

- 1) **FORMATTING INSTRUCTIONS AND APPLICATION PROCEDURE**
- 2) **FREQUENTLY ASKED QUESTIONS**
- 3) **PROJECT DESCRIPTIONS:**

- **Clinical and Population Health**
- **Genetics and Gene Therapy**
- **Infection and Immunity**
- **Neurosciences and Mental Health**
- **Development, Disease and Stem Cells**

1) **Formatting instructions/email application procedure:**

- (a) Email applications should have “PhD Studentship Application” in the subject field, and should comprise:
 - a covering letter
 - CV (**please include grades for all degrees and examinations listed**)
 - a summary/abstract of any research project already undertaken (which should be no more than one side of A4)
 - the names and addresses of two referees.
- (b) Save your application as a single Word document attachment. Name your Word document using your surname first and then your first name, eg Smith John.doc
- (c) Please indicate in your covering letter where you saw the advertisement and also where else you have looked for studentships.
- (d) Please state your nationality and how you will fund international fees if applicable.
- (e) Applications should be sent direct to the Research Degrees Administration Office (ICH.Chratapps@ucl.ac.uk).
- (f) **SEPARATELY**, you should arrange for your two referees to provide a reference once you have submitted your application. Please ask your referees to use the [Reference Form](#) provided (also available via main advertisement) and to send the reference by email to ICH.Chratapps@ucl.ac.uk no later than **2nd January 2013**.

Overseas applicants should also see FAQ1 below BEFORE submitting an application.

Please note that if you apply without following the guidelines given above, your application may not be considered.

2) **Frequently Asked Questions**

Q1. Can students from outside the UK apply?

A. Yes, overseas students have previously been accepted into the programme. The PhD Programme in Child Health Research will fully fund UK/EU students. Non-UK/EU students receive the normal stipend, and the UK/EU component of their fees is paid, but they must pay the extra overseas fees themselves (the current difference for 2012/13 is £15,050 per year and there is normally a 4 per cent increase on fees each year). Furthermore, all candidates who are selected for the Programme must be interviewed, and we unfortunately have no money to pay for overseas students to come to interview.

Q2. Can I come to visit the Institute before the interviews?

A. Yes, there is an Open Day at ICH on Wednesday 21 November 2012 from 2.00pm onwards when prospective applicants will meet the Postgraduate Tutors, existing PhD students, and have the opportunity to take up tours of the facilities. You can also make direct contact with potential supervisors if you wish.

Q3. I have a lower second degree but I am now doing an MSc. Is this equivalent to an upper second?

A. Assuming you passed your MSc, you could be eligible to hold a PhD studentship. Please note, however, that in the previous year's applications nobody with such a background was successful.

Q4. Does my age matter?

A. No.

Q5. I will be away at the time of the interviews (Monday 28th and Tuesday 29th January 2013).

A. Contact us: we may be able to interview early.

Q6. Do I need to have chosen my project from the portfolio, prior to interview?

A. No. The best candidates will be selected and offered studentships and we will then arrange for you to come back to ICH and visit potential supervisors/labs before you make a final decision. However, if only one or two projects would be of interest to you because of your specialist background, please say so in your covering letter.

Q7. I have another PhD offer, which needs a decision before you decide on your studentships.

A. Ask them to wait (they usually will); if not, contact us.

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Quantile inference for extreme value and risk assessment in child health research

Supervisors: Dr Mario Cortina-Borja and Dr Marco Geraci

Hypothesis: Extreme (very high or very low) observations of medical/biological outcomes are found in many studies. These outcomes usually show strong associations with morbidity and mortality risks. Examples include extreme birthweights in perinatal studies (e.g., more than 5 kg or less than 1 kg), extreme blood pressure measurements in cardiovascular research and very low CD4 cell counts in HIV studies. In statistical analyses, such extreme values are often treated as outliers, usually because (i) they come from a population different from that under study (e.g., because of genetic anomalies that cause unusual deviations); or (ii) the statistical model used to describe the data is unable to satisfactorily account for such deviations; or (iii) such extreme values are effectively gross observational errors. In the first two cases, these observations can provide important information about biological processes that, statistically speaking, are out of control or need improved analytical approaches. Common techniques for the identification of extreme values that are suspected to be outliers rely on some practical thresholds that are calculated using methods based on strong assumptions about the data-generating process. However, if the premise is false (that is, the adopted model is incorrect) such rules will lead to a loss of valuable data. Quantile inference [1] provides a framework of analysis in which it is possible to conduct statistical modelling, estimation, and hypothesis testing in the tails of the distribution, that is in regions of data away from the “centre” (i.e., the mean).

Aims and methods:

- 1) To study the statistical properties of sample quantile estimators for the analysis of marginal and conditional distributions;
- 2) To develop confidence interval estimation methods and hypothesis testing procedures for the assessment of the first and second derivatives of quantile functions, in both distribution-free and distribution-based settings;
- 3) To derive practical and easy-to-implement techniques for assessing and testing risk exceedance;
- 4) To develop an R package to be made available to the scientific community at large;
- 5) To apply these methods to available child health datasets from large population-based data collections (e.g. the US Linked Birth and Infant Death Data collection, the 1958 Birth Cohort Study and the Millennium Cohort Study).

The first part of the project will involve literature search and critical appraisal of key papers, review of software packages and preparation of the datasets. Research methods will be based on academic search engines (e.g., Web of Science, Google Scholar, Pubmed), reference management software (Endnote) and statistical software (R, Stata). Report writing will be conducted using TeX typesetting programs (MiKTeX, BibTeX, TeXworks). The second part of the project will focus on the methodological developments. In particular, existing statistical inferential methods for quantiles [1] and extremes [2,3,4] will be studied in depth and new approaches will be devised. Programming in R language will be essential for simulation-based performance evaluation and for package implementation. C/Fortran programming may also be considered. The development of the R package will build on existing software [5]. Given the longitudinal/ multilevel/ spatially clustered nature of some studies, consideration will be given to data correlation [6], weighting in complex survey designs and non-response bias adjustments.

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6. Geraci M, Bottai M. Quantile regression for longitudinal data using the asymmetric Laplace distribution. *Biostatistics* 2007;8:140-154.

The impact of eating disorders on bone health and development in adolescence

Supervisors: Dr Nadia Micali and Dr Jon Tobias

Hypothesis:

- 1) Overall ED behaviours across adolescence will be associated with lower Bone Mass Density at age 17.5.
- 2) Cortical circumference of the bone, bone thickness and density will also be affected. Excessive exercise for weight loss might be protective against low Bone Mass Density in some adolescents. Gender differences will be highlighted. Caloric restriction, fat mass, pubertal timing and genetic factors will be investigated as potential mediators or moderators.

Aims and methods:

Eating disorders have a peak incidence in adolescence¹, a crucial time for physical and skeletal development; when bone mass reaches its highest level. The level of bone mass attained at this age is a key determinant of long-term bone health and risk of osteoporotic fractures in later life². There is some evidence that eating disorders have negative effects on bone development, especially if they onset in adolescence³. The current lack of evidence impacts on available prevention and early treatment for adolescents with eating disorders, this study will allow clear identification of causal biological mechanisms that could help preventative and therapeutic efforts.

Aims:

- 1) To determine the impact of adolescent ED/ED behaviours (measured at age 13, 14 and 16) on bone density at age 17.5 (focusing on hip BMD) and fractures;
- 2) To determine: a) whether associations found with hip BMD reflect an influence on cortical circumference, thickness or density (based on tibial pQCT); b) the role of factors such as caloric restriction, fat mass, pubertal timing, bone turnover, excessive exercise, gender and genetic factors (polymorphisms of RANKL) in explaining the effect of ED on bone health.

Methods:

This is a longitudinal study, based on data prospectively collected as part of the Avon Longitudinal Study of Parents and Children (ALSPAC): a cohort of 14,000 mothers and their children enrolled in pregnancy. Children have been followed up at regular intervals from birth onwards (N=10,000).

Data collected at ages 13,14 and 16 on eating disorders behaviours will be used to predict bone development. The main outcome measure will be Bone Mass Density at age 17; fractures at age 15 and at age 21, and other bone characteristics (such as cortical geometry and strength of the mid-tibia) will be investigated. Collected data will be extracted; new data will be collected at age 20/21 on fractures. Univariate and multivariate logistic/linear models will be used to determine the effect of relevant predictors on outcomes. Risk mechanisms will be investigated using multivariate regression models and where necessary structural equation modelling, in order to develop risk models.

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3. Misra M. Long Term Skeletal Effects of Eating Disorders and Onset in Adolescence. *Ann NY Acad Sci* 1135: 212-218; 2008.

Quality of life and functional vision of young people with visual impairment: development of age-appropriate patient-reported outcome measures for routine use in paediatric ophthalmology

Supervisors: Jugnoo Rahi, Dr Val Tadić and Ms Phillippa Cumberland

Hypothesis:

We hypothesise that it is possible to use self-report questionnaires to measure the self-perceived socio-emotional and functional impact of living with a visual impairment in young people, and to combine this information with objective clinical assessments to improve understanding of visual status and assessment of endpoint outcomes of health care.

Aims and methods:

Background: The recent and increasing trend for development and NHS application of vision-specific patient-reported outcome measures for assessing the functional and socio-emotional impact of visual impairment has largely excluded children and young people,^{1,2} despite the considerable and lifelong impact of sight loss starting in early life. Thus there is presently a significant barrier to engaging children and young people in planning their routine ophthalmic health care and assessing its outcomes and quality. Equally there is limited scope for child-centred assessment of benefit in clinical trials of new treatments.³ To address this gap, we have recently developed a novel Vision-related Quality of Life (VQoL),⁴ as well as a complementary Functional Vision (FV) questionnaire instrument. These allow children and young people to describe and quantify the impact on their everyday lives of both their visual disability and the health care they receive. We developed these for the age-group of 10-15 year olds, chosen for our 'foundation' research, which involved developing de novo both a conceptual framework and child-centred research methods. Our aim is now to adapt our existing instruments to younger (6-9 year olds) and older (16-18 year olds) patients, to produce a suite of age-appropriate instruments that cover the whole paediatric population.

Aim of the PhD: The overarching aim of our innovative programme is to develop a suite of robust and reliable age-appropriate patient-reported outcome measures of Functional Vision (FV) and Vision-Related Quality of Life (VQoL) of children and young people with ophthalmic disorders that lead to visual impairment. To achieve this we propose to adapt our existing FV and VQoL instrument versions designed for 10-15 year old visually impaired children and young people (v.10-15 years) to two further age groups, i.e., younger (v.6-9 year olds) and older (v.16-18 year olds). The proposed PhD investigation will comprise the research activities relating specifically to the older age group of 16-18 year old young people and offers an exciting opportunity to acquire a range of generic and specific research skills and to develop an interest and expertise relevant to future research in young people with disability in a key 'transitional' life period.

Sample and methods: Potential participants will be patients identified through paediatric ophthalmology services at Great Ormond Street Hospital and Moorfields Eye Hospital. The student will apply the theoretical framework and child/young person-centred methodology that we have piloted in our prior research involving 10-15 year olds to follow the standard phases of questionnaire development. This will involve mixed research methods, combining qualitative research approach, psychometrics and population statistics with public and patient involvement. Specifically, the student will adapt the existing items (questions) or generate new items through in-depth qualitative interviews with children and young people. Secondly, he/she will evaluate the relevance and comprehensibility of the new age-appropriate scales by consulting young people as experts. Finally, he/she will test validity and reliability of the new age-appropriate instrument versions by administering them to a national representative sample of children and young people and applying the appropriate psychometric approaches.

Impact of research: This research has far-reaching implications. Rather than a new therapy, we are developing tools that will enhance the quality of life and well-being of children and young people by affording them a voice in decision making and thus enabling them to be partners in the planning and evaluation of their health care, both in routine clinical practice and in the context of trials of new treatments for blinding disorders. The programme will also advance and develop new approaches/methodologies in relevant to a number of areas of child health research.

References:

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2. Pesudovs K, Burr JM, Harley C, Elliot D.B. The Development, Assessment, and Selection of Questionnaires. *Optometry & Vision Science* 2007;84(8)
3. Clarke SA, Eiser C. The measurement of health-related quality of life in pediatric clinical trials: A systematic review. *Health and Quality of Life Outcomes* 2004;2:66:1-5
4. Rahi JS, Tadic V, Keeley S, Lewando-Hundt G. Capturing Children and Young People's Perspectives to Identify the Content for a Novel Vision-Related Quality of Life Instrument. *Ophthalmology* 2011;118(5):819-824

Evaluating the impact of community based nutritional interventions on the agency and empowerment of women in rural Nepal

Supervisors: Dr Jolene Skordis-Worrall and Dr Joanna Morrison

Hypothesis: The success of interventions to improve the nutrition of pregnant mothers and the birth weight of their babies will, in part, be determined by a women's ability to control her own, and her children's' nutritional intake during pregnancy and in the post partum period.

Aims and methods:

A research collaboration between the Centre for International Health and Development at University College London, MIRA Nepal, Save the Children and the World Food Programme is testing the impact of several interventions on the birth weight of babies. One intervention gives food supplements to women, another gives cash transfers to women, and a third intervention supports community women's groups to discuss and take action to improve nutritional health. We believe that one of the main factors affecting whether these interventions are successful or not, is the extent to which they impact on women's ability to control her own, and her children's nutritional intake during pregnancy and in the post partum period. A sub-study designed to explore this hypothesis will use qualitative and quantitative methods. Qualitative methods will include in-depth interviews, focus group discussions and participant observation to explore appropriate measures of agency and empowerment in the context of this trial, and also to understand how respondents interpret and respond to the quantitative questions. A panel survey of mothers in the three arms of the trial will constitute the quantitative elements of the study. The panel survey will aim to measure the extent to which each intervention has impacted the agency and empowerment of the participating mother and also the extent to which any changes in her agency and empowerment has resulted in nutritional improvements for herself and her baby.

We seek a PhD student to join this multidisciplinary team in considering the local relevance of existing measurements of women's empowerment and agency, and measuring empowerment and agency within the context of this nutritional research.

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Statistical models for CD4 cell counts and HIV RNA viral load in HIV-infected pregnant women and children

Supervisors: Dr Claire Thorne, Dr Mario Cortina-Borja, and Dr Claire Townsend

Hypothesis: Statistical models for joint immunological/virological markers in HIV-infected women and their offspring provide important information to aid decision-making regarding treatment as prevention.

Aims and methods: In patients with untreated HIV infection, a decline in the immune system (immunodeficiency) occurs progressively, due to depletion of white blood cells (CD4+ T lymphocytes, or "CD4 cells"). These cells play a critical role in the immune system and are the target of the HIV virus. Treatment usually involves a combination of 3 or more drugs, which prevent HIV replication and allow the immune system to recover. Effective treatment has led to reduced mortality and disease progression worldwide. However, barriers to successful treatment include failure to take medications (i.e. poor adherence), drug resistance and side effects. The two key biological markers used to predict disease progression and evaluate treatment effects are CD4 cell count and the level of replicating HIV virus in the blood ("viral load"). The objective of treatment is to suppress viral load to an undetectable level (1-2), below the assay detection limit (e.g. <50 copies/ml).

There is a close relationship between viral load and CD4 cell count, which is influenced by treatment, adherence, and other factors. Changes in these two markers are usually investigated with separate statistical models (3); but in this project, more sophisticated (bivariate) models (4-5), particularly copulae (6) will be used to analyse them jointly. Data from HIV-positive pregnant women and children living in Europe will be used, and several statistical issues will to be addressed, e.g. modelling within-subject correlation, constructing copula regression models, and using left-censoring models to account for varying assay thresholds. Analysing these data in new ways will lead to a better understanding of the dynamics of HIV disease progression, and response to treatment, and improved clinical management.

The proposed work is methodological in nature, based on "real life" data on HIV positive women and children collected in an on-going European cohort study. This research will provide appropriate and efficient statistical models for analysing markers of viral infections such as HIV. This will lead to a greater understanding of how HIV infection develops in childhood and pregnancy, by describing the elimination and production of the virus, especially during treatment, and at the same time as assessing the impact on the immune system (i.e. CD4 cells). Data patterns that emerge only as a consequence of the joint modelling of CD4 and viral load may lead to improvements in our understanding of HIV disease progression and response to treatment, and ensure that clinical management is optimised. This is particularly important for HIV-infected children, who may start lifelong treatment during infancy.

The successful candidate, based within the Centre for Paediatric Epidemiology and Biostatistics, will develop and implement statistical methods to analyse joint variation in longitudinal CD4 cell counts and VL measurements in HIV-positive pregnant women and children and disseminate these analyses in order to improve the clinical management of HIV-positive pregnant women and their offspring. Note that although the project focuses on HIV-positive pregnant women and children, its methodological findings will apply to other situations; e.g., where no gold standard for a definitive diagnosis exists and results of two immunoassays are used to assess infection status. The project aims to develop complex statistical methods directly addressing real-life problems posed by observational data. This is an exciting opportunity to develop methodological skills whilst also gaining hands-on experience of working on data from a large cohort study with real and useful applications.

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Analysis of the functional interaction between trkA, SHP-1 and p53: New prognostic markers and therapeutic targets for Neuroblastoma

Supervisors: Dr John Anderson and Dr Ximena Montano

Hypothesis:

- 1) To test the hypothesis that trkA activation by p53-dependent repression of SHP-1 expression induces trkA-dependent upregulation of the transcription factor C/EBP α to promote differentiation of neuroblastoma cells.
- 2) To test the hypothesis that the proteomic expression of neuroblastoma cells expressing activated trkA by p53-dependent SHP-1 repression versus not activated trkA, may identify a potential biomarker(s) of neuroblastoma differentiation.

Aims and methods:

In neuroblastoma, the most frequent childhood extracranial tumour¹, the roles of the nerve growth factor (NGF) receptor trkA, the trkA-tyrosine-phosphatase SHP-1 and the tumour-suppressor and transcription factor p53 are not clear and need to be fully defined. trkA and p53 play complementary roles in proliferation and differentiation of neoplastic and non-neoplastic cells, thus suggesting a functional connection between them. This is supported by our experiments demonstrating that p53, in the absence of NGF-stimulation, induces trkA tyrosine (Y) phosphorylation and activation, together with initiation of pathways involved in differentiation of the sympathoadrenergic rat pheochromocytoma PC12 cell line^{2,3}. SHP-1 dephosphorylates trkA-Y674/Y675⁴. Thus we have revealed that, repression of SHP-1-expression by p53 induces trkA-Y674/Y675 phosphorylation and suppression of breast-cancer cell proliferation by promoting a trkA-dependent cell-cycle arrest in the absence of NGF⁵. Moreover, our initial analysis has demonstrated that in neuroblastoma cells lines p53 represses the expression of SHP-1 and leads to trkA-Y674/Y675 phosphorylation.

The aim of this proposal is to determine the molecular mechanisms activated by trkA, through its functional connection with p53 and SHP-1, and their possible roles promoting differentiation of neuroblastoma cells in the absence of NGF stimulation. To test the functional consequences of SHP-1 repression by p53 for trkA activation we will be taking two complementary approaches. Given that the transcription factor C/EBP α has a possible role in neuroblastoma differentiation⁶, we will assess if C/EBP α levels are increased, in a trkA-dependent manner, by p53 repression of SHP-1. We will also identify the signal transduction pathway(s) involved in this increase. We will investigate C/EBP α and the signalling proteins involved in its increase as possible prognostic markers. Analysis will be carried out using immunoprecipitation and Western blotting as well as cell cycle determining techniques such as flow cytometry. We will also identify novel prognostic markers by using neuroblastoma cell lines to test, by proteomic analysis, the outcome of p53-dependent repression of SHP-1, in terms of trkA-phosphorylation and tumour differentiation.

The analysis of proteins involved in this type of trkA activation will determine how they influence neuroblastoma growth properties and be a target for chemotherapy. Together these findings will improve child healthcare as they will provide information to identify best possible treatments and facilitate the development of novel and target-specific drugs.

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Investigating the genetic basis of kidney stones

Supervisors: Dr Detlef Bockenhauer and Professor Robert Kleta

Hypothesis: Urolithiasis (stones in the kidney and urinary tract) is a major public health problem: The life-time risk of kidney stones in the Western world is approximately 10%, resulting in economic costs of about \$5 billion annually in the US alone. The causes for the development of kidney stone are complex, but likely involve a combination of genetic and environmental factors. Obviously, the younger the patient, the more important the genetic factors are. Indeed, in the majority of children with kidney stones an underlying metabolic abnormality can be detected, most commonly an increased excretion of calcium in the urine (hypercalciuria). We hypothesise that excess urinary excretion of calcium (hypercalciuria) can be caused by genetic variants in genes involved in renal calcium handling. Identification of such genes would provide a basis for rationale treatments.

Aims and methods:

The aim is to identify genes involved in excess calcium excretion

Methods:

- 1) At GOSH we have a specialised clinic for children with kidney stones. This provides a unique to obtain DNA from patients and families, as approximately 240 children with kidney stones are seen there annually and the most common underlying metabolic abnormality is hypercalciuria.
- 2) We will perform linkage analysis in families with more than one member affected.
- 3) We will perform whole exome sequencing in approximately 50 patients with hypercalciuria, including the families
- 4) We will perform bioinformatic analysis of the linkage and exome data to identify genes with rare variants in more than 1 patient
- 5) We will then perform assessment of functional consequences of identified variants. The models used for this will depend on the gene identified but will likely involve cell culture of renal epithelial cells.

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Discovering new genetic causes of auto inflammatory disease in children

Supervisors: Dr Paul Brogan and Professor Nigel Klein

Hypothesis:

Using state of the art genetic mapping and next-generation sequencing techniques, it is possible to discover novel monogenic diseases resulting in pathological inflammation.

Aims and methods:

Primary objective: Identify responsible mutations for undefined familial autoinflammatory diseases (first half of project)

Secondary objective: To further characterise the phenotype of the mutation on the patients' cells and in vitro cell culture models (second half of project).

The term autoinflammatory disease was coined just over 10 years ago following the discovery of the genetic cause of familial Mediterranean Fever (Pyrin mutation) (1), followed rapidly by the discovery of the genetic cause of another inflammatory disease called TRAPS (TNF receptor associated periodic fever syndrome, caused by mutations in the TNFR1 gene) (2). Autoinflammatory diseases are distinct from autoimmune diseases since they result in seemingly unprovoked inflammation, in the absence of autoantibodies, and without the presence of autoreactive T cells (3). Many are associated with genetic defects in the innate immune system. We are currently developing an exciting new programme of research at Great Ormond Street Hospital (GOSH) that aims to discover new genetic causes of inflammatory diseases in children. This unique opportunity is possible because of a rapidly expanding clinical service at GOSH that has been developed over the last 10 years in collaboration with the Royal Free Hospital in London: the periodic fever syndrome clinic. In the first part of project, the student will analyze affected individuals belonging to 2 (from a choice of five) consanguineous (i.e. parents first cousins) families suffering from a recessive disorder. Homozygosity mapping will be carried out through high-throughput SNPs genotyping analysis. All homozygous areas absent in unaffected subjects and shared among affected individuals will be identified using the Illumina GenomeStudio software. Good candidate genes which emerge will be sequenced in a conventional manner. The second half of the Ph.D. studentship will study the functional impact of these mutations on the immune system, using in vitro models including transfection of cells in tissue culture. It is likely that the student will focus on one of the two families studied in the first half of the project, although if time permits it may be possible to perform functional studies in both families dependent on the findings.

This exciting Ph.D. studentship will provide training in gene discovery using state of the art genetic mapping techniques, and next-generation sequencing; in addition the student will develop a portfolio of generic scientific and laboratory skills to study the functional implications of novel genetic variants discovered. This unique opportunity is made possible by the recent rapid developments in genetic technology, which now can be applied to a unique clinical cohort of patients with autoinflammatory disease looked after at GOSH. The student will join a vibrant and growing research group working in this area, and will be well supported by two post-doctoral research assistants working in this area, and by the primary and secondary supervisors. In addition, access to UCL genomics and bioinformatics expertise will be provided.

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Identifying the genetic basis of severe human spina bifida

Supervisors: Professor Andrew Copp, Dr Philip Stanier and Dr Nick Greene

Hypothesis: Recent research by the supervisors identified unique genetic (missense) changes in human fetuses with severe spina bifida [1]. The changes affected genes of the 'planar cell polarity' (PCP) pathway, which is known to be involved in early embryo development [2]. *In this PhD project, the student will test the hypothesis that these genetic changes are the likely cause of the spina bifida in the human fetuses.*

Aims and methods:

Spina bifida is a severe birth defect that causes death of the fetus or handicap in surviving children [3]. Genetic factors are known to be important in spina bifida, but few specific genes have been identified [4]. We showed that several of the putative human PCP mutations alter protein localisation, in cell culture. However, we do not yet know if these mutations are the cause of the human NTDs. This PhD project consists of the following studies:

1. *Cell culture experiments.* The student will study whether the genetic changes identified in the human fetuses adversely affect PCP protein function within the cell. Vectors expressing tagged (e.g. GFP, HA) CELSR1 or SCRIB proteins will be co-transfected into MDCK cells along with other tagged PCP proteins, including VANGL1, VANGL2 and PK1.

- Co-immunoprecipitation assays will determine whether CELSR1 or SCRIB can pull down other PCP proteins, and whether this is adversely affected by the missense variants.
- Fluorescence confocal microscopy will be used to determine whether the normal membrane localization of other PCP proteins is disrupted when co-expressed with the mutant CELSR1 or SCRIB proteins.
- Markers of endoplasmic reticulum (ER; e.g. protein disulphide isomerase, PDI) will be used to determine whether the missense variants of CELSR1 or SCRIB cause the proteins themselves, and/or other co-transfected PCP proteins, to be retained in the ER [5].

2. *Creation of new mouse models of human PCP mutations.* The student will introduce several of the genetic changes identified in the human *CELSR1* and *SCRIB* genes into mice, by replacing the normal mouse DNA sequence with the faulty 'human' sequence. This will show whether having two copies of the faulty gene causes NTDs in the mouse embryo. Steps in this part of the study are:

- Homologous recombination and site-directed mutagenesis will be used to introduce knockin mutations into embryonic stem (ES) cells. Targeted ES cell clones will be injected into mouse blastocysts to generate chimeras, and these mice will be bred to achieve germ line transmission. A colony of heterozygous knockin mice will be established, providing a model of the human mutation.
- Knockin mice will be phenotyped to determine the effect of the new mutant gene. Heterozygotes are predicted to be unaffected. Homozygotes will be bred and examined for NTDs at embryonic days 9.5-11.5. Knockin mutations will also be bred as compound heterozygotes with each other, and with known pathogenic mutations at each locus.

Significance: This project could demonstrate, beyond doubt, whether the genetic changes we have identified are truly related to the origin of severe NTDs in humans. This would constitute the first definitive identification of 'spina bifida genes' in humans and could pave the way towards improved genetic counselling and targeting of preventive therapies such as supplementation with folic acid.

Laboratory environment: The student will work in a well-funded lab in which several postdoctoral fellows are available to supervise the laboratory work of early-stage students. UCL Transgenics will provide assistance with creation of knockin mouse strains.

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Gene modification of autologous T cells for gene therapy of X-linked lymphoproliferative disease

Supervisors: Professor Bobby Gaspar and Dr Christine Rivat

Hypothesis: X-linked lymphoproliferative disease (XLP) is a primary immunodeficiency characterised by profound immune defects, affecting in particular T cell responses and the T-dependent responses to pathogens. The aim of this project is to correct the cellular and humoral defects of a relevant murine model of XLP using the adoptive transfer of corrected T cells into the animals.

Aims and methods:

X-linked lymphoproliferative disease (XLP) is a rare inherited disorder characterised by severe immune dysregulation following viral infection, commonly with Epstein-Barr virus. Clinical manifestations include fulminant infectious mononucleosis (60%), dysgammaglobulinemia (40%), and lymphoproliferative disorders, generally B-cell lymphoma (30%).

XLP is associated with mutations in SH2D1A (Xq25), which encodes the SLAM-associated protein (SAP), a small signalling adaptor expressed predominantly in T, NK, NKT cells and some B cell populations. In the absence of SAP, the SLAM-dependent signalling pathways are disrupted, which results in functional defects in multiple lineages. In CD8+ cytotoxic T cells and NK cells, interferon- γ (IFN- γ) secretion and cytotoxicity are impaired, while in the CD4+ helper T cells, the decrease in Th2 cytokine production leads to a defective T-B cell cooperation and consequently to dysgammaglobulinemia and the absence of memory B cells. XLP patients also have a notable absence of the NKT cell subset.

The conventional treatment for XLP is Haematopoietic Stem Cell transplant (HSCT), which can offer a permanent cure although graft-versus-host disease and the complications associated to the conditioning regime are major complications¹⁷. However, the mortality can be high in the absence of a fully-matched donor, and in that context, the transfer of autologous gene-corrected cells would be an attractive alternative for XLP patients.

Several murine models for XLP have been engineered, with a resulting phenotype that recapitulates most of the features of XLP (lack of NKT cell development, abnormal T cell response to immune challenge, defective humoral immunity).

Preliminary results obtained in our group have demonstrated the feasibility of gene transfer into haematopoietic stem cells to correct the phenotype of SAP deficient mice. This includes correction of NK cell cytotoxicity, recovery of basal immunoglobulin levels and correction of T dependent antigen specific responses and germinal center formation. However, with this haematopoietic stem cell (HSCs) approach, all the lineages derived from the modified stem cells will express SAP and the ectopic expression of a signalling molecule in tissues where it is normally absent presents a non-negligible risk of oncogenic transformation, in addition to the possibility of vector-associated insertional mutagenesis.

Therefore, other options are now being considered, and in particular the selective transduction and transfer of T lymphocytes, aiming to correct the intrinsic T cell defects and consequently to restore T cell responses and B-cell help and function. Importantly, in a number of clinical gene therapy studies involving transduction of mature T cells using gammaretroviral vectors for treatment of specific cancers (over 100 patients treated) there has not been any evidence of insertional mutagenesis, suggesting that mature T cells are less susceptible to transformation than HSCs. Our project will consist in defining the optimal parameters for efficient primary T cell transduction in order to transplant SAP knock-out mice and assess the reconstitution of immune functions in these animals.

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Exploring gene therapy approaches for neuroblastoma using a patient's own gamma delta T cells

Supervisors: Dr Kenth Gustafsson and Dr John Anderson

Hypothesis:

Human gamma delta T cells transduced with chimeric antigen receptors targeting GD2 ganglioside will effectively target GD2 expressing neuroblastoma cells.

Aims and methods:

Neuroblastoma is the most common childhood tumour apart from brain tumours. Approximately half of neuroblastoma patients fall into the high-risk category, and this patient group require prolonged, morbid and complex treatments to achieve approximately a 50% chance of cure. Therefore, new effective and less morbid treatment approaches are a research priority.

Immunotherapy using antibodies that target a neuroblastoma cell surface molecule GD2, is known to be effective in neuroblastoma [1]. This project brings together two existing strands of research without our group to explore a new approach to deliver GD2 immunotherapy in neuroblastoma. Firstly, we have developed reagents for gene therapy targeting the GD2. By this approach, a patient's own T cells are genetically modified so that they will permanently be specifically redirected against GD2. This is achieved by retroviral with a chimeric antigen receptor that combines the specificity of an anti-GD2 monoclonal antibody with the signal transduction machinery of T cells [2]. Secondly, we have identified new properties of a subset of human gamma delta T cells [3-6]. We have shown that in addition to their killing properties, gamma delta cells can also control and regulate other cells of the immune system, e.g. by taking up and presenting tumour cell antigens to other T-cells, therefore potentially acting as a vaccine. However, this property is not fully realised unless gamma delta T cells interact with antibodies binding the tumour cell. Our concept ultimately is the genetic modification of neuroblastoma patients' gamma delta cells to recognise GD2 to generate a form of gene therapy that directly kills neuroblastoma but also stimulates a long-term memory immune response against the tumour. As a first step towards combination treatments of anti-GD2 antibody and cell therapy with genetically modified gamma delta T cells, we will test in vitro and in a mouse model whether genetic modification of gamma delta cells in its own right effectively kills neuroblastoma cells and tumour.

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Nanoparticle mediated genetic therapy for spinal muscular atrophy

Supervisors: Dr Stephen Hart and Professor Francesco Muntoni

Hypothesis: Spinal Muscular Atrophy (SMA) is one of the most common inherited diseases with an estimated prevalence of 1 in 10,000 live births. The disease affects cells in the brain and leads to muscle wasting and paralysis and early death although the severity can vary. SMA is caused by a deletion of part of the survival motor neuron gene 1 (*SMN1*) leading to depletion of the SMN1 protein. However, genetic studies have shown that most humans have a closely related gene called *SMN2*, which, has a very similar sequence, and is located very closely to *SMN1* on chromosome 5. Despite their similarities, *SMN2* has a much lower level of activity than *SMN1* because it makes a shorter protein, *SMN2 Δ 7A*, due to differences in exon splicing of the mRNA. However, we have shown that by treating cells with specific oligonucleotides (BAOs), the exon splicing pattern of *SMN2* can be restored to produce a full length SMN2 protein with greatly increased activity level which could overcome the deficiencies of the *SMN1* mutations (1). However, in vivo delivery of BAOs to the affected brain cells in SMA remains a serious challenge. At present the oligonucleotides only work if administered directly to the brain, which is problematic as repeated delivery will be required - an intravenous delivery system would be much better. Nanotechnologies have shown enormous potential for the development of targeted oligonucleotide delivery. We have developed a type of nanoparticle (NP) formulation comprising receptor-targeted peptides with cationic liposomes which have shown great promise for nucleic acid delivery in vivo to a number of tissues (2) including the brain (unpublished). In this project, novel nanoparticle delivery formulations will be developed to deliver novel therapeutic strategies for SMA to the brain by intravenous administration.

Aims and Methods:

1) Optimisation for BAO Packaging and Delivery

The student will optimise nanoparticle (NP) formulations for packaging of fluorescently labelled oligonucleotides with lipids and peptides and then analyze them by fluorimetry (Optima Fluostar) and gel shift assay.

2) Targeted Transfection efficiency in Neuronal Target Cells.

To investigate the motor neuron targeting specificity, different cell types, including human fibroblasts, neuroblastoma cells and primary murine motor neurons, will be cultured and transfected with peptide-targeted NP-BAO formulations that bind to neuron-specific receptors. Transfection efficiency will be detected by fluorescence microscopy and flow cytometry.

3) Efficiency of restoration of *SMN2* splicing

Efficacy of BAOs delivered by RTNs will be tested by correction of *SMN2* splicing in transfections of fibroblasts cultured from SMA patients. Correction of the splicing of *SMN2* will be analyzed at the transcriptional level by quantifying the change in the ratio of full-length *SMN2* to *SMN2 Δ 7A* mRNA by qPCR of cDNA and by western blotting to measure the expression of SMN protein. Dosage, time-course and cellular toxicity will all be optimised.

4) In vivo Delivery of NP-BAOs to Neurons in Mice

RTNs containing fluorescent Cy5-labelled oligonucleotides will be injected intravenously *via* tail vein in wild-type mice. Sixteen hours after injection, mice will be sacrificed and the uptake of the nanocomplexes in brain and spinal cord examined by flow cytometry and compared to other tissues.

5) Rescue of SMA mice

A mouse model of SMA with a mild version of the disease will be tested for in vivo restoration of *SMN2* expression by NP-BAO delivery injected intravenously. Brain and spinal cord will be collected at 24h, 5 and 7 days after injection for analysis of mRNA by *SMN2* human-specific qPCR and for protein by Western blotting using human-specific anti SMN antibody. If effective then more severe models of SMA will be evaluated for rescue of the disease phenotype

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- Other project(s) available: [Novel approaches to achieving long-term gene expression in vivo for use in gene therapy of cystic fibrosis](#)

Improving the treatment for Fabry disease: developing gene therapy and identifying biochemical mechanisms responsible for disease progression

Supervisors: Dr Steven Howe, Dr Derralyn Hughes and Professor Simon Heales

Hypothesis:

Lysosomal storage disorders are a group of inherited enzyme deficiencies affecting 1 in 5,000 people. Fabry disease is one of these disorders and patients suffer from cardiac, renal and neurological problems. Currently, the exact cellular mechanisms responsible for disease onset and progression are not known. It is our hypothesis that in Fabry disease there is loss of mitochondrial function. This disruption of energy metabolism is a critical player in the disease and requires correction by an active and persistent treatment regime. Gene therapy, by replacing the missing enzyme (alpha galactosidase A) in Fabry disease may provide an opportunity for long-term correction of the primary defect and lead to the normalisation of downstream biochemical events, such as mitochondrial function.

Aims and methods:

The aims of this project are to:

- (1) Document, in appropriate cellular model systems of Fabry disease, the effects the associated enzyme deficiency has upon energy metabolism at the level of the mitochondrion.
- (2) Evaluate the ability of gene therapy to reduce substrate accumulation and correct the cellular energy deficit.

A range of state of the art biochemical, molecular and cell culture techniques will be employed. Thus, the student will become fully conversant with enzymatic analysis, mass spectrometry and cellular gene transfer using viral vectors. Whilst based predominantly in the Institute of Child Health, strong interactions will also occur throughout the project, i.e. with the NHS diagnostic laboratories (Chemical Pathology) at Great Ormond Street Children's Hospital (GOSH) and with clinicians both at GOSH and the Royal Free Hospital, London.

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Understanding the genetic mechanisms of diabetes mellitus in children with complex novel syndromes

Supervisors: Dr Khalid Hussain

Hypothesis: Complex diabetes syndromes are associated with new genetic mechanism/s

Aims of the project:

- a) To define the phenotype and genotype relationships of novel syndromes which cause diabetes mellitus
- b) To understand the genetic and molecular mechanisms of diabetes mellitus in these novel syndromes

Background:

Diabetes mellitus (DM) is common in the paediatric population. Type 1 DM is the most common but type 2 DM is also being increasingly diagnosed due to the obesity epidemic. Apart from Type 1 and Type 2 DM there are a large number syndromes associated with DM. Several rare syndromes have been described which are associated with glucose intolerance and or DM. These syndromes include Wolfram, Wolcott-Rallison, Alström, Bardet-Biedl and the Thiamine responsive megaloblastic anaemia syndromes.

As part of an international collaboration in understanding the genetic mechanisms of rare syndromes associated with DM we have identified 12 patients who have a completely new clinical phenotype associated with DM. The clinical features observed in our patients include sensorineural hearing loss, seizures, marked developmental delay, severe early onset weight gain and brain malformations (including cerebellar hypoplasia, Dandy walker malformation and lissencephaly). We have already sequenced the genes (*WFS1*, *EIF2AK3*, *ALMS1*, *ABCC8*, *KCNJ11*, *m.3243A>G* mitochondrial disease) which lead in part to the above mentioned phenotypes (such as Wolfram, Wolcott–Rallison, Bardet-Biedl, Alström, Roger, and Mitochondrial syndromes) and found no abnormal coding variants.

Methods:

This project will involve applying cutting edge techniques in genomics to try and understand the genetic and molecular basis of the DM in these patients. Several simultaneous approaches will be adopted including candidate gene approach, homozygosity mapping analysis in consanguineous pedigrees, whole exome sequencing and whole genome sequencing. Mapping homozygous regions in affected individuals in consanguineous families is a powerful method of localising autosomal recessive genes. SNP analysis will be performed for all affected patients and also for unaffected siblings in order to reduce the number of potential regions for analysis. Whole exome sequencing is a strategy to selectively sequence the coding regions of the human genome to identify functional variations associated with a phenotype or pathology. Whole genome sequencing will be undertaken in those patients where exome sequencing is unsuccessful. Whole genome sequencing will be particularly relevant in those non-consanguineous families with 1 affected child. To understand how the novel variant/s leads to DM the project will involve undertaking immunohistochemical methods for looking at expression of the relevant gene, understanding protein-protein interactions using the yeast two-hybrid system, setting up siRNAs methods to knockdown the gene of interest, establishing Luciferase reporter assays, and the development of knockout mice.

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Development of genetic and biochemical tests to identify children whose epilepsy can be treated with vitamin B₆

Supervisors: Dr Philippa Mills, Professor Peter Clayton, Dr Paul Gissen

Collaborator: Dr. Nicholas Lench

Hypothesis:

Disorders affecting vitamin B₆ metabolism are a more common cause of epilepsy than reported previously.

Aims and methods:

Approximately a third of children affected by epilepsy have seizures that cannot be controlled with anti-epileptic drugs (AEDs). Some seizures that cannot be controlled by AEDs stop immediately when children are given large doses of vitamin B₆. Whilst vitamin B₆ is present in the body as 6 vitamers – pyridoxine (PN), pyridoxamine and pyridoxal and their 5' phosphate esters, only pyridoxal phosphate (PLP) is the active B₆ vitamer. PLP is the cofactor for approximately 140 enzymes many of which are involved in synthesis and catabolism of brain neurotransmitters. We have identified the genetic basis for two of the vitamin B₆ dependent epilepsy disorders, PNPO-deficiency and pyridoxine-dependent epilepsy (PDE)^{1,2}. Individuals with these disorders have mutations in *PNPO* and *ALDH7A1*, respectively. Mutations in these genes affect the amount of PLP available in the brain and result in epilepsy.

We have been investigating a large cohort of patients whose seizures have shown some response to vitamin B₆ treatment. Some of these individuals have mutations in *PNPO* or *ALDH7A1*. For many however, the cause of their epilepsy still remains undetermined. Whilst PNPO deficiency and PDE have been considered rare disorders, more common epilepsies can respond to treatment with B₆³⁻⁷. The genetic basis of these B₆ responsive seizures has yet to be determined although it is believed that they are probably pathophysiologically different from PNPO deficiency and PDE. If we are able to unravel the genetic basis for other B₆-dependent / responsive epilepsies neurologists will be able to rapidly screen for these disorders thereby identifying more easily which patients with epilepsy may benefit from treatment with vitamin B₆.

This project would provide the student with valuable exposure to modern genetic and state of the art mass spectrometry technologies as well as training them in a wide range of functional biochemical techniques. The PhD student will investigate whether vitamin B₆ disorders have 'characteristic B₆-fingerprints' using mass spectrometry based methods⁸. This will facilitate identification of defective step(s) in the B₆ metabolic pathway and will enable rapid diagnosis of future patients and aid in identification of new disorders where B₆ metabolism is not properly regulated. Monitoring levels of B₆ vitamers will help in treatment and may lead to new treatment possibilities.

The student will also exploit next generation sequencing (NGS) methodologies to identify other genes that may be involved in vitamin B₆ dependent/responsive epilepsies. Many enzymes may be implicated in B₆ dependent/responsive epilepsies. A next generation DNA sequencing panel will be developed, using targeted DNA capture and massively parallel sequencing (MPS)⁹⁻¹¹, to enable investigation of all known human genes and human orthologues of genes involved in/or associated with B₆ metabolism in patients who have shown some response to B₆ treatment. We have used similar strategies to investigate patients with other inherited metabolic disorders¹²⁻¹⁴. In patients where no mutations are found using this approach whole exome sequencing will hopefully give insights into these disorders. Proteins in which mutations are identified will be functionally characterised further and the role of these proteins in vitamin B₆ metabolism will be investigated thereby providing a more comprehensive understanding of the mechanism(s) involved in regulating brain PLP levels and ultimately better treatments for these disorders.

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Elucidation of key biomarkers in common complications of pregnancy

Supervisors: Professor Gudrun Moore and Dr Philip Stanier

Hypothesis:

There are measurable molecular changes in chorion villus samples (CVS) that are associated with the three main pregnancy complications: prematurity, pre-eclampsia, and intrauterine growth restriction (IUGR). Some of these molecular biomarkers will be present in the pregnant mother's blood.

Aims and Methods:

Using a combination of high throughput technology and basic molecular techniques, the proposed PhD project aims to elucidate variations in gene expression in the early and term human placenta of normal and complicated pregnancies correlating them with pregnancy outcome.

This study will be divided into three stages as outlined below: Each stage will compromise a year of the PhD.

Year 1: Characterisation of the transcriptome using RNAseq or array based techniques of first trimester placenta (CVS) in four selected groups: IUGR; preterm; pre-eclampsia and normal pregnancies, from a subset of 25-50 of our cohort of >900 CVS samples, and validation of the findings using quantitative RT-PCR. This will be duplicated in term placenta with the same pregnancy complications from our Baby Bio Bank samples which stands at present at >900 trios (Mother; Father; Child DNA/RNA). There are at least 100 sample sets in each cohort from each of the three complications of pregnancy conditions plus many more from normal pregnancies.

Year 2: Subsequently proteomic investigations performed in the state of the art Biological Mass Spectrometer Centre on putative biomarkers will use the same CVS sample cohort and a corresponding sample of mother's blood, obtained during that pregnancy. Data analysis of CVS/maternal blood study will control for the following variables: mother's age; maternal smoking; mother's BMI; alcohol consumption; prescription medication or dietary supplements; diet; mother's birth weight; parity; use of assisted reproductive technology. Data will be analysed using the R statistical package. The student will attend a UCL based course in basic and advanced statistics including R during their first year.

Year 3: In order to clarify whether the biomarker is truly fetal in origin, control maternal bloods from: Those who have never been pregnant; those who have been pregnant in the past (and for whom we have a record of the outcome and birth weight of the pregnancy); those who are currently pregnant (and have provided corresponding CVS samples) will be studied for comparison. Specifically, serum samples will need to be prepared from all 3 groups of women.

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Studying micro RNAs as biomarkers for diagnosis and novel therapeutic targets in muscular disorders

Supervisors: Professor Francesco Muntoni and Dr Jennifer Morgan

Hypothesis:

Muscular dystrophies (MD) are a group of more than 30 inherited conditions characterised by progressive muscle wasting and weakness with variable severity ranging from mild with slow progression to rapidly progressive. The age of onset also differs between the types of MD with some evident at birth and other manifesting in middle or late years. Despite the growing knowledge of novel genes with a role in MD, little is known about the underlying molecular pathways and the secondary factors responsible for the variability in the clinical phenotype.

Micro RNAs are small RNA sequences (~22 nucleotides) that act as post-transcriptional regulators through imperfect base pairing between the miRNA and the target mRNA leading to repression of the target gene by inducing mRNA degradation or translational inhibition. Several studies shed light into the role of microRNAs in the pathological pathways activated in normal and diseased skeletal muscle¹⁻⁴. However, these studies were based on quantifying specific miRNAs mainly in Duchenne Muscular Dystrophy (DMD) and the substantial underlying miRNA expression profile in muscle tissue from a range of MD remain to be elucidated. Further, there is urgent need for developing non-invasive biomarkers for monitoring disease progression, as to date the assessment of the muscle pathology requires an invasive muscle biopsy, which is limited to a single time point and to a single muscle and is not desirable in children.

In this project we will conduct a comprehensive miRNA profiling in muscle tissue and biological fluids, such as serum or urine, from different types of MD in order to gain detailed understanding of the miRNA pathways involved in pathogenic process and the utilization of the dysregulated miRNAs as non-invasive biomarkers.

We have chosen to study 3 types of MD: patients with DMD; congenital muscular dystrophy (CMD) and congenital myopathy (CMYO). The reason is that, in these three different conditions, the pathogenic mutation results in myofibre wasting but, depending on the affected gene, satellite cell function and hence the maintenance and repair of myofibres, is affected in a different way⁵ (reviewed in Morgan et al, 2010). Our hypothesis is that a diverse set of miRNAs will be dysregulated in muscle biopsies from DMD, CMD and CMYO in response to the pathogenic mutation which on the other hand influences different miRNA programs controlling the self-renewal and differentiation of satellite cells. As a result, specific miRNAs will be secreted and released in exosomes from satellite cells into the blood stream and will be detected in serum.

Aims and methods:

The project aims to investigate miRNA pathways involved in the pathogenic process in DMD, CMD and CMYO and to identify specific miRNAs as potential therapeutic targets in future clinical applications. Another objective of the project is to identify non-invasive biomarkers for DMD, CMD and CMYO in biological fluids such as serum or urine. We are going to analyse 30 muscle and 30 serum samples from DMD, CMD, CMYO patients by applying deep miRNA sequencing. MicroRNA sequencing is a novel tool that will allow us to discover previously uncharacterized miRNAs and identify different isoforms of miRNAs. The plausible candidate miRNAs will be studied in detail by analysis in a larger patient cohort and additional experiments in muscle biopsies, cell cultures and animal models.

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Lentiviral gene therapy against HIV

Supervisors: Dr Waseem Qasim and Professr Greg Towers

Hypothesis:

Human TRIMCyp fusion proteins can form the basis of therapy against HIV

Aims and methods:

We are developing new alternatives to conventional anti-retroviral therapy in patients who are unable to tolerate drug therapy, or have developed drug resistance. Genetic engineering of T cells offers the possibility of durable therapeutic benefit and is being tested in a number of clinical trials. We have developed lentiviral vectors derived from HIV-1 which express extremely potent anti-HIV restriction factors based on TRIM5-CyclophilinA fusion genes. These fusion constructs are humanised mimics of anti-HIV genes which have arisen in non-human primates on at least two occasions during evolution. The resulting fusion proteins have prevented HIV-1 infection in these species for thousands of years without evidence of mutagenic escape. We are developing a form of intracellular vaccination against HIV based on the humanised versions of such proteins and anticipate ex-vivo modification and reinfusion of protected cells as a therapeutic option for patients requiring alternative therapeutic interventions. This project will investigate ways to improve how and when TRIM-CypA genes are expressed in target cell populations, and will focus on:-

- i. The design and development of vectors for regulated expression of anti-HIV expression
- ii. Characterisation of immune function of T cells expressing TRIMCyp proteins
- iii. Modification and differentiation of stem cells (T cell precursors) to express restriction factors.

The project will provide training in HIV biology, vector development and cell based therapies in a translational research environment.

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Retinal stem cell therapy: Modulating miRNAs to generate cone precursors from human pluripotent stem cells

Supervisor: Dr Jane Sowden

Hypothesis:

Retinal degeneration involving death of light-sensitive photoreceptor cells in the retina is the leading cause of untreatable blindness in the Western world. Retinal histogenesis is the process that generates all the retinal neurons during development, including the abundant rod photoreceptor (providing night vision) and the less common cone photoreceptor (providing colour and daylight vision). As the adult retina is not able to generate new photoreceptors, replacement of lost photoreceptors by transplanting new cells into the retina is one possible future treatment. Our recent research has shown that new functional photoreceptors can be generated in the retina via the transplantation of immature photoreceptor precursor cells isolated from the developing retina [1, 2]. For clinical therapy, cone photoreceptor precursor cells need to be differentiated from renewable pluripotent stem cell cultures. Human stem cell-derived retinal tissue containing both rods and cones can be generated in vitro [3], but we need to identify what regulates the timing of genesis and maturation of different types of photoreceptor cell. MicroRNAs (miRNAs) are short noncoding RNAs that influence gene expression through post-transcriptional regulation of mRNA translation and degradation and have recently emerged as regulators of neural development that may play an important role in the tightly coordinated process of development of retinal neurons [4]. This project will investigate the hypothesis that miRNA regulation is critical for optimal generation of photoreceptors from human pluripotent stem cells.

Aims and methods:

The aims of this PhD project are:

- 1) to investigate whether miRNAs regulate human cone and rod photoreceptor development and to identify their target genes.
- 2) to differentiate cone photoreceptor precursors from human stem cell cultures and test whether miRNA modulation increases levels of cone generation.

Methods:

- 1) miRNA array analysis will be performed to select miRNA differentially expressed during human photoreceptor development.
- 2) Array results will be validated by RT-PCR, in situ hybridization and immunostaining on embryonic and fetal retina and ES and iPS cell cultures.
- 3) The role of selected miRNA and their targets will be analysed by knock down and gain of function experiments.

These data will establish whether miRNAs play a pivotal role in photoreceptor generation and identify miRNAs involved in human retinal development. The project will explore how miRNAs coordinate neurogenesis in the retina. By characterizing and modulating the expression profile of developmentally regulated miRNA during human retinal development the project will identify whether miRNAs could be used to promote photoreceptor differentiation from stem cells. The long-term aims are to transplant photoreceptor precursor populations isolated from stem cell cultures for retinal repair.

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Role of the WASP EVH1 domain in the control of degradation and the regulation of WASp activation

Supervisors: Professor Christine Kinnon, Dr Austen Worth, Dr Siobhan Burns and Professor Adrian Thrasher

Hypothesis: WASp degradation and impaired WIP binding in patients with Wiskott Aldrich Syndrome causes aberrant podosome formation and dynamics, resulting in dysregulated cell migration and immune responses.

Aims and methods:

Wiskott Aldrich Syndrome (WAS) is a genetic disease in which children suffer with severe eczema, low platelets (and a resultant susceptibility to bleeding), and a severe susceptibility to infection, autoimmune diseases and blood cancers. It is caused by mutations in the gene which codes for the protein WASp, a molecular switch which controls remodelling of the actin cytoskeleton in response to extracellular stimuli. WASp plays a critical role in controlling how blood cells change shape, move, invade tissues and initiate the fight against infection. Patients with WAS are unable to form transient actin rich adhesive structures called podosomes, and we believe this defect underlies their impaired cell migration and cell shape, and underpins their immunodeficiency and autoimmunity.

At rest WASp remains bound to the protein WIP (WASP interacting Protein), which is critical in protecting WASp from degradation and ensuring that WASp activation occurs in the correct sites within the cell. Patients with WAS commonly have mutations which disrupt the binding of WASp to WIP. We hypothesise that disruption of the WASp-WIP complex impairs the formation, maturation and dissolution of podosomes, resulting in cells forming aberrant movements instead of controlled directional migration in response to chemotactic stimuli. This project will elucidate WASp's role in the molecular mechanisms governing how a cell starts to move, and will provide invaluable knowledge of both the molecular pathology of WAS and the mechanisms of cell migration.

In this project the student will make WASp mutants which are unable to bind WIP but are resistant to degradation, to complement a wide range of WASp mutants previously made. These mutants will allow dissection of the function of WASp-WIP interaction, independent of increased WASp degradation. All these constructs will be made fused to Green Fluorescent Protein, allowing us to image the localisation and movement of WASp protein at the adhesive surface of live cells using confocal microscopy. By introducing WIP and actin fused to different fluorophores in the same cell, it will be possible to measure the dynamics of podosome formation and the co-localisation of WASp and WIP complexes to these structures, simultaneously in real time. Using a range of cutting edge imaging techniques and microscopes to image the various WASp mutants in migrating cells, the student will generate and analyse videos of cells initiating movement, dissecting the precise role of WASp in regulating this complex and fundamental cellular process.

This project will provide the student with a fantastic opportunity to gain a wide range of scientific techniques, including molecular cloning, cell biology, cell culture, cell transduction, flow cytometry and a diverse range of microscopy, imaging and image analysis techniques. This work will run alongside the Wiskott Aldrich Syndrome gene therapy program at Great Ormond Street Hospital, and contribute to the world leading WAS and cell migration group within the Molecular Immunology Laboratory. This work will provide essential insights into our understanding of the disease processes in WAS, further informing treatment options (supportive care, stem cell transplant or gene therapy) for patients with this devastating disease, and will contribute to the progressive improvement of the care offered to patients worldwide.

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Developing bacteriophage therapy for *Pseudomonas aeruginosa* infection in patients with cystic fibrosis

Supervisors: Professor Chris O'Callaghan and Dr Martha Clokie

Hypotheses:

- Pre-treatment of human ciliated epithelium with anti pseudomonal phage delivered by aerosol prevents infection and biofilm formation.
- Treatment of established *P. aeruginosa* infection of ciliated epithelium eradicates infection and destroys the associated biofilm.
- Aerosolised anti pseudomonal phage retains its ability to multiply in and kill *P. aeruginosa*

Aims and methods:

Chronic infection with *P. aeruginosa* is the major cause of morbidity and mortality in children and adults with cystic fibrosis (CF). The inability to clear *P. aeruginosa* from many patients despite antibiotic therapy and the emergence of multidrug resistant *P. aeruginosa* poses major health problems. Failure to eradicate *P. aeruginosa* from the lungs is associated with significant morbidity and a marked reduction in life expectancy. It is universally agreed that new therapies and management strategies are urgently required.

Bacteriophages are viruses that infect and kill bacteria and although widely used in Eastern Europe to kill bacteria including *P. aeruginosa* their use has not been adopted internationally. This is due to in part to:

- Lack of information on the ability of bacteriophages to kill the wide spectrum of *P. aeruginosa* strains seen in infected CF patients
- Lack of detailed investigation of bacteriophages to ensure minimal risk of toxicity
- Lack of information on aerosolised drug delivery of bacteriophages
- Lack of bacteriophage manufacture to GLP standards
- A complete lack of appropriately designed clinical trials

The aims of this project are to explore:

- How effective different types of bacteriophages are against *P. aeruginosa* obtained from children with cystic fibrosis cared for at Great Ormond Street Hospital
- Methods of minimising the potential toxicity of bacteriophage therapy
- How phage interacts with *P. aeruginosa* in a relevant environment
- How bacteriophages may be delivered in an aerosolised form to patients and the effect of this on their viability

This work will provide data that is essential prior to establishing GLP production of phage for a phase one clinical trial and a phase two clinical trials of CF patients infected with *P. aeruginosa*.

Methods used in this study will include: human basal and ciliated cell culture; assessment of ciliary function: electron microscopy; confocal microscopy; assays for inflammatory mediators and NO; assessment of delivery of aerosolised bacteriophages to humans.; quantification and analysis of bacteriophages; growth and evaluation of *P. aeruginosa* virulence..

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A leaky barrier: a shared aetiology in skin and intestinal infectious and inflammatory diseases?

Supervisors: Dr Ryan O'Shaughnessy and Dr Mona Bajaj-Elliot

Hypothesis:

Bacterial infections are the most common cause of gastroenteritis worldwide, and the majority occur through the consumption of undercooked poultry or contaminated water. *Campylobacter jejuni* is responsible for ~15% of all known cases of diarrhoea and is responsible for over a million deaths in children <5 years of age in the worldwide. In addition, infectious gastroenteritis makes the gut more vulnerable to post-infectious complications which include Irritable Bowel Syndrome in ~30% of the patients.

C. jejuni and *Clostridium difficile* (an emerging pathogen increasingly affecting children) cause diarrhoea by secreting toxins that break the gut lining. This single layer of cells that is held together by clamp-like structures called tight junctions. In a healthy gut, these prevent water from leaking and at the same time stop bacteria from invading. Bacterial toxins specifically attack these tight junctions.

We have identified a mechanism showing that inhibition of cJun phosphorylation [1] and Rab3Gap1 expression rapidly establishes skin barrier function by forming tight junctions. Enteropathogens such as *Campylobacter jejuni* and *Clostridium difficile* initiate disease by disrupting intestinal tight junction barrier function [2,3]. Currently mechanism(s) involved in these cellular events remain unclear.

In this study we will test the hypothesis that manipulation of the cJun pathway and downstream target genes is a potential therapeutic target for improving gastrointestinal barrier function. Improved GI barrier function has major implications for treating various GI infectious and inflammatory conditions.

Aims and methods:

- 1) To understand the role of cJun dephosphorylation, Rab3Gap1 and tight junctions in the formation of the gut barrier
- 2) To determine the mechanism of gut barrier disruption by *C. jejuni* and *C. difficile*
- 3) To determine whether Jun Kinase inhibition or Rab3Gap1 expression can prevent tight junction disruption by *C. jejuni* and *C. difficile*
- 4) Testing the effect of JNK inhibition and Rab3Gap1 overexpression in gut infection *in vivo* in the mouse

Methods to be used: Gut epithelial cell culture, Immunofluorescence and Microscopy, including confocal microscopy, Physical measurement of barrier function by transepidermal electrical resistance and FITC dextran penetration. Bacterial culture and co-culture with mammalian cells. Bacterial migration assays using transwell assays on cultured gut epithelial cells. Protein analysis including western blot. Also animal model work, gut infection of immunocompetent and immunodeficient mice, and exogenous gene expression by lentiviral/retroviral approaches.

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Chronic Granulomatous Disease (CGD) and inflammation: a role for Myeloid Derived Suppressor Cells

Supervisors: Dr Giorgia Santilli and Professor Adrian Thrasher

Hypothesis:

Chronic Granulomatous Disease (CGD) is an inherited primary immunodeficiency disorder. It is caused by a faulty NADPH oxidase, the enzyme responsible for the production of Reactive Oxygen Species (ROS) and for pathogen killing. Patients with CGD, frequently develop inflammatory complications indicative of exaggerated inflammatory responses that often lead to autoimmune phenomena.

However, the causes for the hyperinflammatory phenotype in CGD patients are still not clear.

Recently, a cell population called Myeloid Derived Suppressor Cells (MDSCs) have been shown to accumulate during inflammation as well as in tumorigenesis. Their role has been better studied in cancer where MDSCs are known to dampen the activation of effector immune cells by a variety of mechanisms. We think that by limiting the immune reaction MDSCs could have a key role in mediating the resolution of inflammation. A link between NADPH oxidase and the immunosuppressive activity of MDSC has already been established in tumour settings [1]. In this project we propose to investigate whether CGD patients have a defect in the development and/or function of MDSC that could explain their hyperinflammatory phenotype.

Aims and methods:

Our aims are:

- 1) To investigate the nature and the immunosuppressive function of MDSCs derived from normal and CGD mice *in vitro*. In the first part of the project MDSCs will be derived *in vitro* from the bone marrow of WT and CGD mice by culturing cells in GM-CSF and IL6 and they will be characterised by surface marker staining and western blot analysis. The immunosuppressive activity of WT or CGD MDSC will be analysed *in vitro* in Mixed Leukocyte Reaction (MLR) assays using the OT II mice system following published methods [2].
- 2) To characterise the phenotype and function of MDSCs during inflammation in a mouse model of CGD. The role of MDSCs in inflammation will be investigated *in vivo* in WT and CGD mice. For this we will imply the air pouch model. Briefly the back of the mice will be inflated with air at day 0 and 3 to create an extra body cavity where to inject the inflammatory stimulus (Lipopolysaccharide). The pouch is a closed system where to study the recruitment of inflammatory cells. The MDSCs recovered from the pouches of WT versus CGD mice will be analysed for their phenotype by surface marker staining. Concentrations of the pro (IL1b, IL6, TNFalpha) and anti-inflammatory cytokines (IL-10 and TGF-b, PDGE2) in the pouch will be measured by ELISA.
- 3) To use *ex vivo* gene therapy to restore normal development and function of MDSCs and a normal response to inflammation. For this part of the project we will use a gene therapeutic approach [3] to restore the NADPH oxidase activity in X-CGD mice and we will investigate the ability of gene corrected mice to develop functional MDSC and to resolve inflammation in an air pouch experiment.
- 4) To investigate the nature and the immunosuppressive function of MDSCs in CGD patients undergoing inflammation. In the last part of our project blood samples from CGD and non- CGD patients under inflammatory conditions will be collected with formal consent and analysed for the presence of MDSCs using conventional markers (CD11B, CD33, CD14, HLA-DR, CD66b). We will separate CD66b cells from PBMC FICOLL of normal and CGD patients using the easysep kit and we will determine the ability of CD66b to suppress T cell proliferation, in MLR assays. Allogenic T cells (from a healthy donor) will be labelled with Carboxyfluorescein diacetate succinimidyl ester (CFSE) and activated by co-culture with dendritic cells, in the presence or absence of the MDSCs identified at different ratio. Cell division will be measured as successive halving of the fluorescence intensity of CFSE.

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Signalling and cytoskeletal remodelling in platelet biogenesis

Supervisors: Professor Adrian Thrasher and Dr Mike Blundell

Hypothesis: The novel thrombocytopaenia caused by mutations in ARL13A is due to defects in the formation of intracytoplasmic cilia which form demarcation membranes in megakaryocytes and subsequently proplatelets.

Aims and methods: To study the signals and proteins required for remodelling the cytoskeleton in platelet biogenesis using thrombocytopaenias as model systems.

Megakaryocytes generate platelets, essential for blood clotting, by remodelling the cytoplasm into proplatelets which transverse through the bone marrow sinusoids into blood (1). Platelet sized particles are generated at the end of long cellular protusions called proplatelets which are then released into the blood via shear stress, adhesion and cytoplasmic remodelling (2) although liberation of platelets from proplatelets has, as yet, never been observed. Megakaryocytes undergo a maturation process where they enlarge and become polyploid via a process called endomitosis (3). This leads to a build up of platelet specific granules, cytoskeletal proteins and an internal membranous labyrinth called the demarcation system (4). This maturation allows the proplatelet extensions to form with platelet like swellings along their length although the specific signals and proteins involved are not yet fully understood. We have recently identified a small kindred with a novel thrombocytopaenia in which a defect in the gene ARL13A has been identified. ARL13A is an ADP-ribosylation factor like protein family member although to date nothing is published on the function, expression or cellular location of the ARL13A protein. ADP ribosylation factors act as molecular switches to regulate protein activity or location (5) and are membrane associated regulators of vesicular biogenesis, intracellular traffic and actin remodelling. The closely related ARL13B is found in primary cilia and in close association with gamma tubulin, which is located in microtubule organisational centres. Multiple centrosomes acting as microtubule organisational centres have been observed in megakaryocytes but how they contribute to platelet formation is not yet known. Mutations in ARL13B lead to Joubert syndrome, a primary ciliopathy (6) indicating a role in cilia and microtubule assembly. There is a 30% homology between ARL13A and ARL13B which is highly conserved between different species, suggesting a similar role for both proteins.

In this project we want to identify the signals and proteins which are important in platelet biogenesis in a recently discovered kindred with ARL13A mutations as well as identify the specific role of ARL13A in platelet biogenesis. Investigation of the role of this protein will improve our understanding of the processes which occur during platelet biogenesis. The characterisation of ARL13A will firstly be investigated as the ARL proteins have only been identified by genome sequencing and although structurally related to ADP ribosylation factors are thought to have no ribosylation activity. Protein interactions will be investigated with ARL13A domain mutants constructed to identify binding partners and identification of domains essential in platelet biogenesis, in order to model the disease, utilising techniques such as immunoprecipitation and mass spectrometry. The sub cellular location and binding partners are to be investigated in a spatial and temporal manner in megakaryocyte maturation. We will use megakaryocyte cell lines which can be induced to form proplatelets and primary cells, of both mouse and human origin, to determine a role for ARL13A in megakaryocyte maturation. This includes the study of endomitosis, demarcation membrane production, proplatelet extension and platelet biogenesis. We have developed lentiviral vectors containing GFP-tagged ARL13A which we will use to infect cells for live imaging. This will allow us to elucidate a role for ARL13A in primary cilia, centrosomes, vesicle and organelle translocation and the microtubule organisation centre throughout maturation of the megakaryocyte in addition to revealing binding partners in fixed cells using confocal microscopy. Using RNAi against ARL13A we will mimic the clinical disease to identify a mechanism for the novel thrombocytopaenia. We will use live imaging to observe the cellular defects and timing of the defects in the megakaryocyte maturation to determine how loss of ARL13A contributes to thrombocytopaenia. We will also use electron microscopy to visualise the demarcation membranes and the role of ARL13A in their formation. GFP-tagged ARL13A will be introduced into megakaryocytes derived from human CD34+ stem cells via lentiviral vectors that have had their endogenous ARL13A knocked down. This will restore ARL13A levels and platelet biogenesis thus providing proof of principle of an alternative treatment for thrombocytopaenia where bone marrow transplantation is not applicable.

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The role of lymphocyte crosstalk in autoimmune myositis

Supervisors: Professor Lucy Wedderburn and Dr Kiran Nistala

Hypothesis:

This PhD will test the hypothesis that in children with juvenile dermatomyositis (JDM) there is dysregulation of B cells, particularly the immature B cell subset which rather than functioning in a regulatory manner, instead promotes inflammation, and so contributes to disease pathogenesis.

Background and Aims:

The overall aim of this PhD will be to understand the contribution of B cells to the pathogenesis of JDM. JDM is a severe, potentially life threatening disease the cause of which is not at all understood (1,2). Evidence from model systems suggest that some B cell subsets may contribute to pathogenesis of autoimmune disease through IL-6 production or autoantibody production whilst others may play a regulatory role in an IL-10 dependent manner (3). Our pilot data suggest aberrations in B cell numbers and function in JDM. Identifying the exact nature of this dysregulation and the mechanisms that underpin it will be central to improving treatment for JDM. At present several powerful immune modulating treatments are used to treat autoimmune disease, yet we do not know exactly how these new drugs work. An example is B cell depletion with the anti CD20 antibody Rituximab, now being used for severe JDM with impressive responses in some patients but not in others (4). Understanding how effector and regulatory B cell balance, and the signalling pathways that govern their phenotype, alter disease expression in JDM will provide valuable insights for autoimmune disease in general and help us to use available therapies more effectively in the future.

Four central questions will be addressed in the PhD:

1. Are the frequency, absolute number and function of regulatory B cells (Breg) in JDM patients abnormal, and if so, does B cell regulatory and effector balance correlate with disease expression in childhood myositis?
2. Are IL-17 producing T cells raised in JDM and if so, do B cells from JDM patients promote the expansion of Th17 cells?
3. Is Blk signalling altered in T and B cells from patients with active JDM?
4. How do B cell abnormalities observed in JDM patients affect myositis in an in-vivo model system ?

Methods:

This project will benefit from being linked to the UK National Cohort study of JDM which is based at ICH UCL, led by L Wedderburn, and has samples and linked data from over 380 children with JDM. The methods to be used in this project will be state-of-the-art cellular and molecular immunology. The Graduate student will learn 7 colour flow cytometry, cell sorting and cytokine secretion assays, sorting of subsets of cells as well as establishing functional assays for immune function, using both healthy control samples and those from children with juvenile myositis (5). They will then gain experience in cell signalling biology through the study of Blk signalling in B cells, and finally will have the opportunity to work on a murine model of myositis that we are establishing. Use of this model will provide the student with the opportunity to learn in vivo work, and to test mechanistically the role of both effector and regulatory B cell subsets in modulating myositis as well as Th17 effects. Together the project will provide an excellent and thorough training in both cellular and molecular immunology, as well as cell signalling and in vivo models of autoimmune disease. The student will benefit from working both in the Wedderburn lab at UCL Institute of Child Health, where there are strong links with the JDM clinic at Great Ormond Street Hospital, and also from the co supervision from Dr K Nistala (UCL), and collaboration with Prof C Mauri, UCL, for expertise on Breg and in vivo modelling work(6).

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Graph theoretical measures of brain disconnection in children with epilepsy

Supervisors: Dr Chris Clark, Professor Rod Scott, Dr Jon Clayden, Dr David Carmichael

Hypotheses to be tested:

1. Children with epilepsy have an abnormal structural connectivity profile with a higher path length and lower cluster coefficient.
2. Graph theoretical measures correlate with measures of cognition.
3. Is structural connectivity uncoupled from functional connectivity in children with epilepsy and if so, is this due to transient or permanent changes to the functional network?

Aims and methods:

The brain can be thought of as a distributed network (like a plan of the railways). New approaches to the understanding of brain function and organisation have used a method called 'graph theory' to describe these networks and how efficiently they work (like how long or how many stops it takes to get between destinations). These new methods have recently been successful in predicting IQ in individuals and have been shown to predict cognitive performance (e.g. how well people can do tests involving language, memory and spatial tasks).

However, graph theoretical approaches to imaging data have yet to be used in the study of children with epilepsy, which is increasingly being described as a disease of neural networks. Building on our previous work three paediatric patient groups will be studied; severe non-lesional epilepsy, temporal lobe epilepsy and convulsive status epilepticus.

Abnormal connectivity of brain networks has been suggested as a way that brain function might be impaired in epilepsy. With the development of these new imaging tools it is now possible to investigate this idea. The purpose of this project is to measure brain networks in children with epilepsy and to determine if such connection measures relate to cognitive performance. If so, these new tools may be used in the future to predict how a child will develop and to test the effectiveness of disease modifying therapies. Because the technology is available now the results of this study would be applicable to patients in the short term.

Training:

This proposal sits at the interface between basic and clinical science. The student will have the opportunity to work on and develop advanced neuroimaging techniques in collaboration with a multi-disciplinary team of physicists, computer scientists and paediatric neurologists. Critically these developments will be tailored toward the delivery of this technology to the clinical population, in this case, children with clinically diagnosed epilepsy.

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Understanding blood flow changes preceding epileptic events in children

Supervisors: Professor Helen Cross and Dr David Carmichael

Hypothesis:

1. Children with epilepsy have measureable fMRI (haemodynamic) responses prior to scalp EEG-defined events in the majority of patients; there will be considerable between subject variability in regions displaying early changes; but common hubs exist across patients (precuneus and piriform cortex).
2. Children with epilepsy have measureable focal haemodynamic changes using intracranial laser Doppler measurements prior to intracranial EEG-defined events.
3. Improved technology and probe tips can improve the reliability of intracranial blood flow measurements

Aims and methods:

Epilepsy is the most common neurological disorder in children living in the UK and it is estimated to affect 60000 children. In up to 75%, seizures are controlled with medication; surgery may be an option for a selected number, estimated at 400/year in the UK [1]. Surgery in childhood can dramatically improve the lives of individuals with epilepsy by cessation of seizures and limiting disability caused by disrupted development [2].

The simultaneous recording of Electroencephalography (EEG) and functional magnetic resonance imaging (fMRI), pioneered at our institution in adults [3] and children [4], allows for haemodynamic changes linked to epileptic discharges to be mapped with good spatial resolution across the whole brain and can contribute to evaluation for epilepsy surgery.

EEG-fMRI has demonstrated blood flow changes can *precede* EEG signs of epileptic activity on the scalp [5]. This is surprising because EEG has a high temporal resolution and is used clinically as the main tool to diagnose epilepsy and determine the areas initiating epileptic activity (based on temporal precedence). Scalp EEG changes may simply be insensitive to underlying electrographic changes explaining pre-event haemodynamic signal changes. Alternatively they may be indexing slower metabolic changes that lead only to electrographic changes at some threshold level. Therefore comparison between scalp and invasive EEG and blood flow driven responses are needed to better understand this relationship.

We aim to compare EEG-fMRI changes to intracranial EEG and laser-doppler blood flow measurements recorded simultaneously in children with focal epilepsy. This data has already been acquired in a few patients – data which is unique to our centre. There is also scope within this project to develop the intracranial measurement methodology. These results will provide valuable information regarding the possible use and location of haemodynamic changes in predicting epileptic activity which might play a role in future localised, acute treatment strategies such as closed-loop brain stimulation devices.

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Postnatal brain growth and development of processing speed and executive function in toddlers born preterm

Supervisors: Dr Michelle de Haan and Professor Neil Marlow

Hypothesis:

Neuroimaging-derived measures of brain growth in the first postnatal months will predict impairments in processing speed and executive function in toddlers who were born preterm.

Aims and methods:

In England and Wales, 8% of live births are born preterm.¹ Cognitive impairments are by far the most common disability identified in survivors of preterm birth, and require expensive long-term educational support throughout the school years.^{2,3} While cognitive impairments have a serious long-term impact on the child and their family, they are typically not identified until some years after discharge from hospital, and often come to light when children experience difficulties with school work. Recent studies show that impairments in specific cognitive skills, namely processing speed and executive functions, underlie these difficulties in academic progress.⁴ If impairments in these specific skills could be identified earlier, it would provide greater opportunity to intervene before problems become entrenched and impact school progress. The challenge is that the tests typically used in the first postnatal years of follow-up after preterm birth are not good at picking up problems in these areas.⁵ The purpose of this investigation is to address this gap in knowledge by studying a group of children born preterm, and their fullterm counterparts, who are part of an ongoing longitudinal study. These children will be 2 years of age at the time of the proposed study, and we aim to obtain detailed measures of their processing speed and executive functions. We will also evaluate socio-emotional and autism-related behaviours to examine how these may relate to any cognitive impairments. By relating this new information to what we will already know about their early brain growth and cognitive development, we will take an important step towards the long-term goal of early identification and treatment of preterm infants a particular risk.

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A developmental model of epilepsy: focal cortical dysplasia and cortical tubers

Supervisors: Dr Thomas Jacques and Dr Jonathan Ham

Hypothesis:

The student will test the hypothesis that abnormal stem cells drive the development of severe childhood epilepsy.

Aims and methods:

The purpose of the project is to understand a common cause of severe childhood epilepsy. Epilepsy is a common disease of children, affecting 58000 children in the UK. Some children have frequent seizures, often starting early in life. Abnormalities of brain development are common in this group of children. Focal cortical dysplasia (FCD) is the most common(1).

We have demonstrated, for the the first time, that a cell with features in common to a neural stem cell can be isolated from the brains of children with FCD(2). The isolation of the cells means that for the first time, we can modify the cells and determine how the disease develops.

The aim of this project is to test the hypothesis that these cells are abnormal stem or progenitor cells and to determine how they contribute to the development of the disease. By analogy, we have previously shown that abnormal stem cells lead to tumour formation(3).

The student will gain a strong foundation in cell biology including cell culture, slice culture, pathology, microinjection, molecular biology and immunocytochemistry and working with human tissue.

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Human speech articulation: Brain functional and connectivity changes during development

Supervisors: Dr Frederique Liegeois and Dr Chris Clark

Background:

Speech is a uniquely human ability that seems to be acquired effortlessly in infancy, with most children achieving near-adult intelligibility levels by the age of six¹. Speed and accuracy of articulation continues to improve throughout adolescence², yet little is known about the developmental changes that occur within speech motor tracts during that period. Some studies have revealed microstructural changes for the corticospinal tract³⁻⁴ up to late adolescence, but none have looked at speech-related tracts separately. Similarly, functional asymmetry for fast articulation has not been investigated throughout development. It is well known that the left hemisphere is dominant for language functions from early childhood⁵, but whether it is also dominant for speech movements remains unexplored. We aim to develop the first neuroanatomical atlases of the human articulation systems during development, and document structural and functional hemispheric differences.

Hypotheses:

We hypothesize that:

- a. Tract sizes will not change throughout development
- b. In contrast, microstructural indices (such as fractional anisotropy and radial diffusivity) will change with age in speech motor tracts
- c. Speech-related tracts will undergo different developmental trajectories in the left ("language dominant") vs. the right hemisphere
- d. There will be left dominant functional asymmetry for complex articulation

Aims and methods:

The overall aim of the project is to determine the neural basis of articulation in typically developing young people, by combining functional and diffusion-weighted MRI. We will focus on changes that occur between three key developmental phases, namely from childhood (8-12 years), to adolescence (13-17 years), to early adulthood (18-25 years).

Twenty children (aged 8-11), 20 adolescents (12-17) and 20 young adults (18-25 years) will be recruited. Participants will perform movements of the tongue (/lala/), lips (/papa/), and complex articulation sequences (/pataka/) while functional MRI data are acquired, allowing us to map the representation of the articulators in the motor cortex. Diffusion-weighted MRI data will then be acquired, to perform tractography of the motor white matter pathways. Language lateralization and competence will also be evaluated using age-appropriate standardised test.

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Dissecting the multiple features of motor-speech problems in a genetic disorder using structural and functional methods of brain imaging

Supervisors: Professor Faraneh Vargha-Khadem and Dr Katrin Schulze

Hypothesis:

It is predicted that affected KE members, who have problems with planning and execution of speech (1), and show both structural and functional abnormalities in motor-related structures (2-4), will also show impairments in (i) phonemic perception, (ii) implicit articulatory learning and (iii) temporal sequencing.

Aims and methods:

We have previously reported on an inherited neurodevelopmental disorder that selectively interferes with articulate speech, and with language function (1). Studies of the three-generational KE family, half of whose 30 members have a severe disorder of speech and language, highlighted the importance of the neostriatal system in the development of fine oromotor skills required for articulate speech, and language learning. We identified the core deficit in the affected KE members as one involving sequential articulation and orofacial praxis (5), viz a form of implicit, procedural motor learning disorder. The identification of the phenotype ultimately led to the discovery of FOXP2, the first gene implicated in the cascade of neurobiological processes that culminate in articulate speech and normal language function. Brain imaging studies of the KE members revealed structural and functional abnormalities in both cortical and subcortical motor-related areas of the frontal lobe, namely, Broca's area, left premotor and motor cortices, and in parts of the neostriatal system (2-4). We now aim to dissect the phenotypic features of the FOXP2 mutation in the affected KE members, by searching for abnormalities in brain systems that subservise (i) phonological perception/processing, (ii) procedural/implicit learning of novel oromotor sequences, and (iii) timing and execution of these sequences (1):

Categorical perception – Given the link between perception and production (e.g. 6), we aim to investigate whether affected KE members, also show an impairment in phonemic perception. This question will be investigated by comparing behavioural standards of categorical perception in the affected KE members relative to unaffected members, and matched controls. In a functional MRI adaptation of the same paradigm, the neural substrate underlying categorical perception processes will be examined in the affected KE members and normal controls.

Implicit articulatory learning – Learning to articulate combinations of speech sounds that form new words from auditory exposures is crucial for the human capacity to produce fluent speech. To date, the procedural/implicit learning ability of the affected KE members has not been documented. We plan to examine this skill which accommodates new learning (7), in an fMRI experiment. Healthy controls and affected KE members will be repeatedly exposed to auditory nonwords, and they will be asked to repeat the nonwords. The aim is to determine how the affected members learn new articulatory sequences, and whether they recruit the same neural network as non-affected controls.

Temporal sequencing – Analysis and production of auditory temporal sequences (or sequencing), relies on the brain circuitry for complex vocal learning inasmuch as it involves special links between the auditory and motor systems. Previous reports suggest that a general deficit in sequencing might be associated with the speech production difficulties of the affected KE members (8). Sequencing abilities and their neural correlates will be studied by presenting members of the KE family with rhythmic patterns for recognition and reproduction. Using fMRI, the neural correlates underlying sequencing will be compared in individuals with and without a FOXP2 mutation.

Longitudinal evaluation – Now that the affected KE members have reached adulthood, their speech and language profiles will be re-assessed and compared with those obtained when they were children to determine the extent of compensation that may have resulted with increased language experience. These longitudinal studies will also assess the integrity of brain pathways in the speech and language network over time. The availability of a new 3T MRI along with the development of new brain imaging techniques providing quantitative measures of white matter integrity (Diffusion Tensor Imaging), tract delineation (tractography), and patterns of connectivity will enable the charting of auditory-motor interactions necessary for fluent speech production.

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Characterising human adrenal stem/progenitor cells and their role in adrenal disease

Supervisors: Dr John Achermann and Professor Gudrun Moore

Hypothesis:

The adrenal glands produce steroid hormones which are necessary for survival. Underdevelopment of the adrenal glands causes life-threatening adrenal failure. This clinical condition is known as adrenal hypoplasia. The molecular basis of adrenal hypoplasia is known in about 50% of cases. We have identified genetic causes of this condition due to disruption of factors DAX-1 (NR0B1), SF-1 (NR5A1) and CDKN1C. All these factors are emerging as having an important role in adrenal stem/progenitor cell expansion and differentiation. Indeed, adrenal cancer can also be associated with alterations in their activity or expression levels. We hypothesise that novel factors involved in adrenal development can be identified by studying patients with adrenal hypoplasia where the cause is currently unknown. Based on our current experience, these factors could be new important regulators of adrenal progenitor cell differentiation. Detailed analysis of the expression of these factors in the developing human adrenal gland would help establish their role in different stages of adrenal cell development. We hypothesise that these factors could be used to differentiate steroidogenic cells in vitro from mesenchymal stem cells, which ultimately could have a role in regenerating adrenal tissue for patients with adrenal failure.

Aims:

- 1) to identify and study novel genetic causes of adrenal hypoplasia in humans that are relevant to stem/progenitor cell development and cell cycle regulation;
- 2) to study expression of these factors in relation to adrenal stem cell expansion and differentiation in the developing human fetal adrenal;
- 3) to investigate the ability of these factors to differentiate immature cells into functional adrenal tissue that might be used to regenerate the adrenal gland for treatment of patients with adrenal failure.

Methods:

This project would involve a range of molecular and bioinformatic techniques. Aim 1 would use exome sequencing of individuals with adrenal hypoplasia to identify novel adrenal hypoplasia genes with detailed bioinformatic analysis of datasets and interpretation of exome sequencing data. Aim 2 would use detailed immunohistochemistry and confocal microscopy to study the spatiotemporal expression of novel proteins in relation to known adrenal factors during critical early stages of human fetal adrenal development. The genetic networks activated in these key cells would be investigated using laser capture of these cells and expression analysis. Aim 3 would be a proof of principle study to assess whether new factors identified might be able to induce a steroidogenic cell lineage from mesenchymal stem cells.

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Assessing the role Endothelial Glycocalyx in the vascular physiology and pathology of children with chronic vascular disease

Supervisors: Professor John Deanfield (Institute of Cardiovascular Science) and Dr Rukshana Shroff

Hypothesis:

We hypothesise that thickness and composition of the endothelial glycocalyx will relate to plasma biomarkers and additional measurements of micro- and macrovascular function in children with chronic kidney disease and type 1 diabetes.

Aims:

The aims of this project will be:

1. To validate, in children, the technique of direct visualisation of the human sublingual microcirculation using a non-invasive imaging device.
2. To determine the role of the endothelial glycocalyx in microvascular physiology and pathology in children
3. To determine the effects of stress (ischemia/reperfusion) on the EG and microvascular function
4. To determine the effectiveness of treatments in improving thickness and function of the EG both cross sectional and longitudinally.

Methods:

Aim 1

Validation and reproducibility of Glycocalyx- SDF Imaging in children.

A non-invasive, semi-automated imaging method to estimate Glycocalyx thickness by SDF imaging has been developed to image the superficial microvasculature. On the basis of the observation that the Glycocalyx limits the proximity of erythrocytes to the capillary endothelial cells, the imaging method uses the erythrocyte–endothelium gap of the capillaries in the image to quantify Glycocalyx thickness (1). These estimations of glycocalyx thickness are proven to be reproducible and correlated with cardiovascular risk factors (2). The majority of the research using this method of microvascular visualisation in humans has been undertaken in adult populations and this project proves a novel opportunity to assess its' applications in children.

Aims 2-4

To assess the physiology and role of the EG in chronic disease and post treatment/intervention, two groups will be investigated cross sectional and longitudinally; children with CKD and adolescents with T1DM.

Microvascular complications contribute significantly to the poor diagnosis of Type 1 Diabetes (T1D) (3). Microalbuminuria (MA) is the earliest clinical marker of diabetic nephropathy and related (renal) microvascular damage (4). Adults with T1D showed reduced thickness of EG and the degradation is more pronounced in patients with MA compared to those without MA (5). This microvascular research programme will be the first to examine the Glycolcalyx in T1D adolescents (age 10-16 yr.). Specifically, the project will investigate if the degree of MA, as assessed by urinary albumin/creatinine ratio (ACR), is associated with a more generalised microvascular derangement and destabilisation of EG.

Children with CKD have significant increased risk for the development of accelerated atherosclerosis, arteriosclerosis, and premature CVD during young adulthood. This is due to a unique combination of uremia-related risk factors and traditional risk factors such as hypertension, dyslipidaemia, hyperinsulinemia and insulin resistance (6). This cross-sectional study to investigate the EG in children with CKD will provide novel insights into the microvascular physiology of this population and provide data for longitudinal follow-up of the effects of surgical interventions (dialysis and transplantation) on the EG in relation to other measures of macro and microvascular function.

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A study on new approaches for reducing neural damage - inhibition of PAD enzymes

Supervisors: Dr Patrizia Ferretti and Professor Charles Marson

Hypothesis: We have evidence that activation of PAD3, a calcium-dependent enzyme that modifies protein structure and function by reducing its positive charges through a process called citrullination, is associated with early neural injury response and extensive tissue damage [1, 2]. We have also preliminary evidence that citrullination increases following hypoxia/ischaemia in an animal model. Therefore we wish to test the following hypotheses:

- oxygen and glucose deprivation (OGD) increases expression of PAD3 and human neural cell death
- OGD-induced cell death can be antagonized by blocking PAD3 expression/activity in a human in vitro model
- a new generation of PAD inhibitors will be more specific and effective in reducing neural damage than a general PAD inhibitor currently available

Aims and methods:

The aim of this study is to investigate further the role of PAD(s) in the damaged nervous system, and test the hypothesis that targeting PAD enzymes may have a broad therapeutic potential in neural pathologies. To this purpose, we will characterize a novel putative PAD inhibitor recently synthesized by the co-applicant using animal (traumatic injury) and human (in vitro neural cell hypoxia/ischaemia) models, and develop new more specific inhibitors that could be of therapeutic value. The study will increase our understanding of mechanisms underlying neural damage and will contribute to the development of novel drugs that will reduce it. A reduction in neural tissue damage will be pivotal to improving functional recovery in children who have suffered mechanical or hypoxic damage.

The student will use a broad range of techniques to investigate PAD role(s) in two neural injury models:

1. *In vitro human model:* Human neural cell lines we have recently generated, primary human embryonic neural cultures and a human neuroblastoma cell line will be used to study PAD3 role in response to hypoxic damage and the effect of a new PAD inhibitor on cell death and PAD activity. The student will develop an in vitro model of human hypoxia/ischaemia reperfusion based on the well-established oxygen and glucose deprivation (OGD) method where reoxygenation after hypoxia results in increased intracellular Ca⁺⁺ and cell death [4, 5].
2. *In vivo animal model:* The chick spinal cord crush injury model [1, 3] will be used to test in vivo the ability of the new PAD inhibitor and related compounds to inhibit citrullination and cell death and reduce cavity size (indicator of the extent of tissue damage), as compared to the currently available inhibitor and to inhibition of PAD3 using siRNA.

Altogether, the student will gain experience in several areas, such as cell culture, neural stem cell biology, experimental embryology, microsurgery, protein and RNA analysis (e.g. immunocytochemistry, q-PCR), enzymatic assays, manipulation of gene expression (siRNA), detection of apoptotic cells, imaging techniques (fluorescence, confocal and time-lapse microscopy). The student will also receive some training in designing new compounds and in chemical synthesis.

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Role of a regulator of the PI3K – Akt pathway and two FOXO target genes in the developing nervous system and childhood brain tumours

Supervisors: Dr Jonathan Ham and Dr Thomas Jacques

Hypothesis:

The aim of this PhD project is to study the role of the *Pik3ip1*, *Mxi1* and *Fbxo32* genes in the developing nervous system and in childhood brain tumours. We hypothesize that each gene regulates specific aspects of apoptosis (programmed cell death) in developing neurons. We also hypothesize that PIK3IP1, Mxi1 and Fbxo32 act as tumour suppressor proteins in the nervous system and that their expression is lost or reduced in paediatric brain tumours.

Aims and methods:

The phosphoinositide 3-kinase (PI3K) - Akt signalling pathway has important functions during normal nervous system development (Ref. 1). However, hyperactivation of this pathway contributes to the development and maintenance of a variety of cancers including brain tumours (2). The binding of growth factors to tyrosine kinase receptors activates Class 1a PI3Ks, which in turn activate the protein kinase Akt (1,2). Akt promotes cell growth and inhibits apoptosis. It can inhibit cell death by phosphorylating and thereby inactivating the proapoptotic BH3-only protein Bad and the transcription factor FOXO3a (1,2)

Developing sympathetic neurons have been an important model for studying the mechanisms by which cell death and survival are regulated in the mammalian nervous system (1). These cells require nerve growth factor (NGF) for survival and die by apoptosis in its absence in a transcription-dependent manner. The binding of NGF to its receptor TrkA on the surface of sympathetic neurons promotes neuronal growth and survival and activates the PI3K-Akt pathway (1). When sympathetic neurons are deprived of NGF, PI3K and Akt activity rapidly decrease. This leads to an increase in the activity of FOXO3a, which induces the transcription of proapoptotic genes, such as *bim*, which encodes a BH3-only protein (3,4). In a recent Affymetrix exon array study with sympathetic neurons we identified many new genes induced after NGF withdrawal, which include *Pik3ip1*, *Mxi1* and *Fbxo32* (5). *Pik3ip1* encodes an inhibitor of PI3K; *Mxi1* codes for a transcriptional repressor and *Fbxo32* an E3 ubiquitin ligase, and both genes are directly activated by FOXO transcription factors (5).

Aim 1: Investigation of the function of PIK3IP1, Mxi1 and Fbxo32 in sympathetic neurons

In immunoblotting and immunostaining experiments, the student will study the pattern of expression of the three proteins in sympathetic neurons dying by apoptosis after NGF withdrawal *in vitro*. The student will then determine the role of the three proteins in NGF withdrawal-induced death using the single cell microinjection technique or recombinant adenoviruses to overexpress and to knockdown each protein in sympathetic neurons (3,4). The student will also investigate the downstream mechanism of action of each protein.

Aim 2: Investigation of the pattern of expression of PIK3IP1, Mxi1 and Fbxo32 during normal nervous system development

The student will investigate the pattern of expression of PIK3IP1, Mxi1 and Fbxo32 in the normal cerebral cortex, cerebellum and superior cervical ganglia during postnatal development of the rat (P1 – adult), in particular during and after the periods of naturally occurring developmental neuronal death. Tissue sections will be prepared and immunohistochemistry performed using PIK3IP1, Mxi1 and Fbxo32 antibodies and an anti-active caspase-3 antibody to identify cells dying by apoptosis.

Aim 3: Characterization of the pattern of expression of PIK3IP1, Mxi1 and Fbxo32 in human brain tumour samples

To determine whether the expression of PIK3IP1, Mxi1 or Fbxo32 is reduced in specific types of childhood brain tumour the student will perform immunostaining of paraffin-embedded human brain tumour sections to detect the expression of the genes of interest at the protein level. Tissue arrays of more than 100 samples per tumour type will be constructed in the GOSH Histopathology Laboratory for the following types of paediatric brain tumour: medulloblastomas; ependymomas; pilocytic astrocytomas / low grade gliomas; glioblastomas; low grade glioma-neuronal tumours.

References:

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Hedgehog signalling in polycystic kidney disease (PKD)

Supervisors: Dr David Long, Professor Peter Scambler, and Professor Adrian Woolf

Hypothesis:

In the UK, there are over 40,000 people with kidney failure who need long-term dialysis and renal transplantation. One of the most commonest diagnosis is PKD¹ which causes morbidity, renal failure and death from before birth through adulthood. Many cellular processes are implicated in cyst formation including altered adhesion, planar cell polarity, dysregulated cell cycle and aberrant structure/function of the primary cilium¹. The commonest form of PKD in adults is autosomal dominant (AD) PKD affecting 1 in 600 people, whilst the recessive inherited form (ARPKD, 1:50,000 births) is an important cause of renal failure during childhood with collecting duct cystogenesis in the second half of gestation¹. Diverse approaches have been proposed to reduce cyst development including cyclin-dependent kinase inhibitors and suppressing the vasopressin system which are undergoing trials in adults with ADPKD. However, it is unclear whether they are practical in babies and children with ARPKD; therefore there remains a need to design new therapies for this disease.

Recent studies in our laboratory have identified the hedgehog pathway as a potential target for novel ARPKD therapies. The three hedgehog ligands; Sonic (*Shh*), Indian (*Ihh*) and Desert (*Dhh*) predominately bind to the receptor Patched 1 (*Ptch1*) leading to derepression of Smoothened (*Smo*) which accumulates in the primary cilia leading to the activation of the *Gli* transcription factors². Our previous work examined renal developmental gene expression in an in-vitro explant model replicating features of ARPKD. In this model, *Ihh* was the most markedly deregulated transcript and *Ptch1* was modestly upregulated³. Furthermore, addition of the generic hedgehog inhibitor cyclopamine prevented cystogenesis³. These observations provide a rationale for examining and manipulating hedgehog signalling in ARPKD *in-vivo*; which will be the focus of this proposal. We hypothesise that inhibition of the hedgehog pathway may slow the progression of ARPKD.

Aims and methods:

The student will:

- 1) Undertake a detailed examination of hedgehog pathway components in two animal models of ARPKD^{4,5}.
- 2) Determine if pharmacological inhibition of the hedgehog pathway alters the progression of ARPKD *in-vivo*.
- 3) Investigate whether haploinsufficiency of *Ihh* alters the progression of ARPKD.

The student will gain a broad experience in the following fields and techniques: understanding mechanisms of kidney developmental and cell biology; working with animal models; explant organ culture of developing kidneys; histology; expression analyses at the protein and mRNA levels (*in-situ* hybridisation, immunohistochemistry, western blotting and quantitative RT-PCR).

References:

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How the skin barrier changes with age and in disease: A study of critical proteins and metabolites involved in skin barrier integrity

Supervisors: Dr Kevin Mills, Dr Wendy Heywood, Ryan O'Shaughnessy and Professor Neil Sebire

Hypothesis:

Diseases of the skin affect 1 in 9 of the population, with the commonest being eczema (atopic dermatitis, AD) and with >80% of patients being affected before the age of 5. However, most patients are no longer affected by adolescence and the cause of AD is still unknown and poorly understood. Data from our laboratories have shown an imbalance in the protease/inhibitor ratio in the skin, which controls the correct maturation, can lead to defects in the barrier function. What is clear is that AD has become more prevalent in the last 40 years, with current thinking that AD is caused by a barrier dysfunction due to excessive use and sensitization to detergents becoming a primary event in the development of the disease. In collaboration with researchers at Great Ormond Street Hospital, we aim to answer these questions by using state-of-the-art proteomic and metabolomic techniques that will enable us to study proteins we have already identified as being potentially critical in the formation of a correct skin barrier formation. A pilot project in our laboratory has demonstrated that patients susceptible to atopic dermatitis have differences in the proteomic make-up of their skin. In these patients, we have found increased amounts of non-specific fragments of proteins which are important structural components of the skin barrier. The origin of these peptides is unknown but we hypothesise that there is an unknown protease(s) involved cleaving these proteins and undermining the important cellular scaffold which makes up the skin barrier.

Aims and methods:

To use state-of-the-art histochemistry to study proteins identified being potentially critical for the correct development of the skin barrier.

Working with Professor Neil Sebire at GOSH Histopathology Department the candidate will be using state-of-the-art immunostaining techniques to study potential biomarkers in skin sections from biopsies of patients with AD. This will provide vital insight into what is what is happening within the actual 3D-architecture during disease.

To use novel 'bait' array technology to study the role of proteases in the correct development of the skin. We would like perform a method previously developed in our lab [1,2] that uses 'bait arrays' to identify potential proteases that are critical for the correct maturation and development of the skin barrier. These arrays consist of beads with a bound bait protein which can be incubated with lysates from the varying layers of skin. Proteins bound to the bait proteins are then identified by in-solution trypsin digestion of the beads and mass spectrometry analysis. Using a skin model present at ICH (Dr Ryan O'Shaughnessy, immunology) we will be able test the role of these critical proteins using mRNAi silencing [3].

These results will give us a greater understanding of the function of proteases and their role in disease mechanisms in the development of the skin barrier.

To study how the skin proteome and metabolome changes with age and disease. In the vast number of cases, children usually grow out of eczema (AD) by the time they become teenagers. The reasons for this are unknown but it is surmised it is due to the development of a mature skin barrier that protects against pathogens and external environment. In this study, we will use cutting-edge technology including Liquid Chromatography Mass spectrometry (LC-QTOF MS) [4] and state of the art MALDI imaging technology, to study the proteome and metabolome of skin from 16 weeks gestation to 16 years of age, in the aim studying how the skin barrier matures and changes with age.

This is a unique opportunity for a student to work hand-in-hand with the histopathology department at GOSH and the omic research labs at ICH. We intend to use a new strategy of histopathology directed omics with cutting edge technology, to look at changes in the molecules that make up the skin and how they change with age and in disease.

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- Other project(s) available: [The determination of the anabolism and catabolism of ceramide trihexoside in Fabry disease: the search for the elusive deacylase](#)

Understanding the molecular genetic basis of ciliopathy diseases

Supervisors: Dr Hannah Mitchison and Professor Philip Beales

Hypothesis:

Identification and functional analysis of genes causing ciliopathies will reveal the function of novel genetic causes of disease, shedding new light on the disease process. Finding key players within the complex cilium structure that affect motility will determine which ciliary pathways are vulnerable in PCD, and how different genes products that cause disease interact with each other.

Aims and methods:

Our long-term aim is to understand the molecular basis of ciliopathy diseases affecting childhood development. In this project we will investigate a rare recessively inherited disease called primary ciliary dyskinesia, focussing on the functional genetics of a large PCD cohort containing consanguineous families with a markedly increased disease risk. We will use the most advanced DNA genomic technologies to screen for inherited mutations affecting the structure and motility of cilia. The normal roles of the encoded proteins and the consequences on their function of inherited mutations will be investigated using a number of approaches available in the lab to look at their localisations in cilia and the effect of loss-of-function of the affected proteins, including by use of zebrafish, fly and trypanosome systems. We will also investigate their protein interactions within ciliated cells. This work will shed new light on PCD disease aetiology and, by revealing novel genetic causes of disease, to improve genetic diagnostics and provide new ideas towards replacement therapies in patients. In the long term this has potential to impact on >50 other disorders affecting the respiratory system and other cilia functions.

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Improvement of the amount of muscle formed by skeletal muscle stem cells

Supervisors: Dr Jennifer Morgan and Dr Silvia Torelli

Hypothesis:

The transplantation of skeletal muscle stem cells (satellite cells) is a possible treatment for muscular dystrophies such as Duchenne muscular dystrophy¹. After intra-muscular injection, donor satellite cells contribute to regenerated muscle fibres and functionally reconstitute the satellite cell pool^{2,3}, but the extent to which they do this depends on factors that are only beginning to be elucidated⁴. We hypothesise that factors driving self-renewal, rather than differentiation, of donor satellite cells are the critical factors influencing their engraftment efficiency.

Aims and methods:

We will determine:

- 1) Whether satellite cells can be driven towards self-renewal rather than regeneration.
In vitro models of mouse satellite cells^{5,6} will be used to determine the effects of factors that either activate or inhibit pathways known to be involved in stem cell self-renewal. Self-renewal, proliferation and differentiation of treated and control cells will be determined by immunostaining⁷.
- 2) The mechanism by which the niche within irradiated host muscle signals to incoming donor satellite cells.
Using microRNA arrays and qPCR, we will determine whether there are alterations in the expression of candidate microRNAs in either muscle fibres or satellite cells derived from irradiated or non-irradiated mdx or control muscles. If they are significant changes, we will determine if up-or down regulating the candidate microRNAs within donor satellite cells affects their differentiation and self-renewal in vitro and in vivo.
- 3) The fate of donor satellite cells if they do not contribute to skeletal muscle fibres or to satellite cells.
We will graft satellite cells from a donor mouse in which a marker gene is ubiquitously expressed to investigate whether donor satellite cells give rise to any other cell type apart from muscle fibres and satellite cells.

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Identification of markers of poor response to treatment in the urine of children with kidney tumours

Supervisors: Professor Kathy Pritchard-Jones, Dr Mark Everard-Weeks and Dr John Timms

Hypothesis:

Proteomics, the study of a cell or organisms entire protein complement, offers the opportunity to discover protein biomarkers produced by different tumour subtypes and to monitor over time the tumour response *in-vivo* to chemotherapy.

Aims:

Paediatric kidney tumours are rare and affect 1 in 10,000 children before the age of 15 years. Wilms tumour (WT) represents 90% (1-2) of all paediatric kidney tumours. Almost all patients with WT are treated within prospective, multicentre trials conducted by SIOP-RTSG (3) (Europe) and COG (4) (formerly NWTSG - USA). SIOP trials use preoperative chemotherapy without proven histology until delayed nephrectomy (5). This PhD project proposes quantitative proteomic characterisation of serial urine (and blood) samples to define differences in circulating and secreted proteins released by chemo-sensitive versus chemoresistant blastemal cells in WT during pre-nephrectomy chemotherapy. Data will contribute to the identification of biological pathways associated with resistance to chemotherapy and should have diagnostic utility.

However the primary aim of the project is to identify urine biomarkers that will be used for non-invasive prediction of the histological subtype allowing for earlier risk-adapted modification of treatment intensity. The capacity of such an approach to improve relapse-free and overall survival would be tested in a prospective clinical trial for which this project is the precursor.

Methods:

Blastemal cell volume (6) is a potential new biomarker that could be used to further refine the current definition of 'high risk' histology. It is premature to introduce this into clinical practice as detailed quantification of residual blastema was only defined on central pathology review, not by local pathologists, and the exact volume threshold at which relapse risk increases sufficiently to justify treatment intensification to 'high risk' histology is not yet clear cut. This programme aims to include the proteomic characterisation of urine from this group of chemo-resistant blastemal tumours to discover the biological drivers and define quantitative markers of 'blastemal volume'.

The Children's Cancer and Leukaemia Group (CCLG) treatment centres were engaged in enrolling patients in the SIOP WT 2001 trial and this process continues with full ethical approval (12-LO-0101). This project will establish robust procedures for the collection and processing of biological samples. For biomarkers to be clinically useful they must be assessed within real time such that results can be fed back to the clinical team for consideration in treatment decisions. This study will establish standard operating procedures to enable the collection of urine and assess the feasibility of biomarker profiling of 'high risk' blastemal type tumours.

Methods for the pre-processing and quantitative proteomic analysis of urine will be developed from methods established over 10 years of research at UCL IfWH Cancer Proteomics Laboratory (7-10). Initially 2 operational methods will be investigated to define the method best suited to the provision of reproducible quantitative data from the samples collected. Thermo Scientific TMT cysteine-reactive Isobaric Mass Tagging Kits enable quantitative tandem labelling of proteins extracted from cells/tissues and biofluids for identification and analysis by mass spectrometry.

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How does the CHD7 control cardiac stem and progenitor cell contributions to normal heart development?

Supervisors: Professor Peter Scambler and Dr Philip Stanier

Hypothesis:

CHD7 regulates the expression of genes important for differentiation of cardiac stem cells and cardiac morphogenesis.

Aims and methods:

The study of cardiovascular development has provided a wealth of information on the roles of intercellular signalling molecules, transcription factors and proteins expressed in specific cardiovascular cell lineages. However, it is increasingly clear that the activities of these molecules are modulated by more general epigenetic effects on transcription at the level of chromatin - the "epigenetic landscape". CHD7 is a chromatin remodelling protein that is mutated in human CHARGE syndrome and in atypical cases of DiGeorge syndrome (1).

Aims:

- 1) The Identification of Enhancers and Associated Genes Regulated by CHD7 During Heart Development
- 2) To Explore Pathways that are Regulated by CHD7

Methods:

Conditional mouse mutants will be used to provide tissue from which RNA will be isolated for genome-wide expression analysis, and chromatin will be isolated for chromatin immunoprecipitation. CHD7 binding to specific, known, cardiac enhancers (2) will be expressed using quantitative ChIP. Once CHD7 target sites have been identified, bioinformatic analyses will be used to identify transcription factors that co-regulate gene expression together with CHD7 (3). This will lead to experiments that investigate whether CHD7 acts as a co-activator or co-repressor with candidate transcription factors, and whether CHD7 recruits those transcription factors to target sites.

The conditions for differentiation of embryonic stem cells into cardiac progenitor cells will be optimised for a cell line that contains a knock-in, encoding a dual tag CHD7 fusion protein. This will provide sufficient material to conduct genome wide ChIP-seq analysis of CHD7 chromatin binding at the earliest stages of cardiomyocyte differentiation. Understanding this pathway will have implications for directed differentiation of ES and iPS cells.

Finally, there is evidence that the closely related protein CHD8 interacts with WNT signalling pathways (4). The student will use in vivo and in vitro tools to examine the possibility that CHD7 has a similar role, especially as CHD8 protein interacts with CHD7.

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The role of the T-box transcription factor TBX22 in craniofacial development

Supervisors: Dr Philip Stanier and Dr Erwin Pauws

Hypothesis:

Understanding the molecular and cellular deficit in a mouse deficient for *Tbx22* will allow the development of improved therapeutic intervention procedures for submucous cleft palate.

Aims and methods:

Mutations in *TBX22* are known to be one of the leading genetic causes of palate defects in humans (1, 2). These range from a complete cleft of the secondary palate to a closed but poorly functioning submucous cleft palate (3). We have developed a mouse model lacking *Tbx22*, which has a submucous cleft palate (4). This appears to result from poor palatine bone development, which in turn affects the soft palate musculature. The mouse has a distinct and reproducible phenotype that strongly mimics the human condition, therefore providing an excellent model to investigate this common phenotype. This project sets out to elucidate the fundamental role of *TBX22* in craniofacial development. Preliminary studies show that *Tbx22* is expressed in the paired embryonic palatal shelves but is down regulated by the time of fusion. Intramembranous bones then develop in the mid and anterior portions of the palate, forming the supportive structures of the hard palate. Lack of palatine bones is the most recognisable structural defect in *Tbx22* mutants. We will investigate if the poor intramembranous bone development is a consequence of insufficient cranial neural crest (CNC), an imbalance with non-CNC mesoderm or indeed an inappropriate differentiation towards a muscle lineage. This will be studied using the ROSA26YFP *Wnt1-Cre* reporter mice crossed to both wild type and *Tbx22*null animals. *Tbx22* expression will then be investigated for co-localisation with the YFP positive cells in the wildtype animals. A similar experiment will also be performed with a mesoderm specific *Cre* such as *Mesp1-Cre* (Yoshida et al, 2008). This experiment will then inform a confirmatory experiment to cross the *Wnt1-Cre* mice or *Mesp1-Cre* to floxed *Tbx22* mice to investigate the phenotype recovered when *Tbx22* is only deleted from the respective cell type.

Expression studies indicate that *Tbx22*null mice undergo an upregulation of muscle development in the palatal shelves prior to the onset of osteogenesis. Therefore, genetic markers of muscle differentiation will be investigated in E10.5 to E14.5 wild type and mutant embryos. The regulation of osteoblastic differentiation into intramembranous bone will also be investigated in E13.5-E17.5 embryos. Analysis will also include expression of *Bmp* and *Fgf* signalling pathways, which are likely to act upstream of *TBX22* and are known to have critical roles in providing instructive cues for normal palatal intramembranous bone development. Next, differential cell proliferation will be investigated in the rapidly elongating palatal shelves (E13.5-E14.5) and at the time of fusion using immunohistochemistry with anti-phosphohistone H3.

An *in vitro* palate culture system will be used to compare palate development between wildtype and mutant mice both in the context of specific pathway inhibitors or modifiers and as a model to investigate repair of palatine bone defects using osteogenically differentiated stem cells (6).

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Identifying the mechanisms of vanadium-induced cytotoxicity in neuroblastoma cells

Supervisors: Dr Andrew Stoker and Dr Alan Burns

Neuroblastoma (NB) is a neural tumour derived from the sympathoadrenal lineage. It is the most common extracranial solid tumour in children, underlying 15% of paediatric cancer deaths [1]. High grades of metastatic disease present great clinical challenges and improvements to chemical and biological treatments for NB are continually being sought. Our group has recently shown that chemical inhibitors derived from the element vanadium can selectively kill 50% of NB cell lines, or induce them to turn into harmless neurons in cell culture (*Clark and Stoker, submitted*). These inhibitors broadly target proteins called protein tyrosine phosphatases (PTPs). PTPs play key signalling roles during neural development, regulating RTK signalling both positively and negatively [2; 3]. This protein family is also increasingly implicated in cancer biology [4; 5; 6], but it has been studied very little in the context of NB. This is therefore a timely opportunity to investigate the role of PTPs in this childhood disease.

The vanadium-based PTP inhibitors have been used in cancer studies for many years [7; 8]. However, due to the potential for long term toxicity, these chemicals may not themselves be ideal for use directly in humans. To avoid using vanadium itself therefore, the proposed project aims to understand the selective, molecular mechanisms underlying the death-inducing properties of the chemical. In this way we will be able to define alternative molecular targets that could be more readily exploited therapeutically in children.

Hypothesis:

We hypothesise that the cytotoxic capacity of BMOV is dependent upon the induction of novel, downstream transcriptional and biochemical changes in NB cells, which in turn either block critical survival-promoting pathways, or directly induce cell death.

Aims and methods:

We will treat NB cell lines with vanadium compounds and use gene expression profiling and bioinformatics with the aim of defining gene expression changes during cell death induction. The key aim will then be to identify and functionally characterise those genes that actually underpin the cell-killing potential of the inhibitors. In the long term this knowledge should allow us to define selective chemical inhibitors of the gene products, for use therapeutically.

The student will pursue the following experimental plan:

- 1) mRNA will be extracted from chemically-treated and control NB cells and the student will perform a microarray analysis of the transcriptional changes that occur early during the induction of cell death.
- 2) A bioinformatics analysis will allow the student to extract candidate genes that show significant expression changes during the chemical treatment. Changes in gene expression will be validated using QPCR and immunoblotting.
- 3) The cellular and biochemical functions of top candidate genes will then be extensively investigated using over- and under-expression methodologies and cell signalling analyses in NB cell lines.
- 4) The expression profiles of candidate genes will also be determined both in primary NB tumours samples and in the developmental context of the sympathoadrenal lineage in mouse and human embryos.

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