



UNIVERSITY OF CAMBRIDGE

Department of Paediatrics

PAEDIATRIC RESEARCH SYMPOSIUM

Tuesday 8th July 2008

9.00am ~ 5.30pm

Howard Building

Downing College

Cambridge

CB2 1DQ

PROGRAMME

- 8.30 REGISTRATION, COFFEE AND CROISSANTS
- 9.00 **Welcome and Introduction**
Professor Ieuan Hughes, Head of Department
- 9.15 **SESSION 1 ~ Chair: Professor Ieuan Hughes**
- 9.15 **Traumatic brain injury induced pituitary dysfunction**
Dr Carlo Acerini & Dr Robert Tasker
- 9.30 **Central Venous Access: Critical Appraisal**
Dr John Biddlestone
- 9.45 **Genetic changes underlying medulloblastoma development**
Dr Martin McCabe
- 10.00 **A sensitive biological marker of sex dimorphism in rodents can be applied to human infants**
Dr A.J. Thankamony
- 10.15 **Cost of Cystic Fibrosis care**
Dr James Jarrett & Dr Richard Iles
- 10.30 **Insulin treatment in the VLBW infant**
Dr Kathy Beardsall
- 10.45 COFFEE
- 11.15 **SESSION 2 ~ Chair: Dr Carlo Acerini**
- 11.15 **Can the fetal genome influence maternal glucose tolerance in pregnancy?**
Dr Clive Petry

PROGRAMME

- 11.30 **The PIND Study – unique surveillance for neurodegenerative disease in UK children**
Dr Anne Marie Winstone & Dr Chris Verity
- 11.45 **Overnight Glucose Control in Children and Adolescents with Type 1 Diabetes: An Overview**
Ms Janet Allen & Dr Gosia Wilinska
- 12.00 **Paediatric Surgery: Genetics of Hirschsprung’s disease**
Dr Adil Aslam
- 12.15 **Studies on mutations in the N-terminal domain of the androgen receptor**
Dr Rieko Tadokoro
- 12.30 **Neuroendocrine stress response to critical illness**
Dr Ricardo Branco & Dr Robert Tasker
- 12.45 LUNCH
- 1.45 SESSION 3 ~ Chair: Dr Robert Tasker**
- 1.45 **Prevention of diabetic complications during adolescence**
Professor David Dunger
- 2.00 **Tidal Volume and Ventilation Perfusion studies**
Dr Richard Iles
- 2.15 **Effect of delayed measurement on blood glucose levels in young subjects with T1D**
Dr Daniela Elleri
- 2.30 **Effects of insulin detemir and insulin glargine on weight gain in adolescent girls with T1DM**
Dr Rachel Williams

PROGRAMME

- 2.45 **Detection and analysis of mutations associated with disorders of sex development**
Dr John Davies
- 3.00 **PICU and Asthma Studies: Smoking inside or out**
Dr Robert Ross-Russell & Jenny Pool
- 3.15 TEA
- 3.45 **SESSION 4 ~ Chair: Professor David Dunger**
- 3.45 **The Study of Tolerance to Oral Peanut (STOP)**
Dr Andrew Clark
- 4.00 **MicroRNA profiling in paediatric malignant germ cell tumours**
Dr Matthew Murray
- 4.15 **Prevention of Childhood Obesity: is infancy too early to start?**
Dr. Ken Ong
- 4.30 **GUEST LECTURE ~ Chair: Professor Ieuan Hughes**
- Prevention of Type 2 Diabetes**
Professor Nick Wareham
Director of the MRC Epidemiology Unit
- 5.15 **CLOSING REMARKS ~ Professor Ieuan Hughes**

Traumatic Brain Injury Induced Pituitary Dysfunction

C. L. Acerini, D. White, R. Branco and R.C. Tasker

Department of Paediatrics, University of Cambridge

Cambridge University Hospitals NHS Foundation Trust, Addenbrooke's Hospital,
Cambridge, UK

Pituitary hormone dysfunction may be an important cause of morbidity in survivors of traumatic brain injury. The hypothalamic-pituitary structures are vulnerable to damage following head injury, and chronic pituitary hormone deficiencies after injury are frequently observed (15 – 65%) in adults. In contrast, little data is available regarding the frequency and impact of this potential complication in children and adolescents surviving TBI.

A prospective, longitudinal observational study of children and adolescents (aged 5 to 16yrs) surviving moderate to severe (GCS < 8) TBI is currently ongoing. The aims are to determine the frequency and natural history of post-TBI related hypothalamic-pituitary dysfunction and its relationships to subsequent brain development and neuropsychological function. 60 subjects will be recruited in the immediate post head injury / PICU period. Assessments, including dynamic anterior pituitary function tests (glucagon and TRH tests) and standard auxology are performed 3 to 6 months post-TBI (baseline) and annually thereafter for a period of 3 years. Cerebral MRI voxel-based morphometry assessment and neuropsychological evaluations are performed at baseline and at end of study.

Sixteen subjects have been recruited to date and preliminary endocrine data (median (range)) from 11 (age at TBI 12.7 (7.5 - 15.5) yrs) with completed baseline assessments are reported: peak stimulation test - GH 29.2 (6.1 – 5.5) mU/L; cortisol 552 (294 – 795) nmol/l. Four subjects (36%) demonstrate abnormalities in either the GH (peak < 20 mU/L) or adrenal axis (peak < 500 nmol/l), or in both axes (n 2). No other endocrine abnormalities have been observed.

Recruitment and follow-up is ongoing with centres based in Edinburgh and Birmingham recently joining the study (2008). These preliminary findings suggest that the frequency of neuroendocrine abnormalities may be as high as those observed in adult TBI studies.

**Central Venous Access:
Critical Appraisal for Evidence Based Practice**

John Biddlestone (1), Madan Samuel (1), Jeffrey Brain (1), Martyn Williams (1), Adil Aslam (1), Ruth Whittlock (2)

1 - Department of Paediatric Surgery, Addenbrooke's Hospital, Cambridge.

2 - Department of Paediatric Oncology, Addenbrooke's Hospital, Cambridge.

Introduction: Longterm paediatric central venous access is achieved through surgical placement of central venous catheters (CVC) and is indicated when delivery of therapeutic medicines, transplantation, and parenteral nutrition are required.

Aim: We present a single-centre, ten-year ambispective study of 2244 CVCs inserted in 2053 consecutive children and analyse the incidence of CVC-related complications stratified for age at insertion, urgency, duration, operative technique, CVC type, primary site of access, tip position and disease process requiring catheter.

Results: Of the 2244 CVC inserted during the study period 129 (5.7%) were subject to catheter related infection (CRI) at 166 +/- 58 days of life of catheter. The incidence density of CRI was 1.1 per 1000 catheter days in all patients. Factors affecting CRI include age at insertion, disease process requiring catheter and CVC type ($p = <0.001$). CRI is shown to be independent of urgency of insertion, site of access, tip position, number of lumens and presence of antimicrobial cuff ($p = >0.05$). Mechanical complications occurred in 62 (2.8%) of all patients; associations between tip placement and age at insertion are demonstrated.

Conclusions: CRI is the most likely complication to necessitate CVC removal. The incidence density of CRI at this single centre is acceptable when compared to published literature. Patients aged less than five years at time of insertion and/or those who require multiple usage of their CVC are shown to be highest risk. Aseptic line management is the most important, controllable factor in reducing complications that necessitate CVC removal.

Identification of Novel Mechanisms for Gene Disruption at the Medulloblastoma Isochromosome Breakpoint Locus

MG McCabe¹, DM Pearson¹, L Liu², SC Clifford³, D Ellison³,
K Ichimura¹, VP Collins¹

1. University of Cambridge Department of Pathology
2. The National EB Diagnostic Lab, St Thomas' Hospital, London
3. Northern Institute of Cancer Research, Newcastle University
4. St Jude's Children's Research Hospital, Memphis, TN

Aims: Isochromosome (17q) is the most commonly reported chromosomal abnormality in medulloblastomas. Its frequency suggests that genes disrupted as a consequence of isochromosome formation may play a role in tumorigenesis. Our aim was to map the position of the isochromosome breakpoints and to identify genes potentially disrupted by isochromosome formation.

Methods: Array CGH was used to examine a series of classic (n=49), desmoplastic (n=1) and large cell / anaplastic (n=1) medulloblastomas. A custom tiling path genomic BAC array giving greater than 90% coverage of chromosome 17 was constructed. To give greater resolution at breakpoint loci, an overlapping fosmid tilepath was included with an average interval between clones of approximately 7 kb.

Results: Array CGH identified three isochromosome breakpoint patterns at two distinct loci. The more common locus was characterised by the presence of two low copy number repeats. The other was located close to the centromere. A region of homozygous loss was identified in one case with a peri-centromeric isochromosome breakpoint. The homozygous deletion spanned 236 kb and encompassed between three and five protein-coding genes and one small nucleolar RNA gene. Its location exactly coincided with the more common isochromosome breakpoint locus. A second case had single copy gain of the same region.

Conclusions: We have accurately mapped the medulloblastoma isochromosome breakpoint loci. In addition, we are the first to show disruption of the more common breakpoint locus by mechanisms other than isochromosome formation – namely homozygous loss and copy number gain. These additional findings suggest that the disruption of genes at the isochromosome breakpoint itself may be more biologically significant than was previously suspected.

A sensitive biological marker of sex dimorphism in rodents can be applied to human infants

Thankamony A, Ong KK*, Dunger DB, Acerini CL, Hughes IA

Department of Paediatrics, University of Cambridge, Cambridge, UK

*MRC Epidemiology Unit, Cambridge, UK.

Anogenital distance (AGD) is defined as the distance between centre of anus to the base of the scrotum in boys and the posterior fourchette in girls. It is sexually dimorphic in rodents and humans, being 2-2.5 fold greater in males. Reproductive toxicologists use AGD as a reliable indicator of prenatal anti-androgen exposure in rodent experiments. Studies of AGD in humans are limited with no longitudinal data during infancy and early childhood.

Objective: To determine AGD longitudinally from birth to two years.

Materials and methods: Subjects were recruited from the Cambridge Baby Growth Study. AGD was measured using vernier calipers and related to birth weight and penile length in males. Data were collected at birth, 3, 12, 18 and 24 months of age. Low birth weight and preterm infants were excluded.

Results: Measurements were recorded in 223 and 217 male and female infants at birth, respectively. Mean AGD (SD) at birth was 2.09 (0.62) cm in males and 0.91 (0.29) cm in females ($p < 0.001$). AGD increased up to 12 months in both sexes and the same significant pattern of sexual dimorphism was maintained. Penile length showed a significant correlation with AGD at birth ($r=0.15$, $p=0.025$).

Conclusion: AGD was sexually dimorphic at all ages studied, and our results replicate cross-sectional data collected solely at birth. We now have reliable reference data on a sensitive biological marker of androgen action to use in human population studies of the effects of chemicals on early genital development.

Cost of paediatric Cystic Fibrosis care in the Eastern Region of the UK

James Jarrett (1,2), Dr. Richard Iles (1,2), Prof. Miranda Mugford (1),
Dr. Erika Sims (1,3)

(1) University of East Anglia, (2) Paediatrics Department, Addenbrooke's Hospital, (3) Optimum Patient Care, Ltd.

Background: As part of a regional network audit of CF care, a database was developed in 1998 to collect information on CF care in the region. Subsequently, data was included from Norfolk, Suffolk and Cambridgeshire from 2004-Present. We are currently collecting patient data in Bedfordshire, Essex and Hertfordshire. As part of the audit in the region, an economic analysis of cost of CF care and cost-effectiveness of NBS would be undertaken.

Aims: The analysis looks at the paediatric cost of illness from 1998-2007 using a retrospective analysis on patient data collected in the Eastern Region CF Database. Unit costs are derived from national sources.

Methods: The viewpoint is the NHS in the Eastern Region. Resource use and costs are reported separately. Cost of care is estimated at the individual level. An accumulation of costs per case method is applied.

Results: We have analysed the baseline 1998 data for all counties. The total cost of caring for 371 patients in the Eastern Region in 1998 was approximately £2.5 million. The median cost per patient is estimated to be £3,996

Conclusion: This study provides an indication of the cost of care in the region and can help to inform decision makers when making decisions on CF care.

Future Plans: When collected, data from all six counties will be compared over time in terms of the cost of paediatric CF care. This is anticipated to be complete by the end of 2008. This data will feed into the cost-effectiveness analysis of newborn screening.

The use of early continuous insulin infusion in very low birth weight infants: effects on glucose control, mortality and morbidity.

Kathy Beardsall

Department of Paediatrics, University of Cambridge, Cambridge, UK

Objective: Studies in adult intensive care have highlighted the importance of insulin and improved glucose control on survival. Very low birth weight (VLBW, birth weight <1500g), infants requiring intensive care often also have hyperglycaemia during the first week of life. Hyperglycaemia at this time is associated with increased morbidity and mortality. This study aimed to investigate the effect of early intervention with insulin replacement and improved glucose control on mortality and morbidity.

Methods: An international multicentre randomised controlled trial of early insulin replacement in VLBW babies. Infants were randomised (n=389) to receive a continuous insulin infusion (0.05 units/kg/h) with 20% dextrose support or standard neonatal care from the first 7 days of life.

Results: Infants in the early intervention arm had lower mean daily glucose level (6.2 (1.4) vs 6.7 (2.2) mmol/l, p=0.007), and less babies had hyperglycaemia for >10% of the first week (22% vs 34%, p=0.008). They had significant increased non protein calorie intake 58(1.0) vs 50 (0.8) kcal/kg/l, p<0.001, and reduction in weight loss in the first week (-0.55 (0.52) vs -0.70 (0.47) p=0.006). However there was no reduction in mortality by expected date of delivery. Secondary end points of morbidity also showed no significant difference between the two arms of the study.

Conclusions: Prophylactic use of early intravenous insulin in very low birth weight infants does not reduce mortality by expected date of delivery.

Can the Fetal Genome influence Maternal Glucose Tolerance in Pregnancy?

Clive J. Petry¹, Mark L. Evans^{2,3}, Dianne L. Wingate¹, Ken K. Ong^{1,4}, Miguel Constância^{3,5}, David B. Dunger^{1,3}

¹Department of Paediatrics, University of Cambridge

²Department of Medicine, University of Cambridge

³Institute of Metabolic Science, University of Cambridge

⁴MRC Epidemiology Unit, Cambridge

⁵Department of Obstetrics & Gynaecology, University of Cambridge

Background: Pregnant women carrying fetuses with Beckwith Wiedemann syndrome (BWS) are at increased risk of developing pregnancy-induced hypertension and possibly gestational diabetes. Mutations in several genes nearby the insulin-like growth factor 2 gene (*IGF2*) that can cause this condition are therefore potentially able to increase fetal growth (macrosomia being a feature of BWS) and worsen both maternal glucose tolerance and blood pressure.

Hypothesis: Variations in fetal growth genes are able to alter maternal glucose tolerance in pregnancy.

Methods: We tested our hypothesis in placental-specific *Igf2*-knockout (p0-*Igf2*; with small offspring) and *H19*-knockout (with large offspring) mice. For the p0-*Igf2* study the experimental group were wild type female mice mated with p0-*Igf2* knockout males and the control group were p0-*Igf2* knockout females mated to wild type males. For the *H19* study the experimental and control groups were reversed. Intra-peritoneal glucose tolerance tests were performed on days 1 (e1) and 16 (e16) of the 21.5 day pregnancy.

Results: There were no differences in glucose tolerances of pregnant mice in either study at e1 or in the p0-*Igf2* study at e16. However at e16 mothers carrying *H19*-knockout pups had significantly worse glucose tolerances than controls: 1845 ± 378 v. 1386 ± 107 mmol.min/L ($p=0.005$, Mann Whitney U test), and lower insulin sensitivities: 32 ± 11 and 73 ± 54 HOMA-%S ($p=0.045$).

Conclusions This study provides the first direct evidence of a fetal growth gene being associated with an alteration in maternal glucose tolerance during pregnancy. This has implications for the aetiology of gestational diabetes.

Studying the spectrum of neurodegenerative disease in UK children. Findings of a prospective national study after 11 years of surveillance.

C M Verity, A M Winstone, L Stellitano

Addenbrookes NHS Trust, HPA, ECDC and the NCJDSU

Aims: To report on the unique data provided by our national study of progressive intellectual and neurological deterioration (PIND), including vCJD.

Methods: Surveillance commenced in 1997 using the BPSU surveillance system to identify UK children with PIND. Clinical information about notified cases, obtained by telephone questionnaire or hospital visit, is anonymised and classified by the PIND Study Expert Group of paediatric neurologists.

Results: By March 2008 UK paediatricians had notified 2420 children who were thought to meet the criteria for PIND. Among them were 6 with probable or definite variant Creutzfeldt-Jakob Disease (vCJD). There were 1025 PIND children with other confirmed diagnoses and in these children there were 115 known neurodegenerative conditions, illustrating the complexity of classifying children with PIND.

In the diagnosed cases the five most common groups were: neuronal ceroid lipofuscinoses (NCL) (n=131), mitochondrial cytopathies (n=108), mucopolysaccharidoses (MPS) (n=97), gangliosidoses (n=96) and peroxisomal disorders (n= 62).

Conclusion: Individually conditions causing progressive intellectual and neurological deterioration are rare but together make up a significant proportion of the work load of some paediatricians. This study provides unique epidemiological data about the distribution of neurodegenerative diseases in the UK child population - invaluable information when considering the differential diagnosis in children with worsening neurological symptoms and signs.

Acknowledgements: Many thanks to all the UK paediatricians who report cases to the PIND Study, to the PIND Expert Group, to the British Paediatric Surveillance Unit and to the Department of Health for funding the study.

Overnight Closed-Loop Glucose Control in Children and Adolescents with Type 1 Diabetes Project Overview

Janet Allen and Gosia Wilinska

Department of Paediatrics, University of Cambridge, Cambridge, UK

The Artificial Pancreas Project at the University of Cambridge (APCam) is approaching the end of the second year of funding from JDRF. Excellent progress has been made on the way to achieve the main objective, i.e. to reduce the frequency of hypoglycaemia whilst achieving normoglycaemia with overnight closed-loop insulin delivery in children and adolescents with type 1 diabetes.

To date two clinical studies have been completed with encouraging results. In the first efficacy study (APCam01) we compared closed-loop (CL) glucose control with the conventional insulin pump therapy (CSII) in 12 children and adolescents with T1D. Closed-loop concentrated glucose in target range 3.9 to 8.0mM (61(45 - 73) vs CSII 35 (19 - 53) %, $p = 0.03$) and improved glucose control while reducing risk of hypoglycaemia. In our second study APCam02 we evaluated the performance of CL system following ingestion of rapidly and slowly absorbed large dinner.

Six children and adolescents with T1D have been studied on two separate nights. In random order, the subjects ate either rapidly or slowly absorbed dinner matched for carbohydrates. Overnight CL resulted in safe and efficacious glucose control following the ingestion of large dinner irrespective of its composition. In our third still ongoing study APCam03 we are testing the closed-loop system in post-exercise conditions.

The results so far have been encouraging. Future plans include progressing from the current manual to an automated mode of operation of the closed-loop system and moving with the clinical studies from a controlled clinical research environment to patients homes.

Expression Analysis of Hirschsprung's disease bowel - Pilot study

Atif Saeed¹, Andrea Loos², Ian McFarlane², Adil Aslam¹

¹Department of Paediatric Surgery, Addenbrooke's Hospital Cambridge
University Hospitals, NHS Foundation Trust. Cambridge

²Genomics Core Lab, National Institute of Health Research Cambridge
Biomedical Research Centre Metabolic Science, Cambridge

Background: The aetiology of Hirschsprung's disease is still not known. It is considered to be multifactorial with multigenetic association, many factors may play role in pathogenesis.

Aim: To study the differences in gene expression between normal and abnormal segments of bowel in Hirschsprung's disease and controls.

Methods: Colonic tissue samples were taken from the diseased segment and the normal colon of Hirschsprung's disease patients and controls. Controls were patients who had their colostomy/colostomy closure for other reasons e.g anorectal malformation. Each Sample was further dissected into mucosa and muscle. RNA was extracted using a standard protocol and then converted to cDNA. Affymetrix protocols and expression array (Gene Chip ®Human Gene 1.0 ST array, Affymetrix USA) were used to compare the genes expression differences between Hirschsprung's disease bowel segments and controls.

Results: Twenty five samples were analysed using micro-array whole genomic chip. Both mucosa and muscle showed good RNA extraction with an average yield of 1.15ug/mg. All samples showed good quality RNA as assessed by Agilent and qRT- PCR. Preliminary data analysis showed out of 28840 expressed genes, mucosa samples showed 509 genes with expression difference of 2- fold and 39 entities with 2- fold change and p-value of ≤ 0.05 . Similarly 408 genes expressed in muscle samples demonstrate 2-fold changes out of which 112 showed p-value of ≤ 0.05 .

Conclusion/Future plans: Our preliminary data showed significant differences in various sets of genes. Further plans are to target specific sets of genes and validate our results using qRT- PCR. This pilot study needs to be extended with further patient and control numbers and additional funding.

Studies on mutations in the N-terminal domain of the androgen receptor

R. Tadokoro Cuccaro, John Davies, Trevor Bunch, Jane Murphy and
Ieuan A. Hughes.

Department of Paediatrics, University of Cambridge

The numbers of N-terminal domain (NTD) missense mutations are low relative to other regions of the AR and the spectrum of the disease milder. We have investigated the functional activity of eight NTD mutations associated with Androgen Insensitivity Syndrome (AIS) patients, to establish pathogenesis and to obtain preliminary data essential for future studies into the molecular mechanisms of NTD dysfunction. The lack of crystallographic structural information for this domain augments reliance on functional data to define pathological cause of disease.

Six missense mutations were investigated; Gln118Glu and five in the Taul region (Ala157Thr, Gly214Arg, Asn233Lys, Gly246Val, Leu270Phe). Two trinucleotide slippage mutations insLeu57 and delLeu57 (insL57 and delL57) identified in patients with MAIS/PAIS were also examined. All mutations were recreated and mutant AR function defined by the plasmid GRE transactivation assays.

Preliminary data indicates that 5/6 missense mutations had reduced activity, the exception was Gly214Arg. Gln118Glu, Gly214Arg and Leu270Phe are in poorly characterised regions of the NTD where structure and function is unknown. The Ala157Thr, Asn233Lys and Gly246Val map close to established RAP74 (TFIIF) binding sites, they may act by reducing the rate of transcriptional initiation. Gly214Arg had wild-type activity in the GRE reporter assay. This was of interest as four patients with Gly214Arg have been diagnosed, they exhibit variable phenotype. The slippage mutations insL57 and delL57 also had wild-type activity. InsL57 and delL57 alter the leucine stretch immediately N-terminal to the AR glutamine repeat. The presence of 3 or 5 leucines relative to 4 in WT had no clear affect on receptor function in this plasmid reporter assay. To obtain equivalent receptor expression a five fold increase of plasmid was required for insL57 mutation relative to WT and delL57, suggesting that translation rates or protein stability were compromised for the insL57 mutation. Analysis of the glutamine repeat RNA structure *in silico* revealed that the leucine codons form a stack at the base of the glutamine repeat hairpin, the length of which was changed by these slippage mutations. Addition of an extra CTG increased stack length, further stabilising the RNA hairpin relative to wild-type.

The study has established a pathogenic role in disease for some of the mutations, other require extended analysis. The compromised receptor mutants described should also be of value to define functional surfaces of the disordered, “molten-globular”, NTD.

Neuroendocrine stress response to critical illness in children

Ricardo G Branco and Robert C Tasker

Department of Paediatrics, University of Cambridge, Cambridge, UK

Paediatric Intensive Care Unit, Addenbrookes NHS Trust, Cambridge

Background: The body prepares for a severe, life-threatening illness by mounting a hormonal neuroendocrine stress response that is essential for survival and recovery. However, this stress response can also have dangerous effects, and modulating this response in critically ill patients may help to improve outcome.

Objective: In our studies we have sought to identify the types of neuroendocrine responses that may influence outcome of critical illness and design an intervention to modulate this response.

Method: To date we have undertaken 3 cohort studies in critically ill children and one interventional physiology study.

Results: In critically ill children with septic shock or bronchiolitis we found that hyperglycaemia (due to insulin resistance) is associated with worsened outcome. In children with septic shock relative adrenal insufficiency (defined as a rise in cortisol in response to ACTH stimulation $<250\text{mmol/L}$) increases the risk of death. Last, in a fixed dosage insulin infusion study (0.1U/Kg/h) to prevent hyperglycaemia we found it to be safe and the physiology suggests that it may reverse the growth hormone resistance present in these patients.

Conclusion: Neuroendocrine dysfunction is seen frequently in critically ill children. It is associated with worsened outcome. We are now planning intervention studies.

**Prediction and prevention of risk for complications
in young people with Type 1 Diabetes**

Professor David B. Dunger

Department of Paediatrics, University of Cambridge, UK

Young people diagnosed with Type 1 Diabetes (T1D) during childhood may expect a reduction in life expectancy of around 17 years, largely related to an increased risk for nephropathy (DN) and cardiovascular disease (CVD).

Although these risks can be reduced with improved glycaemic control, this may be difficult to achieve particularly during adolescence.

Through study of longitudinal cohorts of young people with T1D we have explored hormonal, metabolic, familial and genetic factors which are associated with microalbuminuria, a robust marker of CVD and DN risk.

We have used these observations to design and fund a definitive study to determine whether cardio-renal protection provided by antihypertensive and lipid lowering drugs, in addition to attempts to intensify insulin therapy, will improve the prognosis of young people with diabetes.

The effects on tidal volume and ventilation perfusion mismatch of weaning from continuous positive pressure ventilation to spontaneous breathing in premature infants.

Dr. Richard Iles

(1) University of East Anglia, (2) Paediatrics Department, Addenbrooke's Hospital

Premature infants often require ventilation within the neonatal period. There is an accepted dogma that it is good practice to reduce ventilator support aggressively to minimise potential lung damage. The effects of early reduction in positive pressure support may reduce tidal and lung volume and increase the ventilation perfusion ratio. These changes would potentially influence long term oxygen dependency and consequently the development of chronic lung disease of prematurity (CLD). We will longitudinally track the changes in tidal and thoracic volume and ventilation perfusion ratio that are associated with a transition in positive pressure ventilation from 1) IPPV to CPAP, and from 2) CPAP to spontaneous breathing in oxygen or air by assessing the imbalance of lung ventilation and perfusion ratio by non-invasively using arterial saturation measured during the inspiration of three different concentrations of inspired oxygen, in association with documented changes in lung volume.

Structured Light Plethysmography (SLP) measures changes of the chest and abdominal wall movement by modelling the thoraco-abdominal surface, defined by a projected structured light grid, the movement of which is recorded by digital cameras. Computer processing allows real-time pattern recognition algorithms to identify the changes of the two-dimensional grid intersections and compute a three-dimensional reconstruction. The internal volume of the thorax is computed. The technique measures chest volume changes without connection to the patient.

By correlating both ventilation and perfusion indices with changes in lung volume we hope to gain information regarding the aetiology of CLD and potentially influence ventilator strategies in the premature infant

Effects of delayed measurement on blood glucose levels in young subjects with Type 1 Diabetes

D. Elleri, C.L. Acerini, J. M. Allen, A.F. Larsen, M.E. Wilinska, D.B. Dunger, R. Hovorka

Department of Paediatrics, University of Cambridge, Cambridge, UK

Accurate measurement of blood glucose is essential to assess the performance of continuous glucose monitors and to underpin the development of the artificial pancreas.

The present study investigated the effect of a 3-hour lag-time between blood sampling and glucose measurement in young subjects with type 1 diabetes (T1D). Blood samples were obtained in 6 subjects with T1D during 12 overnight studies at a clinical research facility (M 2: age 14.4 ± 2.5 years; BMI 20.2 ± 2.9 kg/m²; diabetes duration 6.3 ± 3.6 years; HbA1c 7.4 ± 0.5 %; mean \pm SD). Whole blood samples were collected in fluoride tubes every 15 minutes from 16:00 to 6:00 the following day and immediately analysed using YSI Analyser (YSI 2300 STAT Plus Analyser). The samples were then placed on ice and reanalysed 3 hours later by the same YSI Analyser.

The level of agreement between the two measurement methods was assessed by calculating (i) the difference (D) between the immediate and 3-hour delayed glucose measurement and (ii) the relative difference (RD) defined as a ratio between the difference and the mean of the two measurement methods. The Bland-Altman plot assessed the comparability of the two measurement methods. 666 paired-measurements were obtained. The mean D was 0.47 ± 0.41 mmol/L. The mean RD was 7.5 ± 5.3 %. The Bland-Altman plot demonstrated a lack of agreement between the two measurement methods with only 20.7% of RD values within ± 2 SD of the YSI measurement error.

We conclude that a 3-hour time-lag between blood sampling and analysis using fluoride tubes reduces blood glucose by 0.47 mmol/l despite samples being placed on ice.

The Effects of insulin detemir and insulin glargine on weight gain in adolescent girls with Type one diabetes

Rachel Williams¹, Inge Harrison¹, Carlo Acerini¹ and David Dunger¹

¹University of Cambridge Department of Paediatrics, Cambridge, UK

Background: During adolescence, girls with type 1 diabetes (T1D) have increased weight gain when compared to their non diabetic counterparts. This may be exacerbated by increasing insulin doses to optimise glycaemic control in the face of growth hormone mediated insulin resistance. Insulin Detemir (Novonordisk) is a recently licensed long acting insulin analogue comprising the human insulin molecule modified by the addition of a myristic acid residue, resulting in a longer duration of action. In clinical trials, insulin Detemir consistently leads to reduced rates of nocturnal hypoglycaemia, more stable fasting plasma glucose concentrations and less weight gain than that observed with isophane, with equivalent glycaemic control.

Hypothesis: Incorporation of insulin detemir vs. insulin glargine into a basal bolus regime in young women with T1D will lead to reductions in longitudinal weight gain.

Methods: A multicentre randomised open label trial, (n=125) post menarchal women (13 to 20yr) with T1D, of insulin detemir vs. insulin glargine as basal insulin incorporated into a basal bolus regime over a 12 month period.

Primary Outcome: BMI SD score

Secondary Outcomes: regional fat distribution (DEXA), ovarian androgen concentrations, HbA1c, nocturnal hypoglycaemia, blood glucose variability and appetite.

Progress to Date: MREC and MHRA approval obtained. Currently a single centre (Cambridge) recruiting (4 randomised subjects to date). Other regional and national centres (Oxford, Ipswich, Norwich, North Manchester, and Bristol) should be commencing recruitment imminently, following recent adoption by the Medicines for Children Research Network and the Diabetes Research Network.

Future Plans: To finalise recruitment over the next 6 to 9 months, prior to completion of the protocol, analysis of data and preparation of results.

Detection and analysis of mutations associated with disorders of sex development

John Davies, Philippa Prentice, Harriet Miles, Vickie Precious, Trevor Bunch, R. Tadokoro Cuccaro, Norma Coggins and Ieuan A. Hughes.

Department of Paediatrics, University of Cambridge, Cambridge, UK

Mutation research in the Disorders of Sexual Development (DSD) laboratory falls into two overlapping project types: Clinical; this detects patient mutations for defining the aetiology of DSD and to correlate with pubertal outcome. The clinical research creates the unique understanding required for the fundamental research component, which focuses on the structure and function of the mutant Androgen Receptor. The fundamental research has translational potential between bench and patient, but no immediate impact on patient management. At present the clinical research identifies mutations of the Androgen receptor (AR), SRD5A2, 17BHSD and measures the functional activity of mutant AR *in vitro*. The functional activity of the mutated protein is dependent on the location of missense mutation and the amino acid substitution, as will be demonstrated for AR mutations. The function of the mutant AR is correlated to pubertal outcome in PAIS patients and may predict pubertal outcome. Such information could contribute to clinical decisions relating to gender assignment. Examination of the following genes is being planned; SF1 an established cause for DSD. BMP4, FGFR2 and CXorf6/MAMLD1, all of which have mutations described from isolated hypospadias patients. PAIS patients will be screened for FKBP52 mutations. FKBP52 is a strong candidate gene based on known function and a mouse KO model, but not established as a cause of human disease. We utilise AR mutations to investigate the structure and function of androgen receptor. Three receptor regions have been selected for extended study; the N-terminal domain, the surface defined by mutation H917R and a surface antagonist binding site termed BF3. Finally, a project to investigate the activity of the EM5744 agonist on mutant ARs associated with CAIS patients will be described.

PICU and Asthma Studies: Smoking inside or out~ does it make any difference to a child's exposure to environmental tobacco smoking (ETS)?

Jenny Pool & Dr Robert Ross Russell

Children's Services, Addenbrooke's Hospital, Cambridge

Background: 42% of all children in the UK live in a household with smokers and are exposed to ETS. Advice from the DOH and health care professionals recommends that carers smoke outside rather than inside the house if unable or unwilling to give up.

Hypothesis: Smoking outside the house by carers protects asthmatic children from exposure to ETS.

Methods: NicAlert™ is a simple test that can be used to measure cotinine, a by-product of the body's breakdown of nicotine in urine and thus demonstrates second hand smoke exposure.

- 35 asthmatic children (m: 27 f: 8; aged 6-161mths) from smoking families attending Addenbrooke's Hospital Asthma Clinic were recruited.
- Urinary cotinine level was measured using the NicAlert™
- Parents completed a questionnaire: how much they smoked in 24 hours and where they smoked.
- Results were available within 10 minutes.

Results: Urinary cotinine levels from 1- >1000ng/ml cotinine concentration are recordable using the NicAlert™. 7/35 (20%) children had cotinine concentration 1-10ng/ml - no nicotine exposure. 28/35 (80%) children had cotinine concentration 10-100ng/ml - non user of tobacco products but exposed to ETS.

All parents were smokers: 20/35 smoked outside only
12/35 smoked everywhere
3/35 children no information was provided.

7 children had no cotinine in their urine. There were no differences between the amounts or where these parents smoked compared with the children who had measurable cotinine in their urine.

Conclusions Smoking outside does not protect children from ETS.

The Study of Tolerance to Oral Peanut (STOP)

Dr Andrew T Clark, Sr Yvonne King, Dr Sabita Islam, Dr Katherine Anagnostou, Mr John Deighton, Dr Pamela W Ewan.

Cambridge University Hospitals NHS Foundation Trust, Addenbrooke's Hospital, Cambridge, UK

Background: Peanut allergy is common and the most frequent cause of life-threatening food allergy reactions. Patients are at constant risk of accidental reactions, causing reduced quality of life. We investigated a novel disease-modifying treatment.

Methods: 7 subjects with peanut allergy underwent blinded oral peanut challenge to establish threshold reactivity dose. Subjects received daily oral peanut immunotherapy (OIT) with 2-weekly up-dosing (1mg increasing to 800mg peanut protein). Serum peanut-specific IgE and skin prick test weal diameter were measured at enrolment and after up-dosing.

Results: 3 subjects with initial dose thresholds of 50-150mg peanut protein have completed up-dosing and can tolerate 800mg peanut protein (~ 5 peanuts) without reaction; up-dosing is ongoing in 3 children (See table, subjects 4-6). Symptoms experienced during up-dosing included mild facial angioedema and abdominal pain; there were no severe reactions.

Subject	Sex	Age (yr)	Challenge threshold dose*	Current OIT dose	Current dose tolerated?
1	M	9	150mg	800mg (final)	Yes
2	M	12	50mg	800mg (final)	Yes
3	M	13	50mg	800mg (final)	Yes
4	M	12	5mg	100mg	Yes
5	F	12	1mg	6mg	Yes
6	F	14	1mg	1mg	Yes
7	F	13	1mg	0.5mg	Yes

*=dose of peanut protein which caused an unequivocal reaction

Conclusions: Peanut oral immunotherapy resulted in at least a 5-10 fold increase in oral dose threshold and was well tolerated. Future work includes oral challenges to determine high-dose tolerance and a study of frequency and duration of maintenance immunotherapy. Blood work to identify mechanism is underway.

Global Micro-RNA Expression Profiles of Paediatric Malignant Germ Cell Tumours (MGCTs)

Matthew Murray,^{1,2} Roger Palmer,¹ Balaji Muralidhar,¹ Claire Thornton,³ James Nicholson² and Nicholas Coleman¹

¹ Hutchison/MRC Research Centre, MRC Cancer Cell Unit, Cambridge, UK

² Department of Paediatric Oncology, Addenbrooke's Hospital, Cambridge, UK

³ Department of Paediatric Histopathology, Royal Group of Hospitals, Belfast

Background: MicroRNAs (miRNAs) are short, non protein-coding RNAs that regulate gene expression post-transcriptionally and are aberrantly expressed in certain cancers.

Hypothesis: miRNA profiling of MGCTs will distinguish a) malignant tumours from benign tumours/controls and b) between different histological subtypes.

Methods: We studied 48 samples (13 germinomas, 12 yolk sac tumours [YSTs], six teratomas, three embryonal carcinomas, six MGCT cell lines, and eight normal and developmental controls). miRNA profiling was performed using the Exiqon miRCURY™ LNA microarray platform, encompassing 585 known human miRNA sequences. Subsequent data analysis was performed using DNA-chip analyser (dChip) software.

Results: Unsupervised hierarchical clustering segregated the tumours principally by histological subtype. Supervised clustering generated a list of significantly differentially expressed miRNAs which distinguished MGCTs from normal and developmental controls. Similarly, supervised clustering for each individual malignant histological subtype, versus controls, identified unique signatures for each subtype. Furthermore, there were no significantly differentially expressed miRNAs when benign tumours (teratomas) were compared with controls. Direct comparison of YSTs and germinomas, the two main histological subtypes of paediatric MGCTs, revealed 35 significantly differentially expressed miRNAs including the miRNA-302 family. Validation of these array-based results with quantitative-PCR and fluorescent in-situ hybridization (FISH) is ongoing.

Conclusions: Global miRNA expression profiling demonstrates that paediatric MGCTs cluster principally by histological subtype. Furthermore, supervised clustering versus normal and developmental controls has identified potential cancer biomarkers for MGCTs and their individual subtypes.

Future plans: These findings may improve the diagnostic accuracy of childhood MGCTs and, importantly, identify specific miRNA targets for development of new therapeutic agents.

Prevention of Childhood Obesity: is infancy too early to start?

Ken Ong, Raj Lakshman, Celine Druet

MRC Epidemiology Unit, Institute of Metabolic Science & Department of Paediatrics,
University of Cambridge

Background: An association between infancy weight gain and later obesity risk has been observed in studies from diverse populations, but with wide variations in the definitions and results.

Aims: We initiated an international collaboration of birth cohort studies in order to identify precise risk estimates, optimal thresholds, and the predictive ability of infancy weight gain (from birth to 1 or 2y) on childhood overweight.

Methods: We meta-analyzed study and individual-level data from 9 cohorts totalling 36,442 children (age 6 to 14y) and 11,388 adults (age 17 to 66y). Anthropometric data were expressed as age and sex-adjusted SD scores (SDS) against the UK 1990 reference. Optimal thresholds for predictive ability were estimated using the Youden Index method.

Results: Significant associations between infancy weight gain and later overweight were found in all studies. Overall, each +1 SDS rise in weight gain between 0-1y was associated with an OR=1.93 increased overweight risk in childhood, and OR=1.22 in adults. The association was non linear ($p < 0.0001$); estimated optimal prediction thresholds were: +0.87 SDS for weight gain 0-1y, and +0.66 SDS for weight gain 0-2y. The combination of infancy weight gain, mother's BMI and birth weight provided high predictive ability for childhood overweight (ROC curve AUC = 0.73-0.79).

Conclusions & future plans: These findings confirm a consistent association between faster infancy weight gain and later obesity risk and support both population-based and targeted high-risk individual strategies to prevent childhood obesity.

Author Index

- Acerini CL **5**, 8, 19, 20
Allen J **13**, 19
Anagnostou K 23
Aslam A 6, 14
Beardsall K **10**
Biddlestone J **6**
Brain J 6
Branco RG 5, **16**
Bunch T 15, 21
Clark AT **23**
Clifford SC 7
Coggins N 21
Coleman N 24
Collins VP 7
Constância M 11
Davies J 15, **21**
Deighton J 23
Druet C 25
Dunger DB 8, 11, **17**, 19, 20
Elleri D **19**
Ellison D 7
Evans ML 11
Ewan PW 23
Harrison I 20
Hovorka R 19
Hughes IA 8, 15, 21
Ichimura K 7
Iles R 9, **18**
Islam S 23
Jarrett J **9**
King Y 23
Lakshman R 25
Larsen AF 19
Liu L 7
Loos A 14
McCabe MG 7
McFarlane I 14
Miles H 21
Mugford M 9
Muralidhar B 24
Murphy J 15
Murray M **24**
Nicholson J 24
Ong KK 8, 11, **25**
Palmer R 24
Pearson DM 7
Petry CJ **11**
Pool J **22**
Precious V 21
Prentice P 21
Ross Russell R 22
Saeed A **14**
Samuel M 6
Sims E 9
Stellitano L 12
Tadokoro Cuccaro R **15**, 21
Tasker RC 5, 16
Thankamony A **8**
Thornton C 24
Verity CM **12**
White D 5
Whittlock R 6
Wilinska (ME) G 13, 19
Williams M 6
Williams R **20**
Wingate DL 11
Winstone AM 12
- All numbers refer to the page number on which the abstract appears. Bold font indicates the first author of an abstract.

THANK YOU

We would like to thank the following companies for their generous donations in support of our Paediatric Research Symposium.

Novo Nordisk



Pfizer Endocrine



Merck Serono



Ipsen



Ferring



We also would like to thank the Roberts Foundation, University of Cambridge.