomal abnormalities is in progress. Candidate genes are analysed for expression patterns and for mutations in patient DNA. Also, we have initiated array-based CGH (comparative genomic hybridization) analysis in patients with MMC to screen for minor chromosomal abnormalities.

Disruption of Bardet-Biedl syndrome ciliary proteins perturbs planar cell polarity in vertebrates

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The evolutionarily conserved planar cell polarity (PCP) pathway (or non-canonical Wnt pathway) drives several important cellular processes, including epithelial cell polarization, cell migration and mitotic spindle orientation. In vertebrates, PCP genes play a vital role in polarized convergent extension movements during gastrulation and neurulation. Here, we show that mice with mutations in genes involved in Bardet-Biedl syndrome (BBS), a disorder associated with ciliary dysfunction, share phenotypes with PCP mutants including open eyelids, neural tube defects and disrupted cochlear stereociliary bundles. Furthermore, we demonstrate genetic interactions between *BBS* genes and a PCP gene, *Vangl2*, in both mouse and zebrafish; in the latter we show that the augmented phenotype results from enhanced defective convergent extension movements. We also show that *Vangl2* localizes to the basal body and axoneme of ciliated cells, a pattern reminiscent of the BBS proteins. These data suggest, for the first time, that cilia are intrinsically involved in PCP processes.

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Mutations in the endosomal ESCRTIII complex subunit CHMP2B in frontotemporal dementia

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Frontotemporal dementia (FTD) is a common cause of early-onset progressive dementia characterized by personality and/or language changes. FTD loci have already been identified on chromosome 17 and chromosome 9. In addition an autosomal dominant form of FTD in a Danish family has previously been linked to the pericentromeric region of chromosome 3 (FTD3). This project aimed to identify the mutant gene causing FTD3. Haplotype analysis narrowed the disease locus to a physical distance of 15·5 Mb between flanking markers, *D3S3581* and *D3S3690*. Sequence analysis of candidate genes identified a mutation in the 3′ acceptor splice site of exon 6 in *CHMP2B* that segregated with only affected FTD3 family members. This mutation disrupts RNA

processing of *CHMP2B* resulting in the formation of two aberrant splice variants: $CHMP2B^{Intron5}$ due to the inclusion of intron 5 into the mRNA and $CHMP2B^{\Delta 10}$ due to the activation of a cryptic splice site within exon 6. Overexpression of the aberrant CHMP2B isoforms in an *in vitro* cell model showed disruption in the cellular localization of CHMP2B and the endosomal pathway. The identification of mutations in *CHMP2B* in FTD adds to the growing body of evidence in the literature that supports endosomal dysfunction as an underlying mechanism of neurodegeneration.

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