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Abstracts of Scientific Meetings

15th Meeting of the Irish Society of Human Genetics, Monday 3rd September 2012.

Royal College of Surgeons in Ireland.

PROGRAMME:

10.00 – 10.55	Registration / Tea and Coffee.
10.55 – 11.00	Welcome.
11.00 – 12.00	Oral Presentations. Plenary I: clinical research.
12.00 – 13.00	**Keynote address:** 'Old men and selfish spermatogonia: how much do they contribute to the mutation burden?' Prof. Andrew Wilkie, University of Oxford, UK.
13.00 – 14.00	Lunch and Poster viewing.
13.45 – 14.00	Council Meeting.
14.00 – 15.00	Oral presentations. Plenary II: Basic research.
15.00 – 15.45	Tea and coffee / Poster viewing.
15.45 – 16.00	Business Meeting / AGM.
16.00 – 17.00	**Keynote address**: 'How next generation sequencing changes medicine' Han Brunner, Radboud University Nijmegen, The Netherlands.
17.00 – 18.00	Wine reception / Presentation of Prizes/ Meeting close.
19.00 – 21.00	**Dublin City of Science 2012 Public Event** Your Genes, Your Health, Your Future.

SPOKEN PAPERS:

S01. Exome analysis and cardiomyopathy: The Lazarus story

J Casey[1], P McGettigan[2], N Alkazemi[3], P Maguire[3], B Kennedy[3], D Brosnahan[4], E Treacy[5], K Walsh[4], S Ennis[6,7], SA Lynch[7,8]

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We present a study on a non-consanguineous Irish family that includes two siblings (male and female) with dilated cardiomyopathy (DCM) and chorioretinopathy. The children have been extensively investigated by the cardiac, metabolic and genetic teams but the genetic basis of their disorder remains unknown. We aimed to identify the disease mutation by sequencing the exome of the two affected children and their healthy sibling. The data was analysed assuming a recessive model to identify mutations that were uniquely shared by the affected children. One novel heterozygous mutation was identified in the exome data of one patient, but the sequencing coverage was not sufficient to determine the genotype of the second patient. Sanger sequencing was undertaken and showed that both affected children were heterozygous for the maternally-inherited mutation. As a dominant model was unlikely, the entire gene was sequenced and we identified a second paternally-inherited mutation in the patients. Why did the exome analysis fail to identify the compound heterozygous mutation? Retrospective investigations revealed why the candidate mutation was missed in the exome analysis and highlighted potential pitfalls. We have identified a novel candidate gene for this rare phenotype and subsequently investigated defects in Wnt signalling as a possible underlying cause.

S02. Concurrent translocations involving MLL (11q23) and MYC (8q24) in an infant B-cell acute lymphoblastic leukaemia (ALL)

Kelly J[1], Barton L[1], Morris T[1], Smith O[2], Betts DR[1]

[1]National Centre for Medical Genetics, Our Lady's Children's Hospital, Crumlin. [2]Haematology/Oncology Department, Our Lady's Children's Hospital, Crumlin

Acquired chromosomal translocations frequently provide key oncogenic events and many are specific to particular neoplasms. The MLL gene at 11q23 is frequently disrupted by chromosomal translocations in infant onset ALL and is typically associated with a poor prognosis. In contrast, rearrangements of MYC (8q24) are a feature of aggressive mature B cell lymphomas or leukaemias. We describe an infant of 4 months who presented morphologically with B-ALL. The leukaemia had a complex karyotype with structural aberrations including a translocation involving MLL in 95% of cells, with chromosome 19p13.3 as the partner, and a concurrent MYC translocation with chromosome 22q11 in 30% of cells. Therefore, indicating that the MYC rearrangement has occurred as a secondary event in the leukaemia. To our knowledge there has been only one other case describing co-existing rearrangements involving MLL and MYC in infant or paediatric B-ALL. Given that these events typically occur in very different types of haematological neoplasm the significance of a MYC rearrangement in a subset of leukaemic cells in this case presents a dilemma for the prediction of possible

clinical outcome and whether the therapy regime requires any modification.

S03. The complexity of counselling families for double heterozygosity in Inherited Cardiac Conditions.

Nicola Harper, Andrew Green

The National Centre for Medical Genetics, Our Lady's Children's Hospital, Crumlin & The Children's Medical Research Foundation.

Inherited cardiac conditions such as Long QT Syndrome and Hypertrophic Cardiomyopathy can have a major impact on families as the conditions can have a significant risk of sudden death. Clinically, cardiac investigations can identify individuals at an increased risk but due to variable penetrance and expression some individuals at risk may not be identified. Genetic testing can effectively allow identification of family members at risk when a highly likely pathogenic variant is identified. However, these conditions are Heterogeneous and double heterozygosity is reported in 5 to 10% of Hypertrophic Cardiomyopathy and is estimated in 3% of Long QT Syndrome. This leads to added complexity in the Genetic Counselling of families. In this Presentation, we review the Literature regarding Inherited Cardiac conditions and double heterozygosity with particular emphasis on genetic testing and genetic counselling. We present the cases of double heterozygosity seen at the specialist cardiac genetic counselling clinics at the National Centre for Medical Genetics. 9 families have been identified with two variants detected either as double or compound heterozygotes. We discuss the counselling issues that arose regarding segregation analysis, risk assessment and disclosure. This identifies the need for further guidelines on clinical screening for family members and segregation analysis.

S04. NBS Screening for CF in Ireland – First Anniversary Review

Melissa Rogers[1], Karen Meaney[1], Trudi McDevitt[1], Philip Mayne[2], Geraldine Roche[2], David E Barton[1]

[1]National Centre for Medical Genetics, Our Lady's Children's Hospital, Crumlin, Dublin 12; [2] National Newborn Bloodspot Screening Laboratory, Children's University Hospital, Temple Street, Dublin 1

The National Newborn Screening Programme (NBS) for Cystic Fibrosis (CF) began in Ireland on 1st July 2011, employing a two-tier IRT/DNA screening strategy. Blood spot immunoreactive trypsinogen (IRT) is first measured on dried blood spot samples collected between 72 and 120 hours after birth. Babies' samples with IRT in the top 1% are then referred for genetic screening. A total of 628 babies have been genetically screened thus far. They are tested using the xTAG™ Cystic Fibrosis 39 kit v2 for 38 of the most common CF mutations found worldwide. Following analysis, 29 babies with two CF disease causing mutations were identified; 26 had their diagnosis confirmed by means of an elevated sweat chloride result (>60mmol/L), 1 was diagnosed by genetic PND and another diagnosed by the presence of clinical symptoms. The final patient was lost to follow-up. A further 48 babies with 1 CF mutation were identified following screening; 45 of which had a sweat chloride in the normal range, confirming them as carriers only. 1 had a positive sweat chloride and was so diagnosed with CF. This patient subsequently had a second mutation identified following full screening of the *CFTR* gene. Two patients had borderline sweat chlorides, in the range 30-60mmol/L and were referred for full screening of the CFTR gene.

S05. Vascular Ehlers Danlos Syndrome: An obvious diagnosis or not? The Northern Ireland experience and influence of COL3A1 gene testing availability

Donnelly DE[1], Lee B[2], Reid J[2], Kirk S[3], Rea G[1], Stewart FJ[1], McConnell VPM[1]

[1]Northern Ireland Regional Genetics Service, Belfast City Hospital, Belfast Health and Social Care Trust. [2]Cardiovascular Surgery Service, Royal Victoria Hospital, Belfast Health and Social Care Trust. [3]Surgery Department, Ulster Hospital, Dundonald, South Eastern Health and Social Care Trust

Vascular Ehlers Danlos Syndrome (EDS IV) is an autosomal dominantly inherited connective tissue disorder (CTD), associated with COL3A1 gene. The combination of any two of the major Villefranche diagnostic criteria of arterial, intestinal and uterine rupture and family history are considered highly specific. The majority of minor criteria whilst being supportive of EDS IV can be regarded as common features of CTDs. Three of the minor diagnostic criteria; characteristic facial appearance, thin translucent skin and extensive bruising are more pathognomic, fulfilling three of the four eligible criteria for COL3A1 gene testing in the United Kingdom. Four of the five COL3A1 families presented after a severe or fatal vascular rupture with variable clinical phenotype and family history. Three of the COL3A1 mutations result in substitution of other amino acids for glycine residues in the triple helical domain; two not previously reported. Another COL3A1 mutation affected the splice site. The remaining mutation involved a complex contiguous genes deletion including COL3A1 and COL5A2 genes supporting the atypical phenotype. Whilst the intra and interfamilial phenotypic variability in our cohort supports the recognised difficulty in ascertaining the EDS IV diagnosis until a severe or fatal clinical event, the advent of genetic testing brings other issues.

S06. Excess of novel nonsense mutations identified in putative susceptibility genes for schizophrenia and autism spectrum disorders.

Sarah Furlong, Elaine M. Kenny, Paul Cormican, Ciara Fahey, Richard Anney, Gary Donohoe, Aiden P. Corvin, Louise Gallagher, Michael Gill, Derek W Morris.

Neuropsychiatric Genetics Research Group, Dept. of Psychiatry and Institute of Molecular Medicine, Trinity College Dublin, Ireland.

Schizophrenia (SZ) and autism spectrum disorders (ASD) are complex genetic neurodevelopmental disorders that share certain phenotypes (e.g. cognitive deficits), and may share an underlying pathology due to shared genetic risk variants. This study involves next-generation sequencing of the exonic regions of 215 putative susceptibility genes in an Irish sample of 151 cases of ASD, 274 cases of SZ and 287 controls, to identify rare mutations contributing to one or both disorders. A multiplex target enrichment method combined DNA samples using indexes/barcodes followed by enrichment of exonic regions using Agilent SureSelect and paired-end sequencing on an Illumina GAII. Selected genes were categorised as: 1) NRXN1 and interactors, 2) Post-synaptic Glutamate Receptor Complexes (NMDA, mGluR5 and AMPA), 3) Neural cell adhesion molecules, 4) DISC1 and interactors, and 5) Functional and Positional Candidates. Analysis revealed an excess of rare Loss-of-Function (LoF) variants that are predicted to

severely disrupt protein-coding sequence in cases versus controls (27 in 421 cases v 9 in 287 controls; p=0.051, odds ratio (OR)=2.12.) Twenty six of these events are singletons, of which there is a more significant excess in the combined case sample versus controls (21 in 421 cases v 3 in 287 controls; p=0.004, OR=4.97) and the effect is similar for both SZ (13 in 273 cases; p=0.008, OR=4.73) and ASD (8 in 148 cases; p=0.009, OR=5.41, 95%CI=1.28,26.14). Two rare LoF variants occurring in the well established candidate genes for neurodevelopmental disorders; *GRIN2B* and *DISC1* were identified as *de novo* in ASD cases. These results supply new supportive data for known risk genes and identify putative new susceptibility genes for both disorders.

S07. Genetic-Epigenetic Association with Parkinson's Disease.

Kerry Moore[1], Francis O'Neill[1], Jill Kilner[1], Owen Ross[2], David Craig[1], Amy Jayne McKnight[1].

[1]School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Belfast, N. Ireland; [2]Division of Neurogenetics, Department of Neuroscience, Mayo Clinic, Florida, USA.

Studies have highlighted that Parkinson's Disease (PD) is influenced by differential methylation at several loci. We investigated the DNA methylome by conducting an epigenome-wide association study followed by independent replication and integration with novel genome-wide data. Forty-five individuals with extreme phenotypes were matched for age and gender. Blood-derived DNA was bisulfite treated and hybridised to 450K Infinium methylation beadchips (Illumina Inc, USA). Methylation levels were compared between PD individuals and unaffected controls. Twelve top ranked, significantly associated loci were evaluated in an independent replicate population using Sequenom EpiTyper for 200 PD individuals in a cross-sectional case-control discovery design. Cases and controls were also evaluated using Illumina's Human OmniExpress Exome assay, to explore genetic variation associated with these differential methylation profiles. Genome-wide SNP data was analysed using standard quality control and analysis options. Quantitative methylation values were obtained at single-CpG level for 485,577 features. Logistic regression analysis for individuals with PD compared to controls (adjusting for age and gender) revealed twenty unique genes with a sizable difference in methylation ($P_{adjusted}$<0.05, $\Delta\beta \geq 0.2$) after correction for multiple testing. Genome-wide SNP data was analysed for ~ 700,000 SNPs + ~250,000 SNPs focused in exonic regions; preliminary genetic analysis provides support for seven biologically plausible genes. We have identified differences in methylation profiles both globally, and at individual CpG sites, which are associated with PD and supported by SNP-based data.

S08. Whole exome sequencing in Irish pedigrees identifies novel mutations for epilepsy predisposition

Mark McCormack[1], Gerard D O'Connor[2], Erin L Heinzen[3], Kevin V Shianna[3], Judith Conroy[4], Sean Ennis[4], David B Goldstein[3], Norman Delanty[2], Gianpiero L Cavalleri[1]

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Through clinics we actively recruit epilepsy pedigrees, providing a valuable resource for identifying mutations that predispose for epilepsy within the Irish population. As part of a collaborative whole exome study, we identified a rare stop-gain mutation exclusive to Irish cases within *CHRNB3*, a member of the nicotinic acetylcholine receptor family. Upon screening our case cohort, we found the mutation in all five affected members of Pedigree 1. Three unaffected members of this pedigree did not carry the mutation whilst it was not present in over 900 population controls. Two sporadic cases also carried the variant, one of whom shares the variant identically-by-descent with Pedigree 1. In family 2, we performed whole exome sequencing on three affected siblings. We limited our analysis to shared variants that were functional and rare (<3% MAF in European-American population). Our top two variants were both novel heterozygous non-synonymous SNPs in *SLC2A1*, encoding the glucose transporter type-1 (GLUT1). Segregation among the extended pedigree reflected a reduced penetrance model. Screening across our case cohort and population controls is currently ongoing. Through multiple genomic approaches we identified rare mutations in two genes from autosomal-dominant pedigrees which may provide further insight into the predisposition to and neurobiology of epilepsy.

S09. The NTD-associated polymorphism *MTHFD1L* rs7646 may increase disease risk by impacting on microRNA regulation.

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Polymorphisms of the folate related gene *MTHFD1L* have been shown to be associated with the risk of Neural Tube Defects (NTD) in the Irish population. We considered the Single Nucleotide Polymorphism (SNP) rs7646 (A>G), within the 3' UTR (untranslated region) of *MTHFD1L*, as potentially impacting on miRNA regulation. We identified miR-197 which can bind *MTHFD1L* 3'UTR in the position of the SNP rs7646. Allele "G" is predicted to produce an extra matching nucleotide adjacent to the seed sequence of the miR-197. In this study we investigated the binding of miR-197 to the 3'UTR of *MTHFD1L* mRNA and whether the alleles of SNP rs7646 have functional differences in miRNA binding. Results demonstrated that miR-197 specifically binds to the *MTHFD1L* 3'UTR causing a downregulation of the gene in MCF-7 cells. SNPrs7646 significantly changes miR-197 binding affinity, making the repression more efficient for allele "G" than for allele "A". However, the same assays performed in HEK293 and Coriell Lymphoblast cells showed no interaction between miR-197 and MTHFD1L indicating that this effect could be cell, tissue or developmental stage specific. Further experiments are necessary to elucidate the relationship of mir-197 regulation of *MTHFD1L* variants, particularly during early human development and in neural tube defects.

S10. Mutation-specific siRNA therapy for a keratin disorder

Atkinson SD[1], Liao H[2], Szeverenyi I[3], Smith FJ[2], Moore CBT[1], McLean WHI[2].

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Epidermolysis bullosa simplex (EBS) is one of the major hereditary

bullous disorders, characterised by blister formation in response to minor mechanical trauma within the basal layer of the epidermis; it is predominantly caused by mutations in genes encoding keratins 5 or 14 (K5 or K14). RNA interference (RNAi) is a complex naturally occurring cellular process where the presence of double-stranded RNA results in the interruption of the cell's translation of its own mRNA. The cellular process of RNAi can be harnessed to exert user-defined gene silencing with potential therapeutic effect in conditions which show a dominant negative inheritance. A luciferase reporter gene system was developed to assay all 19 possible allele-specific siRNA molecules for two K5 mutations over a standardised concentration range. siRNAs were identified that potently inhibited the mutant allele with little effect on wild-type K5. These lead inhibitors were further tested using epitope-tagged K5 expression constructs, where western blot analysis confirmed that they potently and specifically inhibit mutant K5. In addition, the cellular protein aggregation phenotype was reversed in cultured cells treated with mutant-specific siRNA. This work demonstrates the effectiveness of specifically developed siRNAs in inhibiting the expression of an EBS-causing mutation *in vitro*. If used in conjunction with non-invasive delivery systems which are currently in development, these siRNA molecules demonstrate potential for their use in the treatment of EBS and other disorders resulting from keratin mutations.

POSTER PRESENTATIONS:

P01. Sub-Cortical White Matter Abnormalities due to previously undescribed *de novo* 14q12-13 duplication.

Rea G[1], Stallings RL[2], Mullarkey M[3], McKinstry CS[4], McManus D[1], Morrison PJ[1*].

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Full trisomy 14 has been noted in spontaneous abortions and in live born infants with mosaicism. Partial trisomy of variable segments of 14q has rarely been described. We have identified a previously undescribed *de novo* partial duplication of chromosome 14q in a boy who presented at 14 months of age with neurological abnormalities. This was confirmed by fluorescence in situ hybridization (FISH) and defined by array CGH as dup (14)(q12-q13). Early developmental milestones were delayed. Dysmorphic features include low-set ears, upslanting palpebral fissures with epicanthic folds, a high arched palate and prominent lips. There was general hypotonia and behavioural problems which included self-injurious behaviour. MRI brain showed multiple areas of increased signal intensity in the subcortical white matter of both cerebral hemispheres, most marked frontally and to the vertex. These findings are consistent with hamartomatous lesions, similar to those found to Tuberous Sclerosis (TS). There was no evidence of other features suggestive of TS in this boy and no heterogeneity on TS is suspected as all previously reported cases of TS appear to show linkage to the TSC1 and TSC2 genes on chromosome 9 and 16. The patients clinical features and follow-up over 15 years are described.

P02. Mosaicism and a whole gene deletion in HLRCC.

G Rea[1], S Shanley[2]

[1]The Northern Ireland Regional Genetics Service, [2]Department of Cancer Genetics, The Royal Marsden Hospital.

Germline mutations of the fumarate hydratase (FH**,** fumarase) gene are found in the recessive FH deficiency syndrome and in dominantly inherited Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC) syndrome. We present a pedigree in which the teenage proband presented to dermatology with a painful rash affecting his chest and limbs. Biopsy confirmed multiple skin leiomyomas. There was no family history of note. *FH* sequence analysis failed to detect a mutation but multiplex ligation-dependent probe amplification (MLPA) identified a whole gene deletion. Array Comparative Genomic Hybridisation (CGH) delineated a 668kb deletion in 1q43 which involved *RGS7, KM0, OPN3, CHML, WDR64* and the *FH* gene. Further testing in the family identified that the probands father is mosaic for the deletion, which is present in ~60% of his peripheral blood leucocytes. The father has a large cafe-au-lait pigmented lesion on his left calf and thigh but no recognised features of HLRCC to date. We draw attention to this case to highlight a rarely reported whole gene *FH* deletion and the need to consider mosaicism in apparently unaffected parents. In addition, we review the clinical and molecular aspects of the disease and discuss our recently developed management protocol.

P03. A Retrospective Analysis of the Prevalence of Craniosynostosis on the island of Ireland

Lisa Bradley[1],Tabib Dabir[1] Colm Murphy[2], Dylan Murray[3], Anne McGillivary[3], Sally Ann Lynch[4]

[1]Northern Ireland Regional Genetics Service, Belfast City Hospital, Belfast; [2]Trinity College, Dublin; [3]Children's University Hospital, Temple Street, Dublin; [4]National Centre for Medical Genetics, Our Lady's Children's Hospital Crumlin, Dublin

Craniosynostosis describes the premature fusion of one or more cranial sutures with birth prevalence of 3.5-4.8 per 10,000 live births (LB). Around 15-25% cases are considered to be syndromic. We sought to estimate the prevalence of craniosynostosis within the island of Ireland for cases born within the years 2000-2009. In the Republic of Ireland (RoI), these cases are treated at the Children's University Hospital, Dublin, whilst in Northern Ireland (NI) treatment is at the Royal Belfast Hospital for Sick Children or following onward referral to centres in England. The National Centre for Medical Genetics (NCMG), Dublin and the Northern Ireland Regional Genetics Service (NIRGS), Belfast provide genetic services. We retrospectively reviewed craniofacial/genetic databases, medical/genetic records, X-ray systems and inpatient diagnostic coding data to obtain the relevant information. We identified 208 cases of craniosynostosis in ROI and 99 in Northern Ireland (NI) for the study period. This gave a prevalence of 3.5 per 10,000 LB for the island of Ireland. Syndromic craniosynostosis was noted in 13%. Sagittal craniosynostosis was the most common suture involved in the non syndromic group (37 %) followed by coronal suture (31%). The data was compared with EUROCAT data and published figures, where available. This is the first epidemiological estimate of craniosynostosis in the island of Ireland.

P04. A lesson in the investigation of familial deafness

Lisa Bradley, Tabib Dabir

Northern Ireland Regional Genetics Service, Belfast City Hospital, Belfast

A non-consanguineous family consisting of six prelingually, profoundly deaf individuals across two generations presented to our service. The 56 year old father (and his sister) had a clinical diagnosis of Pendred syndrome, a well recognised autosomal recessive condition characterised by congenital profound sensorineural hearing loss (SNHL), vestibular dysfunction, temporal bone abnormalities and development of euthyroid goitre in late childhood to early adulthood and known to have considerable phenotypic variability even within families. The 49 year old mother's profound deafness had been attributed to infantile measles. The couple's four daughters (aged 12, 15, 17 and 19 years) were all profoundly deaf with otherwise normal clinical examinations. Various modes of inheritance were considered including: maternal autosomal dominant inheritance with incomplete penetrance (both maternal grandparents had normal hearing); alternative autosomal recessive deafness genes in the maternal family with paternal carriage; and maternal carriage of Pendred. The stepwise investigation of this family is presented with interesting results leading to diagnosis in the daughters and mother.

P05. Identifying the genetic basis of Landau-Kleffner Syndrome

J Conroy [1], P McGettigan [2], K Collins [3], B Parry-Fielder [3], M Moran [1], O O'Mahoney [4], D Webb [5], M King [1], S Ennis [2], SA Lynch [6]

[1]Temple Street Children's University Hospital. [2]University College Dublin. [3]The Royal Children's Hospital, Melbourne, Australia. [4]Mercy University Hospital, Cork. [5]Our Lady's Children's Hospital, Crumlin. [6]The National Centre of Medical Genetics, Crumlin.

Landau-Kleffner syndrome (LKS) is an extremely rare form of epilepsy, accompanied by abnormal EEGs and a loss of language in a previously normal child. This results in "word deafness" or auditory verbal agnosia. Additional features may include seizures, behavioural disturbances and cognitive regression. The aetiology of LKS remains unclear. This study was undertaken in two phases. Phase 1 involved genotyping and methylation profiling in two discordant twin pairs and 3 isolated LKS cases. No common disease-causing CNVs or differentially methylated genes were identified. Exome sequencing of the discordant twin pairs and 2 of the 3 isolated cases was performed using the Agilent SureSelect 38Mb enrichment system. Initial data analysis identified no common LKS disease-causing gene. This does not rule out a genetic role in the development of LKS. It is possible that (1) mutations in >1 gene can cause LKS, 2) the exomic region containing the mutations may not have been on the Agilent 38Mb kit and (3) mutations are intronic or intragenic. In order to address these possibilities exome sequencing was performed on an additional 5 LKS isolated patients using the 44Mb Nimblegen enrichment system (Phase 2). Data analysis is currently underway and will be presented at this meeting.

P06. Making matches- linking large Irish Traveller pedigrees as a way of helping gene identification

Sally Ann Lynch[12], Jackie Turner[1], Jillian Casey[2].

[1]National Centre for Medical Genetics, Our Lady's Children's Hospital, Crumlin, D12. [2]National Children's Research Centre, Our Lady's Children's Hospital, Crumlin, D12.

Irish Travellers marry young and traditionally have large families. Pedigree structure is complex. Many individuals have common first and surnames and linking families is difficult. Our research work, on two Traveller families with non-specific microcephaly, illustrates this. Microcephaly is relatively common and proving it is due to a common homozygous mutation is difficult phenotypically. As both families share similar names, we hypothesised that they were distantly related and shared the same disease mutation. Homozygosity mapping in the first family identified high levels of homozygosity (25.3%), with 27 candidate loci containing 1,152 genes. Subsequently, a second family with microcephaly was referred consisting of 5 affected individuals. Comparing the homozygous segments shared by the affected individuals from both families would increase the likelihood of disease gene identification if a common gene was the cause. Four generation pedigree analysis of both families revealed >200 individuals in each pedigree with only 6 surnames in total. This explains the high (25.3%) level of homozygosity found. All 6 surnames are common to both pedigrees. The index case and father from pedigree one share their full name with 6 individuals from pedigree 2. Despite this we have not established a link. We are proceeding to analyse pedigree 2 independently but will also determine if there is any overlap in candidate loci between the two families.

P07. An Interesting Case of Vascular Ehlers Danlos Syndrome due to an Interstitial Deletion of Chromosome 2.

Deirdre E Donnelly[1], Rebecca Pollitt[2], Isabel Nesbitt[2], Christine Quinn[1], Vivienne PM McConnell[1], Mervyn Humphreys[1], Fiona J Stewart[1]

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Our patient presented at 15 years of age with acute dissection of the abdominal aorta at the level of the visceral vessels. Emergency surgery was promptly carried out but, unfortunately, bilateral, through knee, amputation of the lower limbs was required. Twelve days later, further dissection of the mid-thoracic aorta occurred which was managed with an endovascular repair. There is no family history of note. Pathology of the aorta showed marked calcification and intimal wall thickening. Multiplex ligation-dependant amplification (MLPA) revealed a heterozygous deletion of the entire *COL3A1* gene. Subsequent microarray analysis showed an interstitial deletion of the long arm of chromosome 2, with breakpoints at q32.1 and q32.3. This deletion is approximately 8.4Mb in size and contains 27 HGNC genes, including the *COL5A2* gene which is associated with Classical Ehlers Danlos Syndrome. Similar deletions have been reported; common clinical features include mental retardation, behavioural problems, thin, transparent skin, mild facial dysmorphism and cleft palate. Our patient is of above average intelligence and is non-dysmorphic. Extended testing of family members shows that this deletion appears to have arisen de novo.

P08. Classical Galactosaemia- a modifiable Glycosylation Disorder?

Coss KP[1], Doran PP[1], Byrne JC[2], Adamczyk B[3], Rudd PM[3], Treacy EP[4]

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Background: Classical Galactosaemia (Gal) is a rare genetic disorder of carbohydrate metabolism. Treatment, restriction of dietary galactose is life-saving in the neonate. However, long-term complications persist including cognitive impairment, speech and language abnormalities and infertility in females. Gross N-glycan assembly defects in the untreated neonate largely correct on treatment but processing defects persist in adulthood (Coss *et al,* 2012).

Aim: IgG N-glycan profiling to monitor galactose liberalisation and variations in glycosylation in treated adults in parallel with analysis of T-lymphocyte gene expression.

Materials and Methods: NP-HPLC of IgG N-glycans in 27 treated and 5 Gal patients on galactose liberalisation. T-cell RNA gene expression (AffymetrixU133a plus2.0) in 12 adult patients with KEGG analysis to identify dysregulated pathways. Results: Galactose incorporation in IgG was studied with G0/G1 and (G0/G1)/G2 ratios. This identified ongoing N-glycan processing defects in treated Gal patients with significant variability and galactose tolerance amongst patients. Gene expression analysis identified dysregulation of 36 glycan biosynthesis genes by at least 2-fold. Abnormal expression of a number of these genes of physiological relevance including galactosyltransferase B4GALT1 and oligomeric golgi complex member COG1 was validated with qPCR.

Conclusions: Our study suggests Gal is a systemic, modifiable glycosylation defect providing possible new treatment targets.

P09. Optimisation of Modified Methylation Specific Digital Karyotyping (MSDK)-Seq for genome wide DNA methylation analysis

Alan Harrison[1], Elaine Kenny[2], Derek Morris[2], Anne Parle-McDermott[1]

[1]Nutritional Genomics Group, School of Biotechnology, Dublin City University, Glasnevin, Dublin 9. [2]TrinSeq, Institute of Molecular Medicine, Trinity College Dublin.

The methylation the 5'carbon of cytosine in DNA plays an important role in the control of gene expression and repression. This enigmatic enzymatic process has many ties with cell differentiation, human disease, and cancer development. Many methods and techniques have been described to analyse DNA methylation patterns and profiles on both a locus-specific and genome-wide scale. Here we describe an example of the latter. Modified methylation-specific digital karyotyping (MMSDK) results in the generation of a library of short sequence tags to be amplified and sequenced by direct, massively parallel sequencing. This method allows for high-throughput and low-cost genome-wide DNA methylation mapping, and is well suited for the search of new genomic regions that vary their methylation patterns in response to physiological or environmental stimuli. The multi-step nature of this method involves many alternating DNA ligation and restriction digestion steps. Here we focus on troubleshooting these steps, and assessing the coverage that MMSDK-seq provides throughout the genome.

P10. Pharmacogenomics of Valproate induced weight gain

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Sodium valproate (VPA) is one of the most commonly used anti-epileptic medications (AEDs) in the treatment of seizure disorders. Weight gain is one of its known side effects. Up to 70% of people exposed to VPA experience some weight gain while about 10% have significant weight gain necessitating discontinuation of therapy. However, the exact mechanism of VPA-induced weight gain is not fully understood. The aim of the study is to apply the latest generation of genetic mapping techniques to identify genetic and environmental risk factors predicting weight gain induced by this commonly used AED. Blood/ saliva samples for genetic analysis will be collected from 250 children (age 2 – 18 years old) attending the three paediatric hospitals in Dublin who have been diagnosed with epilepsy and are on treatment with VPA for at least six months. Clinical phenotype of weight gain during the treatment period will inform the interpretation of genetic studies (Single Nucleotide Polymorphism (SNPs) and/or Genome Wide Assay Studies (GWAS)). To date 187 patients have been recruited. Phenotype analysis in the first 125 patients is nearly completed. Recruitment to this study is progressing as planned and will be completed by December 2012.

P11. High density imputation of the ASD-associated MACROD2 gene region identifies eQTL for plausible ASD-related genes

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Background: In a recent GWA, the Autism Genome Project (AGP) identified a strong association within the gene MACROD2 and autism (Anney *et al,* 2010). We sought to identify whether additional genotype information could better describe the ASD association signal, and examined whether the ASD associated region was also associated with gene expression in the human brain. Methods: Imputation was performed using BEAGLE in approximately 2900 probands and 2900 pseudo-controls from the AGP ASD sample (Anney et al, 2010) and 193 samples from a human cortical gene expression eQTL dataset (Myers *et al*, 2007).

Results: This association study confirmed the association identified in the 2010 AGP study, and following imputation additional supporting markers were identified. One of the significant trans-eQTL associations supporting the ASD-related MACROD2 association signal locus is between rs439451 and CNTN1, a gene previously implicated in ASD. Discussion: The imputation of the MACROD2 locus in this study demonstrates that imputation can enrich the original association signal and provide additional supporting associated markers. The observation of a trans-eQTL between MACROD2-AS1 and CNTN1 may indicate that molecular follow-up studies should consider exploring the role of these intragenic genes. The authors acknowledge grant support from the HRB and the Autism Genome Project.

References: 1. Anney R *et al.* A genomewide scan for common alleles affecting risk for autism. *Hum Mol Genet* 2010;**19 (20):** 4072-4082. 2. Myers AJ *et al*, A survey of genetic human cortical gene expression. *Nat Genet* 2007; **39(12):** 1494-9.

P12. Risk factors affecting mitochondrial function are associated with diabetic kidney disease

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Small-scale studies suggest that DNA variants in the mitochondrial genome influence kidneyfunction. We sought to explore genetic variants that affect the mitochondria for association with diabetic kidney disease. Common SNPs in the mitochondrial genome were genotyped using Sequenom and Ion Torrent technologies. Additionally, tag and pfSNPs (n=26,766, 113 genes) were analysed for autosomal variation that may influence mitochondrial function. A case-control study was conducted (n_{max}=2,100) where all White individuals had long duration of type 1 diabetes. Case had consistent proteinuria versus unaffected controls. Logistic regression was adjusted for age, duration of diabetes, gender and multiple testing. Replication was conducted in a total of 5886individuals from Denmark, Finland and the USA. Meta-analysis was performed using RevMan software from Cochrane Reviews. mtDNASNP 3243A> was significantly associated with diabetic kidney disease, and a larger effect was observed for end stage renal disease (Padjusted=0.003). 142 unique SNPs were identified with nominal significance in the discovery dataset.An intronic SNP located in the *COX10* gene revealed significant association (P=0.0002) in the meta-analysis where all groups showed effects in the same direction. Additional replication is on-going using independent European cohorts. We have indentified significant SNPs represent important risk factors for diabetic kidney disease.

P13. Association of functional DNMT3B polymorphisms and increased global methylation levels with suicide attempters.

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INTRODUCTION: Recently, a significant epigenetic component in the pathology of suicide has been realised. We investigated candidate functional SNPs in epigenetic-regulatory genes, DNMT1 and DNMT3B, for association with Suicide Attempt (SA) among patients with co-existing psychiatric illness. In addition, global DNA methylation levels between SA and psychiatric controls were examined.

METHODS: DNA was obtained from blood of 79 suicide attempters and 80 non-attempters, assessed for DSM-IV Axis I disorders. Functional SNPs were selected for each gene (DNMT1; N=7, DNMT3B; N=10), and genotyped. Allelic and genotypic tests of association between genetic variants and SA were conducted using Chi squared test of association. Global DNA methylation levels in a subset of patient DNA samples were quantified using the Methylflash Methylated DNA Quantification Kit (Epigentek, USA).

RESULTS: We identified a SNP in the 3'UTR of the DNMT3B gene, which showed evidence of association with SA compared to a non-attempter control group (P=0.004; Bonferroni adjusted P value=0.02). Moreover, haplotype analysis identified a DNMT3B haplotype (TTTAT) which differed significantly between cases and controls (P=0.01). Global methylation analysis revealed that psychiatric patients with a history of SA had significantly higher levels of global DNA methylation compared to controls (P≤0.001, Mann-Whitney test).

CONCLUSION: Our findings support the hypothesis that aberrant DNA methylation profiles play a significant role in the pathogenesis of suicidal acts.

P14. Analysis of the hexonucleotide repeat expansion at C90RF72 in an Irish psychosis case-control sample.

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A hexonucleotide repeat expansion 'GGGGCC' in an intronic region of the C9ORF72 (chromosome 9 open reading frame 72) gene has been found to account for up to 60% of familial amyotrophic lateral sclerosis (ALS) and up to 10% of sporadic ALS. The repeat expansion is located between exon 1a and exon1b of this gene and although function is unknown, it is thought to impact on gene expression. One in seven ALS patients develops frontotemporal dementia (FTD). Analysis of an Irish population-based cohort of ALS identified a higher rate of FTD in ALS patients carrying the repeat compared to those that do not carry the repeat (PubMed ID (PMID): 22305801). Study of an independent FTD sample showed a strong association between C9ORF72 mutations and psychotic symptoms: delusions, hallucinations, paranoid ideation and disordered thinking (PMID: 22300873). Therefore, we sought to screen a large Irish psychosis case-control sample for evidence of association between the repeat expansion and psychosis. Our sample included 742 schizophrenia, 261 bipolar disorder, 162 schizoaffective disorder cases and 1,283 control samples. We used a reverse primed PCR method to amplify the hexonucleotide repeat expansion. Analysis of PCR products was

carried out using a 3130xl Genetic Analyzer and GeneMapper 3.0 software (Applied Biosystems).The pathogenic range of the variant is >30 repeats and the expansion can extend up to 700-1600copies in ALS/FTD sufferers. All samples were genotyped in 96-well plate format and each plate contained two positive control samples that had both previously been confirmed to contain 34+ repeats. Overall the distribution of repeat numbers was very similar for cases and controls. We identified four samples that carried a repeat number approaching the pathogenic range. There were two controls samples (23 and 24 repeats respectively) and two schizophrenia cases (both 26 repeats). Initial reports on this repeat expansion indicated that the normal range of repeats does not usually exceed 23 copies of the hexanucleotide. A small number of apparently normal individuals have an intermediate number of repeats between 24 and 29, the significance of which is unclear. The repeat length in two cases with schizophrenia lies within this intermediate range, raising the possibility of an association between C9ORF72 repeat expansions and psychosis. As expansions may be tissue specific, further studies using Sothern blotting may be warranted to test the hypothesis that some forms of psychosis are linked to C9ORF72 repeat expansions.

P15. Inheritance of chronic kidney disease in men: association with Y chromosome.

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Men have an increased risk of chronic kidney disease (CKD) and end-stage renal disease. Genetic and epigenetic factors on the Y chromosome were explored for association with end stage renal disease. Haplogroup I of the Y chromosome was more commonly observed among 1,361 white European men who developed CKD (20% in cases versus 15% in unaffected controls, p=0.03). Age-adjusted analysis confirms that haplogroup I is significantly associated with an increased risk of CKD (OR=1.36, 1.02-1.83, p=0.03). Association with cardiovascular disease within this CKD cohort was also observed (p=0.008). 238 probes on the Y chromosome were evaluated for differential DNA methylation status in 225 males (151 cases with CKD, 74 age-matched controls with no evidence of kidney disease). Three loci (*DDX3Y*, *NLGN4Y*, and *TTTY14* genes) demonstrated significant association with CKD ($P<10^{-6}$). Two sites flank the SNP defining haplogroup I on the Y chromosome in Europeans, while *TTTY14* is a validated non-coding RNA expressed in the kidney. Male gender is associated with shorter life expectancy for individuals with CKD and with increased cardiovascular mortality in particular. These results suggest that the Y chromosome should be further studied employing large renal disease genetic consortia to explore gender differences associated with risk of CKD.

P16. Rare Copy Number Variation in Neuropsychiatric Disorders: Exploring the Phenotype

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There is emerging evidence that copy number variants (CNVs) provide a new vista on understanding unique and pleiotropic susceptibility to neuropsychiatric disorders such as Autism Spectrum Disorders (ASD) and schizophrenia. Rare CNV and detailed phenotype data were derived from the Autism Genome Project and Irish schizophrenia cases. Patients were classified by whether a rare CNV impacted any genes previously implicated in ASD or Intellectual Disability (ID) or not (0/1), or any genes that are differentially brain expressed (BE) or not (0/1), and association with candidate neurodevelopmental phenotypes were examined. Random forests and mixture models were used to explore whether phenomic features identify CNV-associated sub-groups. No statistically significant univariate associations between CNVs and selected phenotypes were identified for either ASD or schizophrenia. Exploratory analyses suggest sub-phenotypes that might provide good targets for association analyses in future studies, and indicate that distinguishing deletions and duplications is important. Inconsistency of measurements by site in large collaborative studies is a major impediment to assessment of genotype-phenotype associations. Sophisticated modelling suggests that CNV-associated subgroups may exist, however the clinical applicability of these remain to be demonstrated.

P17. Allele specific siRNA as a potential therapy for corneal dystrophy

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The corneal dystrophies are incurable diseases affecting the transparency of the eye. They are predominantly caused by heterozygous, dominant negative mutations in the cytosketetal keratins K3/K12, which provide structural support to the cells; and the TGFBI gene, which is important for corneal development and healing. Here, a siRNA sequence walk and a dual luciferase reporter gene assay was used to determine the best mutation specific siRNAs for the K12 mutation Leu132Pro. Further screening of these siRNAs by standard and dual-tag infra-red western blots as well as pyrosequencing confirmed mutant allele specificity at protein and mRNA levels. 5'Race confirmed a RISC-mediated mode of mutant mRNA knockdown. Cytoskeletal filament aggregation was reduced by mutation specific siRNA treatment in a cultured cell model and no off target effects were observed against closely related keratins in another model epithelial cell line. No immunological response to siRNA via TLR3 upregulation was observed using semiquantitative RT-PCR. Overall this allele specific siRNA approach yields promise for a potential personalised treatment of these disorders via specialised eyedrop formulations.

