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Abstracts

16th Meeting of the Irish Society of Human Genetics, Friday 6th September 2013.

Postgraduate Centre, Belfast Health and Social Care Trust.

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:** "The Unique Network – Supporting Families and Professionals Challenged by Rare Chromosome Disorders." Dr. Beverly Searle, Unique – Understanding Chromosome Disorders.
13.00 – 14.00	Lunch (Provided) and Poster viewing.
13.45 – 14.00	Council Meeting.
14.00 – 15.12	Oral presentations. Plenary II: Basic research.
15.12 – 16.00	Tea and coffee / Poster viewing.
16.00 – 16.15	ISHG Annual General Meeting.
16.15 – 17.15	**Keynote address:** 'Novel strategies for prevention of hereditary and multifactorial retinal disease.' Professor Peter Humphries, Trinity College Dublin.
17.15 – 18.00	Wine reception / Presentation of Prizes / Meeting close.

SPOKEN PAPERS:

S01. The Microcephaly Mystery: Complications of disease gene identification in a consanguineous population.

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Many genetic disorders in the Irish Traveller population follow a clan structure with certain disorders occurring only in specific clans or regions. Identification of disease genes can be simplified by comparing the genetic data of multiple patients with the same condition. However, this can be complicated for heterogeneous disorders whereby the same phenotype can be caused by different disease genes, or different clinical presentations can result from the same mutation. Microcephaly is a common phenotype and proving that it has the same aetiological basis is clinically challenging. Our study involves two clans with non-specific microcephaly and normal brain MRI's. The first clan (family A) includes three affected siblings and the second clan (family B) consists of a proband, four grand-aunts and second cousin (nephew to grand-aunts. Whilst the phenotype is similar in family B, the relationship is distant (3-5 generations) increasing the possibility of heterogeneity. The two clans have similar first and surnames but pedigree analysis of >400 individuals failed to identify a relationship. The grand-aunts share four homozygous regions (H1-H4), two of which are shared by their nephew (H1-H2), one is shared by the grand-nephew (H4) and three are shared by the affected siblings in family A (H1-H3). The nine affected individuals from both families do not share a single homozygous region supporting clinical and genetic heterogeneity. Unexpectedly, the mapping data suggests that the grand-nephew (proband family B) may have a different disease gene to all other eight patients. Our study highlights the complexities of gene cloning for heterogeneous disorders in consanguineous populations.

S02. The exception proves the rule: Male mosaicism in Craniofrontonasal syndrome.

L Bradley[1], R Mabrouk[2], A Paterson[3], D McAuley[3], T Dabir[1]

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Craniofrontonasal syndrome (CFNS), is a rare X-linked dominant disorder (1 in 120 000) caused by loss-of-function mutations of the EFNB1 gene. Paradoxically, heterozygous females have a more severe phenotype than hemizygous males. Phenotypic expression in CFNS females includes frontonasal dysplasia, craniosynostosis, Sprengel shoulder, syndactyly and longitudinal ridges of the nails. In contrast CFNS males show a mild non specific phenotype of ocular hypertelorism and occasional cleft lip. Interestingly, male mosaics for the EFNB1 gene are more severely affected than true hemizygous males and express the 'female CFNS' phenotype. So far only six confirmed mosaic males have been reported. We report a further male case with severe 'female CFNS' phenotype. Mutational analysis revealed *de novo* mosaicism for a duplication of 1 nucleotide in exon 4 of EFNB1 (c.605dupT), confirming the clinical diagnosis of CFNS. The disproportionately female restricted phenotypic expression in an X-linked dominant disorder can be explained either by male lethality or by male sparing as typified

by Rett syndrome and CFNS respectively. X-inactivation results in functional mosaicism in females causing differing expression of EFBN1 in cells leading to abnormal cellular interactions .This mechanism is known as cellular interference and has been proposed to explain the severe female phenotype in CFNS, a process that cannot occur in hemizygous males. Male mosaicism is functionally analogous to female X-inactivation and a similar cellular interference mechanism could account for the severe 'female CFNS' phenotype in mosaic males as highlighted by our case.

S03. The identification of candidate VUR-causative variants by sequencing the whole genomes of an extended family.

MG Dobson[1,2], JM Darlow[1,2,] R Darlay[3], HJ Cordell[3], AJ Green[1], P Puri[2], DE Barton[1].

[1]National Centre for Medical Genetics. [2]National Children's Research Centre (both at OLCHC, Dublin). [3]University of Newcastle, Newcastle upon Tyne, UK.

We are investigating vesicoureteric reflux (VUR), the retrograde flow of urine from the bladder towards the kidneys, in a cohort of 250 families recruited at OLCHC. VUR is a major cause of childhood hypertension and renal failure. It is believed to be monogenic and to be inherited in an autosomal dominant manner in most families. Genome-wide linkage scans have given conflicting results, thus it appears that VUR is highly genetically heterogeneous. We believe that most mutations causing non-syndromic VUR will be found in non-coding regulatory DNA that regulates these genes only in urinary tract development. We sequenced the genomes of nine members of an extended family, including six with VUR. The resulting variants were filtered by inheritance pattern, presence in the previously determined linkage regions for the family, frequency in variant databases, proximity to genes of relevance, evolutionary conservation of the region, and location in open chromatin, in relevant cell types. Approximately 11,000 variants were discovered to be present only in the affected members of the sequenced individuals. After filtration, four of these variants were identified that were in the previously discovered linkage regions, that have a evolutionary conservation score greater than 2 and that were within 100kb of genes known to be involved in urinary tract development (HOXC11 and RARG). These variants are currently being investigated in 592 Irish control DNA samples and in the remaining VUR index cases prior to functional studies in model organisms. Candidate causative variants have been identified in an extended family with VUR using whole-genome sequencing.

S04. Homozygosity mapping in a consanguineous pedigree reveals a novel gene for intellectual disabilities with epilepsy.

M McCormack[1], E Chaila[2], A Shawan[2], J Conroy[3], S Ennis[3], E Heinzen[4], DB Goldstein[4], N Delanty[2], K Caldecott[5], GL Cavalleri[1]

[1]Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland. [2]Department of Neurology, Beaumont Hospital. [3]School of Medicine and Medical Science, University College Dublin. [4]Centre for Human Genome Variation, Duke University. [5]Genome Damage and Stability Centre, University of Sussex.

Beaumont Hospital is the major tertiary referral centre for epilepsy in Ireland and through clinics we have recruited pedigrees with strong family histories of epilepsy, providing a resource for identifying causal mutations in the Irish population. Further, it is estimated that up to 25% of people with epilepsy also have some form of intellectual disability. Clinical evaluation of a large consanguineous family revealed three male siblings with epilepsy, intellectual disability and various levels of ataxia. Dense SNP genotyping was conducted in 10 family members and extensive homozygosity was apparent in all siblings. Mapping analysis revealed a 9Mb region of homozygosity (ROH) located on chromosome 6 is uniquely shared among all three affected siblings, we denoted this to be the candidate causal region. Exome sequencing was performed on the affected siblings and through focusing on rare variants within the candidate causal region we identified 3 potentially disease-causing mutations in two genes. Of these, a putative splice-site variant was the most likely pathogenic variant. No further carriers were found in our cohort of epilepsy patients (n=1363) or population controls (n=830) indicating a private mutation maintained through successive consanguineous marriages. Functional work characterizing the physiological effects of the causal gene in a mouse model and patient cell lines is underway. We provide evidence that erroneous DNA strand breakage is a significant threat to normal neuronal development.

S05. The first four generation Hyperparathyroidism-Jaw Tumour Syndrome (HPT-JT) pedigree identified in Northern Ireland.

VPM McConnell

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The main finding in Hyperparathyroidism-Jaw Tumour Syndrome (HPT-JT) is primary hyperparathyroidism which occurs in <90% of affected individuals with ossifying fibromas of mandible/maxilla occurring in 30-40% and renal lesions (most commonly cysts) in 20%. Benign and malignant uterine tumours appear to be common in affected females. Approximately 100 HPT-JT families have been reported in the literature with prevalence and penetrance not being well established; having implications for identification, counselling, and screening. HPT-JT syndrome, an autosomal dominant condition caused by mutations in the tumour suppressor gene, CDC73 being identified in 50-75% of families where most mutations appear unique to individual families. The 40 year old proband was referred to the NIRGS with a 16 year history of primary hyperparathyroidism and renal calculi. His mother and deceased maternal grandfather have histories of primary hyperparathyroidism from 17 and 22 years respectively. Two maternal uncles, 1 maternal aunt and 1 maternal cousin have chronic histories of renal calculi with earliest age of onset of 17 years. A deceased maternal aunt with complex medical history including significant head injury sequelae had confirmed metastatic uterine sarcoma at 50 years. A pathogenic CDC73 exon 7 deletion mutation was identified in the proband. Genetic testing and investigation in this family revealed other phenotypic features, sometimes in otherwise asymptomatic individuals, further supporting the diagnosis and facilitating appropriate intervention/ monitoring. To my knowledge this is the first reported four generation HPT-JT family with at least nine individuals showing some HPT-JT phenotypic features, providing evidence to further establish the penetrance and prevalence.

S06. Epistatic Expression Quantitative Trait Loci in Four Human Tissues.

DJ Fitzpatrick[1,3], N Shah[1,3], CJ Ryan[1,3], D Greene[2,3], DC Shields[1,3]

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Quantitative phenotypes emerge as the result of multiple interactions, both biological and environmental. However, association studies tend not to take account of interactions between loci (epistasis) when mapping phenotypic variation partially due to the number of

tests required to evaluate interactions. The consequent problem of correcting for multiple testing requires that individual tests achieve minute p-value thresholds which most studies are insufficiently powered to detect. Our study considered interactions amongst distal and proximal SNP pairs where both SNPs asserted a marginal effect on an expression phenotype at a liberal false discovery rate in four human tissues. As such, the search space was reduced from billions to thousands. We report epistatic expression quantitative trait loci in three of the four tissues, 3 in the liver, 2 in the cerebellum and 15 in the pre-frontal cortex at Bonferroni corrected thresholds of $P<2.75 \times 10^{-5}$, $P<4.32 \times 10^{-6}$ and $P<3.51 \times 10^{-6}$, respectively. The results show epistatic regulation for two unannotated transcripts on chromosomes 5 and 19 and for two HLA genes, viz., HLA-DRB5 and HLA-G on chromosome 6. All interactions present are intrachromosomal. However, due to stringent filtering for linkage disequilibrium (LD) between pairwise SNPs ($r^2< 0.01$), we are confident that these interactions do not represent dependent SNP pairs. Some of the interactions map to regions of the genome known to physically interact in lymphoblastoid cell lines. This may be indicative of the importance of long range physical interactions between cis-regulatory elements (CREs) in the regulation of the aforementioned genes.

S07. Assessment of allele-specific gene silencing siRNAs in an *ex vivo* pre-clinical model of lattice type I corneal dystrophy.

DG Courtney[1], SD Atkinson[1,2], EHA Allen[1,2], JE Moore[1], E Maurizi[3], G Pellegrini[3], GC Black[4], FD Mason[4], G Yam[5], WHI McLean[2], CBT Moore[1,2]

[1]School of Biomedical Sciences, University of Ulster. [2]Dermatology and Genetic Medicine, Colleges of Life Sciences and Medicine, University of Dundee. [3]Centre for Regenerative Medicine, University of Modena and Reggio Emilia. [4]Institute of Human Development, University of Manchester. [5]Tissue Engineering and Stem Cell Group, Singapore Eye Research Institute, Singapore.

The aim of this research was to identify a potent and specific small interfering RNA (siRNA) to target the TGFBI Arg124Cys mutant allele while allowing normal wild type expression to persist. The Arg124Cys point mutation is implicated in lattice type I corneal dystrophy (LCDI), a painful and blinding condition resulting from the formation of stromal amyloid aggregates. The only treatment currently available is keratoplasty, a complex surgical intervention that simply delays disease progression. The use of siRNAs in the treatment of TGFBI corneal dystrophies may offer a non-invasive alternative personalized treatment.To identify the best siRNA from a panel of 19 possible mutant allele-specific complexes a dual-luciferase assay was performed. A number of siRNAs were further assessed *in vitro* at the mRNA and protein level by pyrosequencing and Western blot analysis respectively, with up to 50% knockdown of the mutant allele being achieved, while amyloid aggregate formation was also monitored displaying a 56% reduction in the presence of siRNA. An *ex vivo* pre-clinical model of LCDI was established using primary corneal epithelial cells endogenously expressing the TGFBI Arg124Cys mutant allele grown from a limbal biopsy of a patient known to suffer from TGFBI-Arg124Cys related LCDI. Allele-specific knockdown of the endogenous mutant allele was quantified by a combination of pyrosequencing and quantitative RT-PCR analysis of the corneal epithelial cell mRNA, with promising results. This research demonstrates the specificity and potency of a siRNA targeted against TGFBI Arg124Cys *in vitro*. With non-invasive delivery systems currently in development, this siRNA and others targeting specific mutations could be translated into the clinic for personalized patient therapies.

S08. Bioinformatic analysis of transcriptional regulation in diabetic nephropathy.

GJ McKay, DH Kavanagh, AP Maxwell

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Diabetic nephropathy (DN) is a microvascular complication of diabetes. Members of the WNT/ β-catenin pathways have been implicated in interstitial fibrosis and glomerular sclerosis, characteristic hallmarks of DN. These processes are controlled, in part, by transcription factors (TFs), proteins which bind to gene promoter regions attenuating their regulation. We sought to identify known cis-acting transcription factor binding sites (TFBS) over-represented within the promoter regions of WNT pathway members compared to genes across the genome. We used TFBS data from the JASPAR databases on 65 WNT pathway genes to identify known binding motifs. *P*-values were estimated on the hypergeometric distribution for each TF. Gene expression profiles of enriched motifs were examined from DN-related datasets to assess clinical significance. TFBS motifs Transcription Factor AP-2 Alpha (*TFAP2A*), Myeloid Zinc Finger 1 (*MZF1*), and Specificity Protein 1 (*SP1*) were significantly enriched within WNT pathway genes (*P*-values$<6.83 \times 10^{-29}$, 1.34×10^{-11} and 3.01x10-*6* respectively). *MZF1* gene expression was significantly increased in DN in a whole kidney dataset (fold change = 1.16; 16% increase; $P = 0.03$). *TFAP2A* gene expression was decreased in an independent dataset (fold change = -1.02; $P = 0.03$). SP1 was not differentially expressed in any datasets examined. We identified three TFBSs significantly enriched within the WNT pathway genes examined highlighting the use of *in silico* analyses for identifying key regulators of this pathway. Modification of TF binding to gene promoter regions involved in DN pathology may limit progression, making refinement of targeted therapeutic strategies possible through clearer delineation of their role.

S09. Temporary loss of maintenance DNA methylation in differentiated human cells leads to permanent alterations at imprinted loci, but not reprogramming of germline genes.

KM O'Neill, CP Walsh

Transcriptional Regulation and Epigenetics Group, Centre for Molecular Biosciences, University of Ulster, Coleraine.

The phenomenon of genomic imprinting refers to the epigenetic marking of genes in the germ line such that only the allele inherited from the sperm or from the egg is active, depending on the gene. Many imprinted genes play crucial roles in development and loss of imprinting (LOI) plays a causative role in a number of childhood diseases and neurodevelopmental disorders. Imprinted genes are often organised into clusters and co-ordinately controlled through DNA methylation established in the germline at a key control site called the Imprint Control Region (ICR). Previously we generated a hypomorphic series of human non-tumorigenic, hTERT-immortalised fibroblast cells with varying degrees of loss of DNMT1. However, due to the adverse effects of DNMT1 reduction on cell viability a proportion of cells continued to proliferate likely due to silencing of the shRNA-expressing construct. We show here that despite recovery of DNMT1, DNA methylation was unable to be re-established at both paternally (*H19*) and maternally (*SNPRN*) methylated ICR; this is consistent with a need for passage of the ICR through the germline in order for the imprint to be reset. Levels of the epigenetic modification at the non-imprinted germline genes and at selfish DNA elements were comparable to the parental cell line. Ultimately, such data highlights differences in the susceptibility of imprinted and non-imprinted loci to DNA methylation reprogramming events in human, differentiated cells. These hTERT-lines will provide a unique resource for examining

the effects of loss of maintenance methylation on human imprinted ICR and for further mechanistic studies to gain insight into the control of imprinting in human cells.

S10. Meta-analysis of European ancestry individuals with Autism Spectrum Disorder reveals genome-wide significant association at the astrotactin 2 (ASTN2) gene locus on chromosome 9.

R Anney[1] on behalf of The Psychiatric Genomics Consortium - Autism Spectrum Disorder Working Group

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

Autism Spectrum Disorder (ASD) affects social communication, social interaction and is accompanied by restricted and repetitive behaviour. Twin-studies have highlighted a strong genetic underpinning in these disorders. Recent estimation of SNP-heritability also suggests that common variation harbours some of the liability to ASD. We conducted a large high-density meta-analyses combining data from multiple studies including 6500 individuals with ASD. The Psychiatric Genomics Consortium (PGC) Autism study comprised of data from six studies across five different genotyping arrays. All studies included in these analyses were family-based, containing genotype data on parent-child trios. These trio data was converted to case and matched pseudo-controls using PLINK_v1.07. Imputation was performed using SHAPE-IT/IMPUTE routines against the 1000 genome project (build v3.Aug2012). The primary analyses explored individuals with any ASD diagnosis restricted to "European" ancestry (n=5305 individuals). Secondary analyses explored association with stricter autism diagnosis, cognitive ability, verbal status and gender. Association was tested for using logistic regression of imputed dosages. All association analyses were performed using PLINK v1.07. Fixed effect meta-Analysis was performed using METAL, weighted for the inverse standard error of the effect. In the European ASD GWAS we observed a genome-wide significant association for the ASTN2 SNP rs7026354 (OR=1.17; p=6.7x10-9). Each of the five studies reported an association in the same direction. In addition to the association signal observed at ASTN2, other strong associations were observed within previously implicated ASD genes, EXT1 (rs7836146; OR=0.85; p=9.16x10-7) and MACROD2 (rs6079556; OR=0.88; p=2.18x10-6). Using an independent dataset of 1500 cases and 51000 controls from 5 distinct datasets, we observed 73% same-direction effects for the 26 SNPs passing $p < 1x10-5$. This accumulation is significantly different from chance (p=0.014). We observed a single genome-wide significant finding at the ASTN2 locus on chromosome 9 in the largest genome-wide association analyses in ASD to date. ASTN2 (astrotactin 2) is a cell adhesion molecule expressed in the brain and is thought to have a role in neuronal migration. ASTN2 has been previously implicated in ASD via the observation of rare copy number deletions. In a replication study we show a significant direction effect for highly associated SNPs. These data along with those supporting SNP-heritability indicate that common variation studies will be important in explaining genetic liability in ASD.

S11. The PAR2-linked *SPRY3* gene is regulated by X chromosome-linked promoters in an autism susceptibility region.

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1. Dept. of Biochemistry and 2 Dept. of Anatomy, University College Cork.

Sprouty proteins are key regulators of cell growth and branching morphogenesis. Unlike the mouse gene, which is X-linked, human *SPRY3* maps to the pseudoautosomal region 2 (PAR2); however, the human Y-linked allele is not expressed due to epigenetic silencing. We report that *Spry3* is highly expressed in neural ganglion cells, including cerebellar Purkinje cells, postnatally in mice, with conservation of this expression pattern in the human. Transient over-expression or knockdown of *Spry3* in cultured mouse superior cervical ganglion cells inhibits and promotes, respectively, neuronal growth and branching. A 0.7 kb gene fragment spanning the human *SPRY3* transcriptional start site recapitulates adult ganglion cell expression in LacZ reporter transgenic mice. In the human and mouse the *SPRY3* core promoter contains an AG-rich repeat. We identified nine alleles of the human *SPRY3* core promoter repeat in normal caucasians, and found virtually identical allele frequencies in a set of 120 autism families. We characterised multiple *SPRY3* transcripts originating from three start sites in the X-linked *F8A3 – TMLHE* locus, a region recently implicated in autism causation. Our results implicate *SPRY3* as a candidate gene for autism, particularly in cases with altered patterns of neuronal or brain growth.

POSTER PRESENTATIONS:

P01. All in the eyes - a lesson in diagnosis.

AC Magee

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A 28 year-old man was reviewed on request when his mother contacted our department asking for a copy of the publication which featured her son in 1991. He presented at age 18m, with short stature, cleft palate, fused ectopic kidneys and tricuspid valve prolapse. Downslanting palpebral fissures, prominent ears, microstomia, oligodontia and fused incisors were noted. Mum was concerned as she had taken Debendox (pyridoxine/doxylamine) during early pregnancy. A clinical diagnosis of Eastman-Bixler (facio-cardio-renal) syndrome was made, and the case published. He went on to develop frequent infections, always had feeding problems, and had salivary ducts reimplanted. Isolated growth hormone deficiency at age 5 responded to treatment. Type 2 diabetes was diagnosed at age 25. As Eastman Bixler was thought to be recessive, parents decided to have no more children. Mum underwent sterilisation. Shortly afterwards, the marriage failed. At review consultation, Mum expressed her disappointment that the published photos had eyes blacked out as she felt he had beautiful eyes. His features suggested Kabuki syndrome and genetic testing confirmed a nonsense mutation in MLL2. Review of clinical photographs shows the typical long palpebral fissures of Kabuki syndrome, often the diagnostic gestalt for this syndrome. The family describe a mixture of emotions: sadness that the reproductive decisions were not actually needed while accepting these were made on the information available at the time: relief that their older son's future children will not be at risk: relief that this son's medical problems are consistent with the diagnosis and management can be organised.

P02. Fabry Disease in Northern Ireland.

FJ Stewart[1], A Muir[2], J McOsker[2], T Jardine[2], A Wilson[1], P McKeown[2]

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Fabry disease is a lysosomal storage disease due to a deficiency of alpha galactosidase. It is caused by mutations in the GLA gene which is on the X chromosome. It differs from many X-linked disorders in

that females frequently show clinical features. Affected individuals may show the classical form or a more attenuated form. Treatment for this condition is available in the form of enzyme replacement therapy. The incidence has been estimated at approximately 1:50 000 males (Desnick 2001). However a newborn screening study suggested an incidence of 1:3000 with an 11:1 ratio of individuals with the more attenuated phenotype (Spada 2006). Northern Ireland has a population of 1.7 million. To date we have found 11 families who have Fabry disease within which are 46 individuals with a GLA mutation. All individuals with a mutation are followed up on an annual basis at our multidisciplinary Fabry clinic. Three families have the p.A13P mutation. All other mutations are seen in only one family and they are Exon 1 deletion, p.R392SfsX2, p.R220X, c.144delG, p.W209X, p.A13T, p.D313G and c.802_804delCA. 10 patients are aged under 18. The eldest female is aged 79. The eldest male is 75.These figures suggest an incidence of at least 1:37,000 in our population. We believe this may be an underestimate of the true incidence. We feel that family follow up of newly diagnosed cases and genetic testing of at risk relatives is essential as clinically asymptomatic individuals may miss out on the opportunity for monitoring and treatment.

P03. On the origins of renal cell carcinoma, vesicoureteric reflux and C (Opitz trigonocephaly) syndrome: A complex puzzle revealed by the sequencing of an inherited t(2;3) translocation.

JM Darlow[1,2], L McKay[3], MG Dobson[1,2], S Scala[4], DE Barton[1],I Winship[5,6]

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The co-incidence of an inherited balanced translocation through 2q37.3 in patients who developed renal cell carcinoma (RCC) with the finding of genetic linkage in the same chromosomal band in a study of vesicoureteric reflux, an inherited developmental disorder, suggested the possibility that there might be a gene involved in urinary tract development in that band that could account for both findings. We have identified the breakpoints. On 3q, the gene *CD96* is bisected in an intron, while on 2q the break-point i between *CXCR7* and *COPS8*. *CXCR7* is involved in kidney development, and expression has been reported to be elevated in >50% of RCC, but it was not consistently expressed in the tumours of our patients. *COPS8* interacts with the von Hippel-Lindau protein. This suggests that the t(2;3) translocation brought an enhancer or suppressor of *CD96* into proximity to *COPS8*, causing it to be mis-regulated, leading to a risk of RCC. Mutation of *CD96* was thought to be the cause of some cases of C Syndrome because a t(3;18) translocation bisecting *CD96* was found in a case and no gene was found at the breakpoint on chromosome 18. However, none of our patients with t(2;3) have any features of C Syndrome. The other breakpoint in the C Syndrome case was in 18q12.1, which is now known to contain the desmocollin and desmoglein gene clusters, responsible for tight junction formation in epithelia, and it seems likely the mis-regulation of these genes is the true cause of the syndrome.

P04. What's in a name? Long term follow up of infantile myofibromatosis.

L Bradley[1], L Jeffers[1], C Thornton[2], PJ Morrison[1]

[1]N. Ireland Regional Genetics Service, Belfast City Hospital. [2] Department of Histopathology, Royal Victoria Hospital, Belfast.

Infantile myofibromatosis (IM), defined by Chung (1981), was first described as 'congenital fibrosarcoma' (1951) and 'congenital generalised fibromatosis' (1954). Although only ~300 cases are reported, it is the most frequent fibrous tumour of infancy and should be considered in infants with multiple subcutaneous nodules. Long term follow up is rarely documented. We report a case of IM and follow up after 30 years. The literature and recent discoveries regarding pathogenesis are reviewed. A male infant was referred because his mother reported that she had been diagnosed as an infant with neurofibromatosis. Her mother described when holding her as 'like holding a bag of marbles' due to the presence of prominent nodules on the tongue and left side of her neck with multiple subcutaneous nodules on the back, near joints and on the soles of the feet, noticed shortly after birth. Family history was non-contributory. Examination showed no stigmata of a neurocutaneous disorder. Following the consultation, old notes were located. These described an infant girl born at 38 weeks, weighing 2.3kg, with multiple subcutaneous nodules, tongue nodules and small cystic areas in both kidneys. Additional neonatal problems included disseminated intravascular coagulation secondary to gastrointestinal bleeding requiring multiple transfusions. By 8 months, the lesions were reported to be resolving. Her paediatric chart referred to assessment at 3 years (a few lesions behind the ears) and gave the diagnosis as 'disappearing neurofibromatosis'. The original biopsy specimen (reporting 'fibromatosis') was reviewed and confirmed what we now refer to as 'infantile myofibromatosis' supporting the original genetic opinion.

P05. KEDS- Disseminating knowledge and educating health care professionals about new developments in Genetic research in Ireland.

SA Lynch[1], A Ward[1], J Turner[1], M Byrne[2], J Casey[3]

[1]National Centre for Medical Genetics. [2]School of Medicine and Health Sciences, University College Dublin. [3]National Children's Research Centre Dublin.

The translation of research findings on rare disorders from the laboratory into hospital practice can be challenging. Taking it one step further, translation into primary health care is even more problematic, as rare disorders are not a priority for primary health care professionals (HCPs). We were funded to implement an educational programme translating our research findings and informing HCPs in midwifery and primary care. As our research is focussed on genetic disorders in the Traveller population, we are targeting HCPs who care for these families. We have included information about Cystic Fibrosis testing as newborn screening was recently introduced. We have liaised with GPs and midwives and have created an educational package to help manage common genetic disorders. In addition, we have produced a series of common clinical scenarios that HCPs might encounter to help with advising on relative risk and genetic testing procedures. We are developing a microsite in conjunction with UCD which will host these educational packages. We will publicise our educational tools in a series of articles (GP and midwifery journals) and by hosting regional workshops. We are running a two day laboratory workshop to update our staff to facilitate translation of our research into our diagnostic laboratory. Access to a local laboratory that offers carrier testing for common disease genes in Irish Travellers is essential if population carrier testing is to be achieved. Ultimately, we are hopeful that carrier testing will be available for CF and common disorders found in the Traveller population and that this will be arranged through their local HCPs.

P06. A Tale of Two CTs: Evaluation strategy for genetic hearing loss.

T Dabir[1], J Turner[2], J G Toner[3]

[1]Medical Genetics Department, Belfast City Hospital. [2]Dept. of Radiology, Ulster Hospital. [3]Dept. of Otolaryngology, Belfast City Hospital.

Congenital hearing loss is the most prevalent and genetically heterogeneous sensorineural disorder. Genetic counselling and risk assessment depend on accurate determination of the specific genetic diagnosis. Autosomal recessive non syndromic hearing loss related to GJB2/GJB6 gene (Connexin 26/30) is the commonest cause of prelingual deafness. In the absence of a specific diagnosis, empiric recurrence risk, coupled with connexin 26/30 testing results is used for genetic counselling. We present two cases of rare genetic hearing loss highlighting the importance of temporal bone CT scan in accurate diagnosis, targeted gene mutation analysis, counselling and management. This useful but inconsistently requested investigation should be an essential part of evaluation of genetic hearing loss prior to referral to genetics service

1: A 25 year old female was re-referred to genetics. She was thought to have non syndromic autosomal recessive hearing loss as a child and parents were counselled accordingly. Temporal bone CT was not done previously. Connexin 26/30 testing was normal. Temporal CT identified inner ear abnormalities leading to SLC26A4 gene mutation analysis which confirmed the new diagnosis of Pendred syndrome.

2: A 3 year old male child with prelingual deafness and his pregnant mother were referred to genetics. Pending the results of temporal CT scan an empiric recurrence risk of 1 in 10 was suggested on the basis of one affected child and normal connexin 26/30 results. Her second son was found to have hearing loss on newborn screening. Temporal bone CT identified inner ear abnormalities leading to POU3F4 gene mutation analysis confirming the rare X-linked deafness.

P07. The effect of folic acid supplementation on DNA methylation patterns in pregnant women.

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DNA methylation plays an important role in gene regulation. Although changes in methylation patterns are known to occur over time, evidence for the environment's influence on this warrants further exploration. Attention has tended to focus on folate, given its role in 1-carbon metabolism and the generation of S-adenosylmethionine, the methyl donor for methylation reactions. Data demonstrating this association have arisen from animal studies, and remains to be fully elucidated in humans. We sought to identify Folate-Sensitive Differentially Methylated Regions (FS-DMRs) in blood samples from a folate intervention trial. During the second and third trimesters of pregnancy, 119 women were supplemented with either folic acid or a placebo. DNA was extracted before and after intervention. Methylated DNA was enriched using immunoprecipitation from a subset of these samples and hybridised to NimbleGen's Human DNA Methylation 2.1M Deluxe Promoter Array, covering ~30,000 gene promoters. Analysis of the array data has found that 32 loci exhibited a change in methylation, and are potentially FS-DMRs. The most significant of these will be assessed over the rest of the cohort using a method based on methylation-specific PCR. Although folic acid supplementation has very well established health benefits during gestation, the molecular mechanism still remains elusive. For the first time in humans, a folic acid intervention study has identified potential FS-DMRs that may explain this.

P08. siRNA mediated allele specific knockdown of mutant Keratin 12 in a preclinical *ex vivo* MECD model.

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Meesmann's epithelial corneal dystrophy (MECD) is a hereditary disease of the ocular surface resulting from an accumulation of keratin aggregates in the corneal epithelium. The pathology is due to a point mutation in either *KRT3* or *KRT12* resulting in a destabilisation of corneal epithelial integrity. We previously reported a severe MECD phenotype caused by the keratin 12 (K12) Leu132Pro point mutation. Due to the dominant negative pathomechanism of MECD, RNA interference therapeutics and specifically short-interfering RNAs (siRNAs) present a viable alternative to current surgical based treatments. Here we demonstrate the specificity and potency of K12-Leu132Pro-9, a siRNA previously identified by our group, in an *ex vivo* pre-clinical model of MECD, generated using a corneal limbal biopsy from a patient with the K12-Leu132Pro mutation. To replicate a more realistic model of MECD, allowing for the assessment of both wild type and mutant K12 in the same cell, a dual Flag/Strep tag quantitative infrared immunoblot was performed and confirmed K12-Leu132Pro-9 potency and specificity with a 91% silencing of mutant protein achieved. A modified 5'RACE method was then used to confirm siRNA-mediated cleavage of K12-Leu132Pro mRNA at the predicted site. Using a combination of pyrosequencing and quantitative RT-PCR, the K12-Leu132Pro-9 siRNA was shown to achieve a 63% silencing of the endogenous mutant allele in the *ex vivo* pre-clinical model of MECD. Further assessment of this promising siRNA in animal models alongside development of effective delivery will allow this personalised approach to treating corneal dystrophy to translate to the clinic in the near future.

P09. Investigation of the Functionality of Non-Synonymous Polymorphisms within the Human DHFRL1 Gene.

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DHFRL1 (Dihydrofolatereductase like-1) is a newly discovered reductase enzyme that participates in *de novo* thymidylate synthesis to support DNA replication in the mitochondria. While non-synonymous variants in the parent gene, DHFR, are rare, the databases report a number of non-synonymous polymorphisms within DHFRL1. We sought to initially confirm the presence of these genetic variants and test their potential impact on DHFRL1 function. A total of 3 non-synonymous DHFRL1 polymorphisms were identified in dbSNP (rs17855824, rs61739170 and rs114936057) and 2 were confirmed by Sanger sequencing. Recombinant DHFRL1 was generated using Gateway® cloning and confirmed polymorphisms were produced using site directed mutagenesis. Recombinant proteins were purified and confirmed

by SDS-PAGE and Western blot and were assessed for enzyme activity. We confirmed rs17855824 and rs61739170 as definitive polymorphisms with an allele frequency of 7.5% and 15% respectively based on sequencing 40 alleles. Induction and purification protocols are still being optimised but preliminary results indicate that while the Km values for NADPH are similar for all recombinant DHFRL1 enzymes, the Km values for dihydrofolic acid differ for both polymorphisms. Our preliminary data indicates that DHFRL1 variants are in fact functional. The rs61739170 polymorphism results in a Pro>Ala change, while the rs17855824 polymorphism results in a Val>Ile change. These preliminary findings remain to be confirmed but are potentially exciting as it is the first time a human reductase enzyme has been demonstrated to vary in its enzyme activity due to common non-synonymous genetic polymorphisms. These may be important in relation to human disease risk.

P10. miRNAs are associated with diabetic kidney disease.

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Circulating and urinary miRNAs have been associated with diabetic kidney disease (DKD). This study evaluated DNA methylation in a White European type 1 diabetes (T1D) population with (cases) and without (controls) DKD. This project had full ethical approval. An initial case-control study was conducted for DKD with blood-derived DNA methylation status at ~ 480,000 sites determined using Illumina's HumanMethylation450K BeadChip. Cases (n=150) were defined as diabetic individuals with persistent proteinuria, retinopathy and hypertension, while controls (n=100) had no evidence of diabetic kidney disease and were not prescribed antihypertensive medication. All individuals were diagnosed with T1D before the age of 31 years of age. *In silico* replication was evaluated in 96 case and 96 control individuals using publicly available data (GEO: GSE20067). Independent *de novo* replication was determined in 100 cases and 150 controls using SequenomEpiTyper analysis. Stringent quality control was employed and association assessed accounting for age, gender and duration of T1D; data from the discovery collection was adjusted for multiple testing. Fourteen miRNAs were selected for replication on the basis top-ranked CpGs in the association analysis ($P<10^{-6}$) with previously reported association in the literature, more than 1 CpG top-ranked for each miRNA, or top-ranked promoter associated CpG sites. One miRNA was excluded as it was located on the X chromosome and one failed Sequenom design. Six CpG sites were replicated with $P<0.05$, most significantly were miR663 and miR375.DNA methylation associated with miRNAs is associated with diabetic kidney disease in individuals with type 1 diabetes.

P11. Development of therapeutic siRNA for anepidermolysisbullosa simplex causing mutation in keratin 5.

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Epidermolysis bullosa simplex (EBS) is a rare incurable, inherited skin disorder predominantly caused by dominant-negative mutations in the genes encoding keratins K5 or K14. In the case of a dominant-negative mutation, the mutant allele disrupts the function of the wild-type allele, resulting in the disorder. EBS is characterized by blister formation within the basal layer of the epidermis in response to minor mechanical trauma. RNA interference, specifically small interfering RNA (siRNA), offers a potential therapy route for this disorder by selectively silencing the mutant allele; allowing the wild-type allele to function normally. A systematic screening method was used to develop mutation specific siRNAs for the Glu475Gly mutation identified in a family with EBS. A luciferase reporter gene system was used to test 19 allele-specific siRNAs; covering all possible positions of the mutation within the siRNA. The specificity of the lead siRNAs was confirmed by knockdown of endogenously expressed mutant allele in patient cell lines at the RNA and protein level; by pyrosequencing and western blot respectively. The potency of the lead siRNA was analysed over time and demonstrated no off-target effect on closely related keratins. The lead siRNA selected for this particular mutation demonstrates its ability to target and knockdown the mutant allele with little or no effect on the wild-type allele within patient cells. This approach demonstrates that it is possible to design highly potent siRNA, which will only inhibit the mutant allele. Efforts are now focused on non-invasive delivery system to allow development of siRNAs as a treatment of patients suffering from EBS and other keratin disorders caused by dominant-negative mutations.

P12. Telomere length, genotype and methylation association studies for chronic kidney disease.

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Shorter telomere length has been associated with chronic kidney disease (CKD) in relatively small cohorts. This study sought to identify biological markers associated with telomere function in a White European population with (cases) and without (controls) CKD. This project had full ethical approval. Review of published literature revealed 376 genes that influence telomere function. Genotype data was extracted for 1,200 case and 1,200 control individuals based on data from 2.4 million SNPs where original genotyping was performed on Illumina's Omni1-quad, OmniExomeExpress and as part of the WTCCC3. Additional 'look-up' data was extracted from published genome-wide studies for CKD. Methylation status for relevant CpG sites was extracted from Illumina's Human Methylation 450K BeadChip for 600 cases versus 200 controls. Telomere length was established by SYBR green assays on a 7900HT (Life Technologies) for 2,653 individuals. Stringent quality control was employed and association assessed accounting for relevant covariates; data was adjusted for multiple testing using the Benjamini Hochberg approach. No SNPs were observed with genome-wide significance ($P<10^{-8}$), however differential methylation at CpG sites in multiple genes were significantly associated with CKD, including clusters of differential methylation in *TERT* and *MALD1* genes. Our data suggests that studies evaluating telomere length for association with disease should also consider the influence of genetic and epigenetic contributions on observed, age-related telomere length. Several biologically relevant markers affecting telomere function have been identified that may represent important risk factors for CKD, as well as improving our understanding of biological mechanisms that contribute to chronic kidney disease.

P13. Review of Statistical Methodologies for the Detection of Parent of Origin Effects

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Many genome-wide association studies have failed to explain the full heritability of complex diseases. This may in part be due to the fact that these studies typically do not consider the functionality of the alleles to be dependent on parental origin. The inheritance of an allele from one parent may not result in the same effects as if this allele had originated from the other parent. Usually these effects come under the term parent-of-origin effects and can be explored when family data (parents and at least one affected offspring) are available. A number of models have been proposed to detect parent-of-origin effects, including effects such as imprinting and other effects such as maternal effects. These models vary in complexity, ease of application (availability of software), and in their power to detect particular effects. The aim is to review these different approaches; to explore the different mechanisms of parent-of-origin effects that are detectable with the different models and tests and to compare the approaches. This is important as these are a number of tests available, some more appropriate than others, depending on the type of data and knowledge of the suspected underlying disease causing mechanisms that are available. Simulation studies are undertaken to explore which tests may be most appropriate under particular circumstances for family trio data.

P14. Analysis of the hexonucleotide repeat expansion and founder haplotype at C9ORF72 in an Irish psychosis case-control sample.

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A hexonucleotide repeat expansion 'GGGGCC' in an intronic region of the C9ORF72 gene has been found to account for up to 70% of amyotrophic lateral sclerosis (ALS), and this locus also being implicated in the pathogenesis of frontotemporal dementia (FTD). Study of an independent FTD sample showed a strong association between C9ORF72 mutations and psychotic symptoms. We sought to screen a large Irish psychosis case-control sample for evidence of association between the repeat expansion and psychosis. We carried out haplotype analysis on this region, due to reports of a founder haplotype. Our sample included 1,165 cases and 1,283 controls. We used a reverse-primed PCR method to amplify the hexonucleotide repeat expansion. Haplotype analysis was carried out using available GWAS data for these samples. 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 >30. There were two controls samples (26 and 25 repeats respectively) and two schizophrenia cases (27 and 28 repeats). Haplotype analysis found that for the 512 samples that carried more than 7 repeats, 482 (94%) carried the founder haplotype. The significance of the intermediate number of repeats (between 24 and 29) is still unclear. Haplotype analysis showed a clear association between repeat number and the founder haplotype. It would appear that this haplotype is not unique to ALS-FTD cases, but its presence predisposes this region to the repeat expansion, and in turn this may lead to a greater risk of molecular instability.

P15. Analysis of rare variants at MACF1 in psychosis cases and controls.

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Psychosis (schizophrenia (SZ) and bipolar disorder) are complex neurodevelopmental disorders influenced by several genetic and environmental factors that affects approximately 1-2% of the population. The heritability of the disorder has been estimated at 80% and it has been shown that common and rare variants could give an increased risk to SZ. During a previous next-generation sequencing study performed in my lab, a rare loss-of-function mutation was discovered in a SZ case in the MACF1 gene, which interacts with NRXN1, a known risk gene for SZ. To further study mutations in that gene, we selected three other rare and potentially functional missense variants in MACF1 and genotyped them in 1,252 cases and 1,732 controls using TaqMan. We found that the variant rs139995582 is associated with psychosis (p=0.02). Using available GWAS data, we performed a haplotype analysis with Plink and Haploview and found that all three mutations display a founder effect as each is carried on the same haplotype background in all carriers. Further study is required to test for cryptic relatedness between these individuals, which could possibly uncover large affected pedigrees. Interestingly, three cases carried both variants rs139995582 and rs150229499. Haplotype analysis, confirmed by an allele-specific PCR, showed that those two variants are most likely on the same haplotype and inherited from a common ancestor.

