National Institute for Health Research

The NIHR Biomedical Research Centre at Great Ormond Street Hospital for Children NHS Foundation Trust and UCL Institute of Child Health

NIHR GREAT ORMOND STREET HOSPITAL / UCL INSTITUTE OF CHILD HEALTH BIOMEDICAL RESEARCH CENTRE

Translational Clinical Research
3-year Full-time Non-clinical PhD Studentships

Application Instructions

1. Download a copy of the application form and save your completed application as a single Word document attachment. Name your Word document using your first name and surname, eg John Smith.doc

2. Send your application by e-mail to the BRC Management team at BRCStudentship@gosh.nhs.uk no later than **5pm on 11th November 2013**.

3. Separately, you should arrange for your two/three referees to complete a reference form to accompany your application. This can be found at http://www.ucl.ac.uk/ich/education-ich/phd-prog/phd_studentships/brc-phd-studentships-2013

Please send a copy of the form to your referees or ask your referees to download the Reference Form from the ICH website. They will need to send the reference by email to BRCStudentship@gosh.nhs.uk no later than 5pm on 11th November 2013.

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

National Institute for Health Research

The NIHR Biomedical Research Centre at Great Ormond Street Hospital for Children NHS Foundation Trust and UCL Institute of Child Health

NIHR GREAT ORMOND STREET HOSPITAL / UCL INSTITUTE OF CHILD HEALTH BIOMEDICAL RESEARCH CENTRE

Translational Clinical Research PhD Studentships 2013

Frequently Asked Questions

What is a PhD studentship?
Research studentships enable graduates with good honours degrees to undertake a training programme under the guidance of named supervisors.

The student will complete a three year research project which includes training in research methods, scientific communication and other personal skills, designed to lead to the submission of a doctoral thesis and the subsequent award of a PhD.

All students will be registered to University College London (UCL) for their degree, and will be based at UCL Institute of Child Health. As such, they will be expected to participate in departmental activities, such as seminar programmes, etc.

Successful candidates for a studentship will receive an annual stipend for the duration of their three year research project. Stipend amounts are reviewed periodically to take into account inflation, cost of living, etc. For example, the stipend for the 2013 GOSH / UCL BRC PhD studentships is £15,740 for the first year and with annual increases thereafter.

Why should I apply for a GOSH / UCL BRC PhD studentship?
GOSH and ICH combine to provide a stimulating research environment and a comprehensive programme of support for graduate students.

This scheme will be for talented graduate students committed to undertaking translational biomedical research focussed on the clinical impact of patients at Great Ormond Street Hospital and worldwide. The NIHR BRC scheme funds patient-focused early phase translational clinical research (commonly referred to as experimental medicine), the aim of which is to pull basic scientific discoveries into clinical research, and through to benefits for patients and the NHS.

Together with its partner, Great Ormond Street Hospital for Children NHS Foundation Trust, UCL ICH contains the largest concentration of research expertise in the scientific basis of child health in Europe. GOSH and UCL ICH have long been at the forefront of paediatric medical research and GOSH / UCL BRC aims to build on this by undertaking experimental medicine research in across four research themes; The Molecular Basis of Childhood Diseases; Diagnostics and Imaging in Childhood Diseases; Novel Biological Therapies for Translation in Childhood Diseases; and Gene, Stem and Cellular Therapies. Further information on the objectives of GOSH / UCL BRC and the remit of each of the four research themes can be found on the GOSH website at http://www.gosh.nhs.uk/research-and-innovation/gosh-ucl-biomedical-research-centre/

What is the eligibility criteria?
Applications are invited from recent graduates or final year undergraduates who hold or expect to gain a first/upper second class honours degree or equivalent from any recognised university worldwide.

How do I apply for a studentship?

1. Download a copy of the application form and save your completed application as a single Word document attachment. Name your Word document using your first name and surname, eg John Smith.doc
2. Send your application by e-mail to the BRC Management team at BRCStudentship@gosh.nhs.uk no later than **5pm on 11th November 2013**.
3. Separately, you should arrange for your two/three referees to complete a reference form to accompany your application. This can be found at http://www.ucl.ac.uk/ich/education-ich/phd-prog/phd_studentships/brc-phd-studentships-2013
4. Please send a copy of the form to your referees or ask your referees to download the Reference Form from the UCL ICH website. They will need to send the reference by email to BRCStudentship@gosh.nhs.uk no later than 5pm on 11th November 2013.

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

How much is the stipend?
Successful candidates for a studentship will receive an annual stipend for the duration of their three year research project. Stipend amounts are reviewed periodically to take into account inflation, cost of living, etc. For example, the stipend for the 2013 GOSH / UCL BRC PhD studentships is £15,740 for the first year and with annual increases thereafter.

Can students from outside the UK apply?
No nationality restrictions apply. Overseas students have previously been accepted into the programme. This PhD Programme 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 2013/14 is £15,850 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.

I have a lower second degree but I am now doing an MSc. Is this equivalent to an upper second?
Assuming you passed your MSc, you could be eligible to hold a PhD studentship. Please be aware that previously, nobody with such a background has been successful when applying to previous ICH PhD programmes.

Do I need to have chosen my project from the portfolio, prior to interview?
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 the Research and Career Aspirations section of the application form.

National Institute for Health Research

The NIHR Biomedical Research Centre at Great Ormond Street Hospital for Children NHS Foundation Trust and UCL Institute of Child Health

NIHR GREAT ORMOND STREET HOSPITAL / UCL INSTITUTE OF CHILD HEALTH BIOMEDICAL RESEARCH CENTRE

Translational Clinical Research PhD Studentships 2013: Project Portfolio

Molecular Basis of Childhood Disease

1. Molecular mechanisms of asthma: Hedgehog signalling in epithelial/lymphoid cross-talk

(Tessa Crompton)

Project Aim

The purpose of this study is to translate our basic research findings on Hedgehog (Hh) signalling in mouse asthma to the human system, in order to increase understanding of the molecular mechanisms of childhood asthma, and to improve treatments for this disease.

Asthma, is a common chronic disease of childhood, and represents a major economic and health burden in Western society, with over 1.1 million children in the UK currently treated for asthma, and ~30 children per year in the UK dying of asthma. Asthma is an immune-mediated allergic disease predominantly involving T-helper 2 (Th2) immunity. Therapy tends to involve steroids, which have significant side effects, and some patients develop dependency or are steroid-resistant. Therefore, new asthma treatments are urgently needed, including strategies to specifically target Th2 responses and to modulate the lung-lymphocyte interactions driving disease. The purpose of this study is to investigate the molecular cross-talk between lung epithelium and T-cells in asthmatic lungs, and to test the hypothesis that Hh signalling in the lung drives and exacerbates asthma. A deeper understanding of the molecular mechanisms of disease induction and resolution will inform development of new therapies, and specifically the possibility of using Hh pathway inhibitors to treat severe asthma.

Plan of Investigation

- In order to test the hypothesis that secretion of Hh proteins by human lung signals to T-cells and directs their differentiation to the Th2 lineage, thus driving asthma, we will undertake the following objectives.
- We will test the hypothesis that expression of Hh proteins is upregulated in asthmatic human lung, and that T-cells in asthmatic lung are responding to these signals.
- We will test the hypothesis that Hh proteins are highly expressed in the lung as it grows and forms in infancy and childhood, and that this expression may in part account for the high prevalence of asthma in childhood.
- We will test if Hh-treatment biases human CD4+ T-cell differentiation to Th2.
- We will test if increased Hh signalling to immune cells (in the absence of full Hh pathway induction in cilia-bearing cells and subsequent negative feedback) accounts for the high incidence of asthma in Bardet-Biedel syndrome (BBS).

Clinical Relevance

This project will apply our expertise in the basic science of T-cell differentiation to the clinical setting of childhood asthma, both in the general population, and in BBS. Therefore, we will be working directly at the interface between basic and clinical science.

2. Identifying the mechanism of vanadium IV-induced cytotoxicity in neuroblastoma cells
(Andrew Stoker)

Project Aim

We aim to identify novel gene and protein targets in neuroblastoma (NB) cells that could be exploited therapeutically. The targets of interest are effectors of cytotoxic, vanadium-based chemicals in NB cells. We will define how vanadium-based chemicals alter the expression and function of these effectors and how these effectors regulate NB cell survival and cell death. This will provide better understanding of tumour cell survival control, identifying targets that could be exploited pharmacologically.

Plan of Investigation

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. We aim to identify and characterise these downstream effectors, allowing us to formulate new pharmacological approaches for this disease.

- They will extract mRNA from BMOV-treated NB cells and controls, and perform a microarray analysis of the transcriptional changes that occur early during the induction phase of cell death.
- They will carry out bioinformatics analysis and extract a panel of candidate genes that show significant up or downregulation during the cell treatment, and fit these genes into known signalling pathways using network analysis software.
- Changes in candidate gene expression during BMOV treatment will be validated using QPCR and immunoblotting.
- The cellular and biochemical functions of a small set of top candidate genes will then be investigated using molecular genetic approaches. In particular we will seek to drive their up/downregulation in cells, inducing cytotoxicity and confirming their potential as surrogates for BMOV.
- 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.

Clinical Relevance

In order to develop novel therapeutic approaches for neuroblastoma treatment, research into the basic control of tumour cell survival must continue to be tested in culture models. By determining how BMOV triggers cell death in NB cell lines, we will provide potential targets for future drug development. The objective of this research is to identify novel genes and proteins in neuroblastoma tumours that are critical for the aberrant survival of the tumour cells. This will provide better understanding of growth and survival controls in neuroblastoma cells and at the same time pinpoint new genes and proteins that could be directly targeted pharmacologically, hopefully leading to new or improved therapies.

3. Discovering new genetic causes of inflammatory disease and arthritis in children
(Paul Brogan/Nigel Klein)

Project Aim

Great Ormond Street Hospital comes into contact with children with severe genetic fever syndromes who present with severe fevers and inflammation affecting virtually any organ in the body from very early on in life, many below the age of 1. Some of these children will die of the disease if left untreated. The aims of this study are to identify responsible genetic mutations for undefined familial autoinflammatory diseases and to further characterise the phenotype of the mutation on the patients' cells and in vitro cell culture models.

Plan of Investigation

Based on our experience, the first 18 months will identify the mutated gene(s), although it is possible that candidate genes may be identified well before this time point; the second half of the Ph.D. studentship will study the functional impact of these mutations on the immune system. 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.

We have established several systems to achieve this, including knockdown and transfection experiments in cell lines in vitro (including HeLa and B cell knockdown and transfection experiments) examining basal and stimulated cytokine production assessed at the mRNA and protein level, since excessive pro-inflammatory cytokine production is central to the pathogenesis of most autoinflammatory diseases. Having established the functional basis of these conditions, the student will use silencing RNA to knockdown the expression of the gene of interest and/or synthetic inhibitors of downstream signalling molecules to explore the potential therapeutic benefit of blocking the pathological cytokine signals in vitro.

Clinical Relevance

Using basic science, we anticipate that the findings of this project will be rapidly translated into diagnostic tests for these families and for children with similar diseases, and could lead to novel therapeutic approaches based on the findings of the functional studies, as has been the case in previous studies of this nature (3;5-10).

4. Somatic mosaicism and new gene discovery in NLRP3 mutation-negative CAPS patients
(Paul Brogan/Nigel Klein)

Project Aim

Cryopyrin associated periodic syndromes (CAPS) comprise a spectrum of periodic fever disorders, consisting of 3 overlapping syndromes of increasing disease severity: familial cold autoinflammatory syndrome (FCAS), Muckle-Wells Syndrome (MWS), and Chronic Infantile Neurologic Cutaneous Articular syndrome (CINCA), also known as Neonatal Onset Multisystem Inflammatory Disease (NOMID). The latter is the most severe form, exhibiting continuous disease activity with neonatal onset of fever, urticarial rash, dysmorphic features, deforming arthropathy, chronic meningitis, papilloedema, hearing loss and growth and mental retardation.

Most CAPS patients are heterozygous for germline mutations in the NLRP3, indeed to date the only identified cause of CAPS has been variants of NLRP3. More than 80 different disease causing mutations have been identified, mostly clustered in NLRP3 exon 3, but also described in exons 4 and 6 (2,3,4), and are either “de novo” or transmitted in an autosomal dominant manner. The aims of this study will be to investigate the relative contribution of somatic mosaicism for NLRP3 mutations versus the possibility of entirely novel genes causing CAPS in “mutation negative” CAPS patients at GOSH and the National Amyloidosis Centre (NAC).

Plan of Investigation

A preliminary study of six GOSH and three NAC patients with mutation negative CAPS, that will take two main lines of investigation. The first will attempt to identify somatic mosaicism as the cause of their disease. In parallel the second line of investigation will attempt to identify novel candidate genes using whole exome sequencing of DNA extracted from whole blood. Functional analysis of any new mutations found will be performed, most likely in subsequently funded studies since these are often complex and require setting up new assays to perform them. The nature of these studies will ultimately be determined by the novel sequence variants identified and potential pathways involved.

Clinical Relevance

The timely diagnosis of NLRP3 somatic mosaicism can increase the rate of genetic diagnosis of CAPS to 80% or higher, and novel genetic mutations could account for the remainder. If new causative genes for CAPS emerge from this research, this discovery would be a completely novel breakthrough, and would impact on other children with mutation negative CAPS elsewhere in the world.

Findings from this study could also protect these children from having their expensive therapy withdrawn, a significant concern given the current economic climate and changes to the NHS. Furthermore, with this research we will try to ascertain the best methodology to approach cases of clinically diagnosed CAPS who are NLRP3 wild-type through conventional screening methods, and to establish it at ICH/GOSH, making screening for somatic mosaicism and other new mutations routinely available for future patients referred to the clinical service.

5. The role of ARNT2 and related proteins in hypothalamo-pituitary disease in humans
(Mehul Dattani)

Project Aim

The aim of this proposal is to further our understanding of human conditions affecting the hypothalamo-pituitary axis, with particular focus on the hypothalamus. We have recently identified mutations in the gene encoding the aryl hydrocarbon receptor nuclear translocator 2 (*ARNT2*) in a family showing a highly unusual phenotype displaying several features of hypothalamic insufficiency including cortisol deficiency, central hypothyroidism, abnormal growth in conjunction with obesity, and central diabetes insipidus. Arnt2 is involved in xenobiotic metabolism, circadian rhythm, response to hypoxia, and CNS growth and differentiation. To our knowledge, this represents the first mutation in a human gene leading to a complete failure of the neuroendocrine hypothalamus. However, the mechanisms underlying these phenotypic features are not very well understood and it remains to be established whether mutations in *ARNT2* or related proteins involved in the same pathway during normal development are mutated in our cohort of patients suffering from hypopituitarism.

Plan of Investigation

- Screen our large and unique cohort of patients with hypothalamo-pituitary disease for mutations in the gene encoding the transcription factor ARNT2 and related genes including *OTP*, *SIM1*, *SIM2* and *BRN2*, which participate with ARNT2 in the development of the neuroendocrine hypothalamus in animal models.
- Study the expression patterns of *OTP*, *SIM1*, *BRN2* and *SIM2* in relation to that of *ARNT2* in human embryos using the Developmental Biology Human Resource (HDBR) based at ICH.
- Perform conditional targeted mutagenesis of *ARNT2* in the hypothalamus and pituitary and to determine the mechanisms by which mutations in *ARNT2* lead to a phenotype.

Clinical Relevance

We hope that as a result of these studies, a further proportion of our patients will have an explanation underlying the phenotypes present. Given the heterogeneity of the phenotypes, which often include diabetes insipidus and obesity in a large proportion of the patients with hypopituitarism, it is highly likely that defects in hypothalamic development may underlie these phenotypes. The pathophysiology of these and other associated features may also be elucidated, such as the CAKUT identified in patients with *ARNT2* mutations. Genetic counselling of patients will also be facilitated.

6. Does defective BLK signaling account for impaired immune tolerance in childhood myositis?

(Lucy Wedderburn / Kiran Nistala)

Project Aim

The purpose of the project is to explore mechanisms for immunological abnormalities in Juvenile Dermatomyositis (JDM), to correlate these to clinical disease course and to identify new therapeutic targets in the pathways found to be implicated.

Key questions

Our pilot data show that JDM patients produce lower levels of the immunoregulatory cytokine IL-10, from circulating B cells when compared to healthy children. In this project we will test if JDM B cells fail to regulate T cell responses, and will ask if the B cell signaling molecule, BLK, which has a genetic association with JDM, accounts for this abnormality.

Plan of Investigation

- B cell cytokine production will be assessed directly *ex vivo* and following stimulation with anti-CD40. Cytokines will be detected by intracellular staining (TNFα, IL-6, IL-10, IFNγ) together with B cell surface markers by flow cytometry. In parallel the student will measure actual serum cytokine levels.
- The expansion of pathogenic T cells in active JDM observed in pilot data, including Th17 cells, needs to be confirmed. The magnitude of B cell IL-10 production will be correlated with the frequency of T cell cytokine subsets, to see if reduced B cell IL-10 is associated with an increased frequency of effector T cells ex-vivo.
- To test the suppressive capacity of JDM B cells, PBMC will be sorted by magnetic beads and B cells will be co-cultured with autologous memory and naïve CD4+ T cells. T cell expansion and cytokine expression will be assessed by flow cytometry using CFSE and cytokine staining. Transwell and antibody blocking experiments will be used to test the role of contact dependent /independent signals. B cell regulatory function will be correlated with patient clinical outcomes.
- Although the BLK allele linked to JDM has been shown to reduce expression of BLK in B cells, the functional consequences of this alteration remain unclear. The student will be ideally placed to investigate the role of BLK signaling in JDM. He/she will examine expression of BLK at a message and protein level in JDM B cells and see if this correlates with IL-10 production or is associated with specific autoantibody profiles. Using kinase inhibitors, they will be able to test if blockade of BLK signaling alters B cell function in terms of both cytokine and antibody production. If initial results are promising, the student will be able perform further mechanistic studies in vivo using BLK knockouts, in collaboration with Prof Mauri, UCL.

Clinical Relevance

There is an emerging therapeutic armamentum targeting B cells (including anti-CD20, Rituxmab) in autoimmune disorders. Data from this PhD will provide a mechanistic understanding for the defects in B cell function in JDM and for the first time offer a scientific basis for choosing lymphocyte and cytokine targeted therapy in JDM. It is likely

that results from this proposal will be of direct benefit to patients as clinical trials (of Bayesian design) are currently being planned for novel therapies in JDM.

7. Investigating the pathogenesis of JAK-1 immunodeficiency
(Siobhan Burns/Adrian Thrasher)

Project Aim

We have recently identified a novel immunodeficiency associated with a loss of function mutation of Janus Associated Kinase 1 (JAK1). The purpose of this project is to determine how JAK-1 deficiency leads to impaired function of immune cells in humans.

Plan of investigation

- To generate of JAK1 deficient and mutant cell lines.
- To examine the relative importance of JAK 1 for signalling through Type I, Type II and IFN cytokine receptors.
- To investigate the importance of JAK1 in myeloid cell function.
- To investigate the importance of JAK1 in anti-viral protection.

Clinical Relevance

This project has arisen from the identification of a novel gene defect causing human immunodeficiency and susceptibility to mycobacterial disease. The project utilises a basic science approach to understand the functional relevance of the mutation and aims to generate results that will feed back to the clinic: informing our understanding of disease pathogenesis, facilitating identification of other patients with JAK1 deficiency and informing patient care.

8. How does the CHARGE syndrome gene CHD7 control cardiac stem and progenitor cell contributions to normal heart development?
(Peter Scambler / Phil Stanier)

Project Aim

To assess the action of CHD7 on embryonic cardiac enhancer elements and gene expression during cardiac morphogenesis.

Plan of investigation

- Identification of Enhancers Regulated by CHD7 During heart Development.
- Functional analysis of CHD7 gene regulation.

Clinical Relevance

A long term, potential benefit is to use cells capable of forming different structures during repair or regeneration strategies. This is because the use of cellular treatments of events like heart attacks may involve instructing cells to recapitulate what happens in development. Conversely, isolation and expansion of cells to be used in such therapies may rely on reprogramming one cell type into another, a process that requires alteration of expression in a manner similar to that controlled by CHD7. This is termed direct reprogramming, and can be effected via viral vectors.

9. Regulation of Autophagy-Inflammasome crosstalk by the Wiskott-Aldrich Syndrome protein-cytoskeleton axis
(Adrian Thrasher / Mona Bajaj-Elliott)

Project Aim

To test the hypothesis that in innate dendritic cells and macrophages, Wiskott-Aldrich Syndrome protein (WASp)-mediated cytoskeletal rearrangements provide a conduit for crosstalk between the autophagy machinery and the Inflammasome complex.

Plan of investigation

- To elucidate the role of WASp in macrophage and DC-mediated starvation and microbial-mediated autophagy.
- To elucidate molecular mechanism(s) responsible for the aberrant NALP3-mediated inflammasome activation observed during WASp deficiency.
- To determine how WASp-cytoskeleton axis may fine-tune crosstalk between the autophagy process and the Inflammasome?

Clinical Relevance

Protein platforms such as the 'Inflammasome' and the 'Autophagy' machinery are so integral to cell homeostasis that mutations in these systems inadvertently results in disease. Defects in the inflammasome and autophagy function are implicated in a variety of auto-inflammatory and autoimmune diseases, ranging from Mediterranean Fever syndrome, Inflammatory Bowel Disease, Diabetes and Multiple Sclerosis. Patients born with defects of the immune system, such as the Wiskott Aldrich Syndrome, are susceptible to recurrent infections and exhibit severe inflammatory manifestations. Here, we suggest for the first time that Wiskott-Aldrich syndrome patients exhibit dysregulation in inflammasome function. Using WAS as a model, we aim to gain better understanding of why the autophagy machinery in WAS patients is unable to keep a check on the inflammatory capacity of the inflammasome. Importantly, our findings may help to improve current and advance future therapeutic intervention for patients with defects not only in the immune system but in other organ-specific inflammatory/autoimmune conditions.

Gene, Stem and Cellular Therapies for Childhood Diseases

10. Gene therapy for HIV

(Waseem Qasim / Greg Towers)

Project Aim

We have generated human mimics of naturally occurring TRIM-Cyclophilin restriction factors and are investigating if these could be of therapeutic benefit in patients with HIV-1 infection. Such restriction factors have proven effective during non-human primate evolution, arising naturally on at least two separate occasions- in both Old and New World monkeys, retrotransposition of the HIV-1 binding factor cyclophilin A (CypA) into the TRIM5 gene locus has resulted in the production of novel fusion proteins that are powerful inhibitors of HIV-1.

The project aims to (i) Investigate promoter systems capable of mediating regulated TRIMCypA expression in T cells (ii) Develop strategies for site specific integration of TRIMCypA within the TRIM5 locus and (iii) Characterise the effects of TRIMCypA expression on endogenous TRIM expression and T cell function.

Plan of investigation

- Regulating vector mediated TRIMCypA gene expression.
- Targeting the TRIM5Cyp insertion to the endogenous TRIM5 locus.
- Characterising TRIMCypA modified immune cells.

Clinical Relevance

The project will help to develop new treatments against HIV-1 infection. There are over 400 children in London with HIV infection, and as they get older, newer forms of treatment are needed. This student will join an existing program of work relating to T cell gene therapies, with a specific focus on HIV. The project will provide research training in molecular biology, virology and immunology techniques and has direct translational implications.

11. Gene therapy with anti-CD19 chimeric antigen receptor gene-modified autologous gamma/delta T cells for paediatric lymphoma.
(Kenth Gustafsson / John Anderson)

Project Aim

We propose to investigate the use of V9Vδ2 cells as effector cells for CD19-CAR (chimeric antigen receptors) based T cell adoptive transfer. We will make use of four receptors (already generated with codon optimisation and full humanization) containing the anti-CD19 single chain variable fragment (scFv) in combination with different cytoplasmic portions, i.e. CD3ζ, CD28, or OX40.

Plan of investigation

- Optimization of the transduction of purified healthy donor γδT cells.
- Evaluation of CAR-equipped V9Vδ2 cell cytotoxicity to CD19-positive tumour cell lines *in vitro.*
- Functional studies *in vitro*: Assessment of the release of IL-2 and IFN-γ cytokines, granzyme B and perforin cytolytic granules by effector T cells following their co-culture with paired antigen positive and negative target cells.
- Evaluation of *in vivo* efficacy and safety of adoptive transfer of CAR-transduced γδT cells in lymphoma tumour-bearing xenograft models following infusion of CAR expressing human γδT cells.

Clinical Relevance

This project is intended to translate our recent work into clinical applications by combining work in two areas. CAR constructs have been made already and are being developed for clinical use. In another area of work we have discovered novel aspects of γδT cells that strongly suggest that these would be suitable targets for CAR gene therapy resulting in presentation of tumour cell antigens to other T-cells in addition to their enhanced tumour cell direct killing effect as a result of CAR expression. CD19+ B-cell lymphomas are common forms of childhood cancer and so would benefit from novel forms of immunotherapies in combination with available therapeutic options.

12. Gene modified bone marrow stem cells therapy for inherited skin diseases
(Wei-Li Di / Waseem Qasim)

Project Aim

This studentship will build on our progress in the development of novel therapeutic strategies for the treatment of inherited skin diseases). In this project, we will focus on the debilitating skin disease recessive dystrophic epidermolysis bullosa (RDEB), an inherited blistering skin disorder caused by defective expression of COl7A1. The student will investigate the feasibility of systemic cell therapy based on gene modified autologous marrow derived cells for the treatment of the skin disease.

Plan of investigation

- Development of Lentiviral gene transfer system.
- Assessment of gene modified (mensenchymal stem cells) MSC in epidermis-like cells in 3D culture.
- In vivo human-murine chimaeric skin grafting model.
- Evaluation of therapeutic efficacy in (haematopoietic stem cells) HSCs and MSCs obtained from RDEB patients.

Clinical Relevance

If pre-clinical studies are successful, we have the necessary expertise and infrastructure to initiate phase I clinical trials of gene based therapy for RDEB.

13. Bioengineering approaches for the repair of congenital craniofacial malformations with patient-derived somatic stem cells
(Patrizia Ferretti)

Project Aim

To harness the plasticity of autologous paediatric human adipose tissue-derived stem cells (hADSCs) and use them to construct bioscaffolds consisting of different tissue types for more effective craniofacial reconstruction in children with congenital abnormalities.

Plan of investigation

- Learn basic techniques (e.g. cell culture, differentiation protocols, microscopy, histological analysis, immunohistochemistry, TUNEL protein and RNA analysis); develop and test new decelluarisation protocols and composite scaffolds.

- Manipulation of hADSC to induce epithelial phenotypes. Comparison of decellularised composite scaffolds with nanoscaffolds. Test additional nanoscaffolds if appropriate

- Select most promising scaffolds for the different hADSC-derived tissue types alone or in combination (e.g. cartilage alone, cartilage with epithelial lining) and test in the CAM-graft model.

Clinical Relevance

This study will increase our understanding of the plasticity of hADSC and their value for autologous cell therapy in craniofacial reconstruction in children with congenital craniofacial abnormalities. Crucially it will determine which of the two proposed approaches for bioscaffold construction (scaffolds based on "reconstructed" decellularised tissue and nanoscaffolds) can better support formation of hADSC-derived cartilage and bone, and would be more suitable for scaling up to move from the bench to the bed-side. Therefore, findings from this study will be relevant to the management of craniofacial abnormalities.

14. Collection, characterization, banking and reprogramming of human amniotic fluid stem cell lines for use in novel clinical stem cell therapies
(Paolo De Coppi / Anna David)

Project Aim

The purpose of this proposal of investigation is to isolate and grow human amniotic fluid stem (hAFS) cells under Good Manufacturing Practice (GMP) conditions to create a bank of AFS cells for potential use in fetal and neonatal therapies. Amniotic fluid stem cells are broadly multipotent adult stem cells that hold great promise in the field of regenerative medicine. Because of their unique characteristics we believe that it could be advantageous to bank hAFS cells during pregnancy or at birth. They could thus be used to create personalized autogenic or allogenic cell therapies that could be translated into clinical use. In addition, we aim to generate induced pluripotent stem (iPS) cells from hAFS cells for using in regenerative medicine, pharmaceutical screening, and in disease modeling.

Plan of investigation

- Preparation of amendments of the protocol, information and consent form sheets (to show to the parents) for the permission to bank hAFS cells and Ethical Committees approval.
- Develop and optimize the procedures by focusing on collection (in closed systems), transport, selection, expansion, cryopreservation and thawing of hAFS cells following specific GMP. Test and validation of the GMP process.
- Characterization of hAFS cells to check their quality and stem cells potential with specific markers.
- Collection of different hAFS cell lines for banking in GMP.
- hAFS cell reprogramming to iPS cells for diagnosis and preclinical application. Setting up the protocol that guarantees the maximum efficiency, viability and safe of iPS colonies**.**

Clinical Relevance

This project aims to collect and bank human AFS cells under GMP conditions to be released for use in pre-clinical and translational clinical research. This will provide also the opportunity to better investigate AFS cell populations and characteristics for potential use in fetal and neonatal therapies. Furthermore, iPS cells developed from AFS cell samples with congenital diseased will be studied. This could lead to the identification of new biomarkers or behaviour of pathologies, with the potential development of new therapeutic strategies.

Novel Therapies for Childhood Diseases

15. Studying micro RNAs as biomarkers for diagnosis and novel therapeutic targets in muscular disorders
(Francesco Muntoni / Jenny Morgan)

Project Aim

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 muscular dystrophies (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.

Plan of investigation

- Patient material and RNA isolation from muscle samples and serum/urine.
- Small RNA sample preparation.
- Bioinformatics analysis.
- Validation of new and candidate biomarkers miRNA.
- The functional relevance of the selected differentially expressed miRNAs will be investigated in cultured human myoblasts.

Clinical Relevance

The project aims to further understand the additional element of regulation of muscle function mediated by miRNAs in different types of muscular dystrophies and to discover miRNAs that can be utilised as non-invasive biomarkers for monitoring disease progression. The discovery of dysregulated miRNAs in different MD will also help to identify candidate miRNAs as potential therapeutic targets for future clinical applications.

16. Usher syndrome – towards therapy
(Maria Bitner-Glindzicz)

Project Aim

Investigate antisense therapies for treatment of Usher syndrome mutations.

Plan of investigation

- Identify further patients with splice mutations amenable to manipulation by antisense therapy.
- Design a 'genomic capture' method and examine RNA transcripts from patients with monoallelic mutation.
- Verify the effect of novel intronic mutations on splicing by RNA analysis of nasal transcripts.
- Determine whether aberrant spliceforms can be reverted to wild-type transcripts by the addition of antisense oligonucleotides (AONs).

Clinical Relevance

It will be a 'proof-of-principle' study of a novel therapy for Usher syndrome using a 'personalised medicine approach'. As yet there is no treatment for the retinal degeneration in this condition. Usher genes are often large and gene therapy poses significant technical problems. This offers a potentially more straightforward therapeutic approach and will benefit from local expertise using similar methods in neuromuscular diseases.

Other Improvements in outcomes are:

- More complete molecular diagnosis through a CPA accredited laboratory, which will include complete custom capture of Usher genes to include intronic as well as usual exonic regions.
- Better quality of information available to family and health professionals regarding diagnosis.
- Facilitation of Cochlear Implant and audiological care planning based upon accurate diagnosis.

17. Targeting phosphorylation of the H3.3 chaperone DAXX as a new treatment for paediatric glioblastoma
(Jonathan Ham / David Michod)

Project Aim

The key goals of this research are to determine whether DAXX phosphorylation can be used as a biomarker in paediatric glioblastoma and to establish whether targeting DAXX phosphorylation can be used as a therapeutic approach to treat paediatric glioblastoma.

Plan of investigation

- Determine DAXX, HIPK2, DEK, calcineurin expression and DAXX phosphorylation level in paediatric glioblastoma.
- Examine response of paediatric glioblastoma to drugs modulating DAXX phosphorylation.

Clinical Relevance

The research will have an impact on the understanding of paediatric glioblastoma aetiology, with a particular focus on a recently highlighted epigenetic pathway which is frequently mutated in paediatric glioblastoma. Moreover this research might have an impact on the improvement of paediatric glioblastoma treatments.

Current treatments of paediatric glioblastoma rely mostly on radiation and chemotherapeutic treatment. Improving knowledge of the biology of this brain tumour will lead to the discovery of targeted drug treatments with fewer side effects.

18. Airway inflammation in Primary Ciliary Dyskinesia
(Chris O'Callaghan / Steve Hart)

Project Aim

In this project we will investigate the mechanism behind the marked inflammatory response Primary Ciliary Dyskinesia (PCD) and evaluate treatment strategies aimed at reducing it.

Plan of investigation

- Obtain ciliated epithelium from healthy individuals and patients with PCD caused by inner and outer dynein arm defects.
- RNA interference in healthy cells targeting genes that are known to cause PCD.
- Test and develop methods to mechanically stress ciliated cells in culture.
- Test effects of exposure of cultured cells to Azithomycin, clarithromycin and corticosteroids.

Clinical Relevance

This work will impact on the treatment of children suffering from primary ciliary dyskinesia (PCD). PCD causes defective mucociliary clearance with children suffering from recurrent chest and sinus infections and many from hearing deficit. Current treatment is based on antibiotic therapy when needed and physiotherapy. It has not been appreciated that children with this condition may also have chronic inflammation as a result of reduced or absent ciliary movement. If the treatments evaluated in ciliated cell culture are successful we will develop clinical trials to evaluate their clinical effect. This project will also resolve questions relating to the huge level of inflammation, independent of infection, that we have seen in children with PCD.

19. Circulating microparticles as therapeutic targets of plasma exchange in antineutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV)
(Paul Brogan / Despina Eleftheriou)

Project Aim

We will investigate the hypothesis that microparticles (MPs) are an important therapeutic target of plasma exchange (PE) in AAV. This will be an ancillary study of an international trial of therapeutic PE in AAV, the PEXIVAS trial

Plan of investigation

- Identify the soluble and MP plasma components that are removed from patients by PE.
- Characterisation of plasma MP by flow cytometry.
- The influence of PE on MP removal.
- Influence of PE on soluble plasma inflammatory mediators.
- In vitro assessment of MP subpopulation pathogenicity.
- Investigate the relative pathogenic contribution of soluble and MP plasma fractions from patients.

Clinical Relevance

In the short term (<5 years) this project will provide a better understanding of how therapeutic PE works in AAV, and will explore a potentially important novel therapeutic target, circulating MPs. In the longer term these findings could lead to safer and more targeted therapy for AAV, and could lead to individualized PE prescription based on monitoring of effective MP removal.

20. Gene therapy for Duchenne Muscular Dystrophy using zinc finger nucleases
(Francesco Conti / Francesco Muntoni)

Project Aim

We propose here to test a novel gene therapy technology, based on the use of zinc finger nucleases (ZFNs), to specifically correct duplications in the dystrophin gene. ZFNs are enzymes that cleave specific DNA sequences and appropriately designed ZFNs can be used to precisely remove duplicated regions of DNA. As a first step, the repair of duplications will be tested in cells isolated from patients affected by DMD and, where possible, expression of a functional transcript and protein will be determined. These results will inform us on the efficacy of this approach, and form the basis of future studies aimed at repairing duplications in other regions of dystrophin.

Plan of investigation

We will evaluate the effectiveness of selected ZFNs repair dystrophin duplications in cells lines form patients with DMD. More specifically, we propose the following aims:

- Design ZFNs to target a range of duplications in the dystrophin gene.
- Determine the efficacy of ZFN cleavage in control cells.
- Evaluate correction of duplications in cells from DMD patients.
- Validate the restoration of dystrophin expression in patient cells and following injection of cells into dystrophin-deficient mouse models.

These experiments will provide proof-of-principle for the strategy of using ZFNs to correct gene duplications in dystrophin.

Clinical Relevance

The proposed research is aimed at correcting duplications in the dystrophin gene, which are one of the causes of Duchenne Muscular Dystrophy, a condition that affects approximately 1:1500 males. The project represents the first step in the development of a novel gene therapy, as at present effective approaches to correct genetic duplications are not available. Cells isolated from the patients in which dystrophin expression has been restored, may be transplanted in dystrophin-deficient muscle (cell transplantation therapies are currently under development by members of our and other laboratories) or via systemic injection of the therapeutic agent following preclinical testing in animal models.

21. Investigation of the molecular mechanisms of Nephrotic syndrome
(Aoife Waters)

Project Aim

To define the molecular mechanisms underlying steroid resistant nephrotic syndrome (SRNS).

Plan of investigation

- To characterise Notch and Glucocorticoid (GC) pathway expression in human SRNS.
    - Genotype-phenotype characterisation of a large NS cohort
    - Immunohistochemical analysis of NS patient material
    - Analysis for Notch and GC pathway expression in human podocyte cell culture

- To determine whether genetic and pharmacological inhibition of Notch could rescue GC pathway expression and responsiveness in human podocytes derived from SRNS patients.
    - Genetic inhibition of Notch in SRNS patient cell lines
    - Pharmacological inhibition of Notch

- To assess the mIRNA profile of urine and serum of NS patients

Clinical Relevance

The aim is to target those patients with NS for whom no further treatment options are available other than dialysis and renal transplantation. Furthermore, as SR can be a significant problem of several other diseases that rely on GC-based treatment strategies and given the extent of pre-clinical and early phase clinical trials on the efficacy of Notch inhibitors in the field of cancer biology and diseases such as Alzheimer's Disease, I anticipate that there may be interest from the pharmaceutical companies that are already developing these drugs and seeking new indications for their use. Should this be the case, then this may have wider beneficial consequences on cost of in-patient care and overall disease burden in addition to improving quality of life for affected patients and their families.

Diagnostics and Imaging in Childhood Disease

22. Elucidation of key biomarkers in common complications of pregnancy
(Gudrun Moore)

Project Aim

There are four major complications of pregnancy; intrauterine growth restriction (IUGR), prematurity, pre-eclampsia, and recurrent miscarriage. 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. This particular study will only analyse the first three complications listed above as they all regularly involve access to placental tissue post 14 weeks gestation.

Plan of investigation

- 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.

- Subsequently proteomic investigations will be performed in the state of the art Biological Mass Spectrometer Centre led by Dr Kevin Mills. Biomarkers will be identified using the most appropriate use of 2D-DIGE/PAGE, ProteinChip technology and mass spectrometry-based screening of 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.

- 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.

Clinical Relevance

The proposed study will enable us to explore the placenta of normal and pathological pregnancies before the clinical manifestations of diseases. A healthy placenta is crucial for a successful pregnancy. To be able to know which genes are differentially expressed in the placenta of fetuses that will be growth restricted, born preterm or pre-eclamptic will enable us to help elucidate further the pathophysiology of these common complications. The differentially expressed genes might be provide key biomarkers for the earlier management of the patients or potential drug targets for new and better medical treatments.

23. The determination of the anabolism & catabolism of ceramide trihexoside in Fabry disease: the search for the elusive deacylase
(Kevin Mills)

Project Aim

Fabry disease is an X-linked lysosomal storage disorder caused by abnormalities in the *GLA* gene which results in a deficiency of α-galactosidase A. This leads to a progressive intracellular accumulation of neutral glycosphingolipids primarily ceramide trihexoside (CTH) in the lysosome of the cell. Organs that are primarily affected by Fabry disease include the kidney and heart. CTH and more recently lyso-CTH, a deacylated form of CTH are the only available biomarkers. However; each has its own limitations and the identification of new and improved biomarkers are essential to improve diagnosis, monitor treatment and disease progression in Fabry disease.

By understanding the mechanism of the breakdown of CTH to lyso-CTH we will gain greater insight into the disease mechanisms involved in Fabry disease. The identification of the elusive deacylase that creates the highly toxic lyso-CTH, will allow the development of chemical inhibitors to target the enzyme. Finally, this work is extremely important from a perspective that enzyme replacement therapy does not cross the blood brain barrier and new treatments will be required.

Plan of Investigation

- Develop cell culture models and learn subcellular fractionation/ mass spectrometry techniques (training provided by K. Mills, W.Heywood & S.Eaton)
- Refine techniques/model, study CTH tracer metabolism in cell culture models (training provided by K. Mills, W. Heywood & S.Eaton)
- Proteomic analysis of cell culture models to look at disease mechanism and pathways affected by CTH / lyso-CTH metabolism. (training provided by K. Mills & W.Heywood)

The interface between basic and clinical science

Using clinical family history more cases of Fabry disease are being diagnosed in childhood. This creates an opportunity for clinicians to manage and treat patients well before clinical symptoms manifest. This research will provide understanding of the metabolism of CTH in Fabry disease which will improve diagnosis and provide further avenues for clinical treatment.

24. Interaction between diet, gut microbiome, gastrointestinal immunology and genetic factors in the development of coeliac disease in children

(Kenth Gustafsson / Nigel Klein)

Project Aims

- To investigate the interaction between genetic/host factors, diet and gut microbiota on the development of coeliac disease
- To assess whether differences in these interactions results contributes to the heterogeneity of children with coeliac disease, with reference to serological markers and response to dietary elimination.
- To inform as to potential mechanisms through which human gastrointestinal GD T-cells differ may contribute to gut mucosal inflammation.
- To clarify the optimal sample in studies planning to assess gut microbiota in children.

Clinical Relevance

There is limited information on the interaction between diet, gut microbiome and mucosal immune function in man and in particular, the pathogenesis of coeliac disease. The data from this study will provide valuable insights into these interactions and may provide information as to future diagnostic and therapeutic strategies, with a particular focus on gamma-delta T-cells.

25. Identification of new biomarkers for the early diagnosis and monitoring of Epstein Barr Virus lymphoproliferative disease in immunosuppressed children
(Judith Breuer / Nigel Klein)

Project Aim

- Using next generation whole viral genome sequencing, to investigate Epstein Barr virus evolution in immunosuppressed children at risk of PTLD.
- Using Ion Torrent sequencing of the VDJ region to interrogate the T cell repertoire in children with EBV infection at risk of PTLD and compare the results with conventional assays of T cell function.
- To develop next generation sequencing methods for early diagnosis and monitoring of PTLD.

Clinical Relevance

The research will lead to increased understanding of the pathogenesis of EBV lymphomas in subjects undergoing solid organ and stem cell transplant recipients, children with primary immunodeficiencies, and patients with HIV/AIDS. The research will lead to the identification of biomarkers that predict evolution of malignancy and reduce unnecessary treatment of subjects with high EBV viral loads but at low risk of LPD and improve the early diagnosis of those with disease. identification of somatic mutations which drive tumour formation will provide insights into therapeutic targets. In addition the project will develop novel protocols for efficient high throughput sequencing of B cell repertoires. The data will inform our understanding of the host pathogen interaction in development of viral driven lymphomas

26. A proteomics approach to identification and clinical application of biomarkers to improve treatment of high risk childhood kidney cancers
(Kathy Pritchard-Jones / Mark Weeks)

Project Aim

This project tests the hypothesis that proteomic analysis of sequential urine and serum samples taken during pre-operative chemotherapy for childhood renal tumours will reveal characteristic protein signatures that predict the underlying tumour subtype and its differential *in-vivo* response to chemotherapy. Combination of these data with parallel molecular analysis of the tumours themselves will allow better categorisation of Wilms tumour according to the biological pathways that are disrupted and should signpost the molecular drivers to be prioritised for novel targeted therapeutic approaches in future clinical trials.

Plan of investigation

- Establish sample collection, storage protocols and limits of proteomic analysis
- Establish SOP's and proteomic analysis of urine/serum
- Define urine proteomes for sample subtypes
- Analyse poor prognosis samples with urine/serum. Match to high blastemal cell count post-chemotherapy
- Sample analysis to define disease/control differences
- Validate urine based biomarkers alongside existing prognostic markers in serum establish overlap and functional application.
- Blinded comparison of biomarkers through of defined 'high risk' patients with those defined by histological, genetic and scanning technology.

Clinical Relevance

The development of urinary biomarkers will offer a non-invasive, easily applied approach to improving diagnosis, risk stratification and response monitoring in childhood renal tumours treated with pre-operative chemotherapy.
The expected benefit to patients is to improve relapse-free and overall survival and quality of survival through better risk-adapted use of existing therapeutic regimens. This project also aims to contribute to better definition of the biological pathways involved in high risk Wilms tumour, which may can be adaptable to more routine histopathological testing on the excised tumour pathology specimen.

27. Developing novel diagnostic markers and therapeutic targets in childhood epilepsy

(Thomas Jacques / Jonathan Ham)

Project Aim

The purpose of the project is to understand a common cause of severe childhood epilepsy, validate novel diagnostic markers for the disease and provide a method for testing new therapeutics. Epilepsy is a common severe neurological 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.

Plan of investigation

- Identification of diagnostic markers in FCD.
- Testing of therapeutic targets in FCD.
- Differentiation of BCs after restoration of mTOR signalling.
- Differentiation in response to integrin signalling.
- Differentiation of BCs isolated in co-culture.
- Differentiation of BCs isolated in 3D cultures.

Clinical Relevance

The project will have an impact on children with chronic neurological disability in particular those with epilepsy. In the short term, the project will improve the diagnosis of children with epilepsy. For example, in the work leading up to this project we have already identified a novel diagnostic technique that we have incorporated into routine use in the histopathology laboratory at GOSH. This test allows us to distinguish some children with a genetic cause for their epilepsy. It is anticipated that these further studies will refine and extend these sorts of diagnostic tests and this will ultimately allow optimisation of the treatment of these children. In the medium term, these cellular models will provide the first system in which test novel therapies for this disease.

28. Development of genetic markers in paediatric/adolescent Inflammatory Bowel Disease (IBD) to determine disease outcome, prognosis and response to therapies
(Mona Bajaj-Elliott/ Fevronia Kiparissi / Sarah McCartney)

Project Aim

NOD2/CARD15 was the first gene defect identified in IBD in 2001. Since 2009, this family of proteins (NLR family; 22 members) has expanded and continues to be implicated in a variety of complex inflammatory diseases including diabetes, arthritis, cardiovascular disease and cancer. At present no studies have defined the impact of defects in other NLR family members on IBD pathogenesis. We wish to test the hypothesis that mutations in this family (e.g. the inflammasome complex) in combination with established defects (Autophagy & ER stress) contribute not only to intestinal pathogenesis, but may also impact on extra-intestinal manifestations and co-morbidiity.

Plan of investigation

The principal outcome measures will be to link gene defects (in NLR/inflammasome family, autophagy/ER function) with disease presentation, progression and response to treatment. This will include linking disease distribution and development of penetrating illness (strictures) to a gene defect (miRNA analysis). We will also look at co-morbidities like vascular events like strokes and myocardial infarctions and atherosclerosis, both having been recently reported in Paediatric IBD. Lastly we will look at colectomy and surgical rates and quality of life issues.

Clinical Relevance

We are in a unique position of being able to look retrospectively but also prospectively at the natural history of our patients with IBD, as the main applicant is the named Consultant in Transition for Paediatric Gastroenterologist and works both at GOSH and UCLH seeing them until the age of around 21. One of the collaborators is an Adult Gastroenterology consultant at UCLH who then follows the patients up as adults. This provides us with a 10-20 year time frame that can look at how IBD has progressed in the individual.

By linking progression to identified genes, one could then predict on a patient level which patient will develop co-morbidities and which ones will have little or no problems. Those who have a poor prognosis treatment could be intensified early, *for example,* if poorly controlled a stem cell transplant could be offered early.

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

(Faraneh Vargha-Khadem / Dr Katrin Schulze)

Project Aim

With this project we aim to (I) dissect the FOXP2-related phenotypic features of verbal and orofacial dyspraxia in the affected KE members, (ii) develop a model of motor speech learning and control, and (iii) characterize the features of speech impairments, such as dysarthria and dyspraxia. Functional imaging paradigms and structural imaging protocols will be used to relate impairments in different aspects of motor speech to dysfunction in the speech and language network, and to indices of grey and white matter integrity.

Clinical Relevance

Speech and language problems affect a substantial number of children and cause serious psychological and emotional upset for those affected and their families. Evidence suggests that persistent speech and language problems have a genetic basis affecting the neurodevelopmental processes of brain structure and function. Against this background, studies of the KE family, half of whose members carry a mutation of the FOXP2 gene, provide a window of opportunity for understanding how the speech and language circuit develops in the presence of genetically-induced brain abnormality. This understanding can in turn aid diagnosis of the components of the system that might be affected in other cases, and may also provide guidelines for appropriate support and education of affected children.

30. Organisation of the memory circuit in childhood epilepsy: Implications for outcome

(Faraneh Vargha-Khadem)

Project Aim

The aim of the proposed project is to develop a functional magnetic resonance imaging (fMRI) task for children undergoing epilepsy surgery to assess hippocampal-dependent episodic recall of everyday events. The proposed fMRI task (i) will be ecologically valid (e.g. relevant to everyday activities of children); (ii) can be performed irrespective of level of intelligence (i.e. within the repertoire of low functioning children); (iii) will not be language specific (i.e. can be performed by children from any linguistic background); (iv) will activate not only the hippocampal/medial temporal areas of the long term memory circuit, but also map interactions with brain circuits involved in speech and language, and visual perception (including object recognition, spatial location).

Plan of investigation

Neuroimaging investigations: MRI scanning will be carried out on a 1.5 T Siemens Avanto scanner at Great Ormond Street Hospital, and will incorporate:
Volumetric MRI data. This will be used to evaluate tissue and lesion volumes, and lesion location (pre-operatively).
Functional MRI. Investigations of memory will be performed as part of pre-operative investigations. The recall portion of the episodic memory task will be administered as an event-related design, to allow us to test item-specific characteristics of episodic recall. Laterality of memory representation will be determined. In addition, we will investigate the cortical and subcortical regions involved in the episodic memory network using diffusion weighted imaging and tractography.

Statistical analysis: We will compare study groups (patient, healthy controls) for all relevant outcome variables using analysis of variance. We will use regression analyses to evaluate the moderating influence of predictor variables (such as lesion location, seizure focus, anti-epileptic drug (AED) use, and change in fMRI lateralisation) on the difference in pre-operative memory scores.

Clinical Relevance

This research will impact on diagnostic evaluation of memory function for patients who are candidates for epilepsy surgery. The findings will identify the functional organisation and the mapping of core brain networks involved in memory, specifically episodic memory, crucial for day-to-day functioning, in relation to language dominance and visual processing. The output of this research will advance our understanding of how these brain areas become lateralised or reorganized in the face of early onset of epilepsy, and pave the way for surgical planning and remedial interventions post surgery.

31. Profiling Novel Biomarkers for Juvenile Onset Systemic Lupus Erythematosus
(John Ioannou / Lucy Wedderburn / Clarissa Pilkington)

Project Aim

Profile novel biomarkers relating to oxidative and nitrative post-translational modification of autoantigenic proteins that may have both prognostic and diagnostic clinical utility for the management of children and adolescent patients with juvenile onset systemic lupus erythematosus (JSLE).

Plan of investigation

- Profile role of NHis (nitrated tyrosine residues on histone3) assay as a biomarker in JSLE.
- Assess pathogenicity of nitrated nucleosome through in-vitro / ex-vivo studies.
- Profile clinical utility of assay for measuring reduced ß2GPI in predicting the presence of anti-DI antibodies in JSLE.

Clinical Relevance

This research will impact on health of children with the rheumatological condition juvenile onset SLE. There have been no formal epidemiological studies in children, but given that 20% of all patients with SLE develop SLE as a child, we can infer an incidence rate of 4 to 30 children per 100,000 / year with prevalence rates of around 30 (white) to 80 (African American) per 100,000.

At present there no tests that can tell us which patients will develop active disease in the future, which patients will develop antibodies such as anit-s2GPI antibodies that can lead to a stroke or pulmonary embolism, which patients have or will develop neuropsychiatric lupus, nor do we understand what precisely drives the production of pathogenic autoantibodies in the first place. The projects aims to test the clinical utility of optimized novel biomarkers in addressing this need and also explore at a mechanistic level novel concepts of pathogenicity that may open the door to new preventative therapeutic strategies.