

20th Meeting of the Irish Society of Human Genetics



Friday 15th September 2017 Croke Park, Dublin

ORAL PRESENTATIONS:

S01. DEVELOPING AN MDT MODEL IN NEUROFIBROMATOSIS TYPE 1 (NF1) AS A PARADIGM FOR ENTRY INTO A EUROPEAN REFERENCE NETWORK (ERN).

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Neurofibromatosis (NF1) affects 1/2500 people throughout the world. Children with NF1 require a multidisciplinary service ideally, delivered on a single site. NF1 is a very variable condition with children requiring the expertise of genetics, paediatricians, ophthalmologists, dermatologists, neurologists and other specialities as required. Building such a service concentrates expertise, facilitates coordination of care and fosters ideal opportunities for research.

Aims: 1) To develop a service ensuring children had access to a multidisciplinary clinic on an annual basis. 2) Hold monthly clinics offering ophthalmology, medical, developmental and dermatology follow up. 3) To create a registry of patients which captures the incidence and prevalence of NF1 in Ireland. To offer best possible care for the children attending the service by following international consensus guidelines. 4) To liaise with NF1 Association, families and research authorities.

Methods: 1) Appointment of a CNS/CNM2 in Neurofibromatosis as funded by the NCH Foundation. 2) Visit to the complex NF1 Clinic in Manchester's Children's Hospital and learn from their service, MDT and guidelines. 3) Establish links with genetics, oncology, radiology and orthopaedic depts. in OLCHC. 4) Create a referral pathway for HCPs to ensure children with NF1 are referred to most appropriate service in a timely fashion. 5) To register the service on Orphanet and gain entry into an ERN as a multi-site service in conjunction with OLCHC.

Results/Conclusion: To date, the service has been running for 9 months. The CNM2 provides telephone service and coordinates clinics. The Clinic has been registered in Orphanet and the process has begun to create a patient registry and enter the service in the ERN.

S02. TUMOUR RISKS AND GENOTYPE-PHENOTYPE ANALYSIS IN AN IRISH COHORT OF PATIENTS WITH GERMLINE MUTATIONS IN THE SUCCINATE DEHYDROGENASE SUBUNIT GENES SDHB, SDHC AND SDHD

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Germline mutations in the succinate dehydrogenase subunit genes SDHB, SDHC and SDHD are the most frequent causes of inherited pheochromocytomas and paragangliomas. Patients presenting with these tumours are usually offered genetic testing for these and other genes as part of standard clinical investigations. However, the information regarding penetrance and phenotype genotype correlations associated with SDHB/C/D mutations is variable, making it difficult to determine an optimum management strategy for this group.

In order to address this issue we undertook a retrospective cohort study of patients who underwent genetic testing for SDHB, SDHC or SDHD. 195 patients were identified through the Irish Genetics laboratory electronic database as having had a genetic test for SDHB, SDHC or SDHD and referral source, referral reason and genetic test outcome were analysed.

Analysis of penetrance and phenotype presentation was determined through a Clinical Genetics chart review of 147 patients from 40 separate families. Analysis of age-related tumour risks according to relevant gene and mutation type (for SDHB and SDHD) provided estimates of penetrance and genotype-phenotype correlations.

Increased knowledge of the molecular basis of phenotypic variability commonly observed in individuals with germline SDHB/C/D mutations will facilitate the development of age-appropriate management protocols based on gene specific tumour risks.

S03. CATALOGUING INHERITED DISORDERS AMONGST THE IRISH TRAVELLER POPULATION

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Irish Travellers are an endogamous, ethnically Irish population of ~40,000. Consanguinity is common. Knowledge of Traveller disorders exists but mainly in specialised Irish centres. Most Traveller disorders are published but ethnicity is not explicit, hampering diagnoses, particularly if the patient is overseas where knowledge about this population is poor.

Aims: To catalogue inherited Irish Traveller disorders through identifying the disorders, detailing mutations, use of coding, (OMIM, Orphacodes & ICD10), publications, and help develop a database to facilitate diagnoses.



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Methods: A literature review was undertaken. Key national and international Clinician/scientists were contacted to identify relevant disorders and publications. Laboratory and clinical databases were searched to retrieve disorders & mutations. Annotations were updated. An Excel database was established listing each disorder, its appropriate code, associated mutation and relevant publication.

Results: 86 distinct rare genetic disorders resulting in 75 phenotypes were identified; 78/86 were autosomal recessive; 4 of these were dominant disorders presenting only in the recessive state. Seven dominant disorders with no recessive phenotype were included as > one affected individual existed. One common 17q12 duplication was included, presenting in two unrelated families. Homozygous mutations were found in all recessive disorders bar one. The genetic basis of 78/86 was established. A further 2/76 have common haplotypes; the genetic basis of six disorders remains unclear. Linkage disequilibrium was observed in 4 families with co-existing McArdles disease and microcephaly & 11 individuals have co-existing Friedreich's ataxia & galactosemia.

Conclusion: Our work is the first step towards cataloguing inherited Irish Traveller disorders. Future challenges include development of an online mutation database.

S04. MUTATIONAL ANALYSIS IN A COHORT OF ADULTS WITH A BIOCHEMICAL DIAGNOSIS OF TRIMETHYLAMINURIA ATTENDING AN IRISH METABOLIC UNIT

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Primary Trimethylaminuria (TMA)(OMIM 136132), is an autosomal recessive rare disorder which results in diminished capacity to oxidise the dietary derived amine trimethylaminuria to its odourless metabolite Trimethylamine-n-oxide (TMA-n-Oxide). Severe primary TMA has been defined as the percentage of unmetabolised free TMA in urine being >40% and mild/moderate TMA range is 10-39%. More than 30 variants of the Flavin monooxygenase 3 (FMO3) have been reported to cause primary TMA. Diagnosis of primary TMA has implications for management of the patient in relation to treatment and genetic counselling.

We sequenced the entire FMO3 gene coding region in 10 patients who had a biochemical diagnosis of TMA made in the past 5 years. Three of the patients had severe TMAU (% TMA range 39.4 to 45), (Group A) and 7 had mild to moderate TMAU (%TMA range 10-30), (Group B).

We identified causative (loss of function) in 5/10 individuals. Homozygosity for loss of function mutations was detected for 2/3 cases with severe TMAuria (Group A).

3/7 of the patients with mild to moderate TMAuria biochemically had a genetic diagnosis. Two were homozygous for Glu158Lys/Glu308Gly and the other was compound heterozygous for P153L and A232T.

Primary TMAU is rare in Ireland and mutational analysis should not replace biochemical diagnosis. The rate of detection of pathogenic

mutations was low using the recommended biochemical cut-offs. The E305X mutation the first *FMO3* mutation described in OMIM (136132.0001) in an Irish Australian family may be an Irish Mutation.

Two new apparent *FMO3* mutations are described in this Irish population. A cut-off of free TMA levels higher than that suggested on the Gene Utility card may be more beneficial in directing genotyping.

S05. TARGETED NEXT-GENERATION SEQUENCING FOR THE MOLECULAR CHARACTERISATION OF HEREDITARY RENAL DISEASE

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Background: As part of the Irish Kidney Gene project, 2000 people with renal disease were surveyed and >30% of participants reported a family history for their condition. This strongly suggests an underlying genetic component for the development of kidney disease. Blood and urine tests as well as kidney biopsies are frequently used to inform on aetiology of the disease. However, in around 10% of cases, aetiology is simply unknown, making it difficult for physicians to provide a clear diagnosis or prognosis to these patients.

Aim: This project aims to utilise genomic sequencing to stratify patients with hereditary renal disease (HRD). In doing so we seek to aid clinical diagnosis, provide insight into pathogenesis and in some cases point to specific therapies.

Methods: We developed a custom, targeted NGS panel for inherited kidney diseases which we have applied to 48 HRD patients. The panel includes 11 genes which are established causes of polycystic kidney disease, von Hippel Lindau syndrome, renal cysts and diabetes syndrome and Alport syndrome. The NimbleGen Heat-Seq kit was used for library preparation and samples were sequenced using an Illumina MiSeq platform at Beaumont Hospital. Data was analysed using a custom bioinformatics pipeline and variants were classified according to the ACMG guidelines.

Results/Conclusions: To date, this panel has identified candidate pathogenic variation in a third of samples studied. Future work in this project will include the development of a larger targeted panel including >100 known renal disease genes.

S06. IDENTIFYING NOVEL INHERITED BREAST CANCER MUTATIONS IN AN IRISH POPULATION

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Breast cancer is the most common female malignancy worldwide. Up to 10% of cases are the result of an inherited monogenic mutation, while a further 25% appear in familial clusters. Only 30% of hereditary breast cancers are attributed to mutations in *BRCA1* and *BRCA2*, identified as high-risk genes through linkage analysis. While *BRCA* mutational status is highly informative, and allows clinicians to modify surveillance, prevention and therapeutic strategies, the risk conferred by mutations in other genes is more difficult to define in light of variable penetrance. Next-generation sequencing has been rapidly evolving to advance testing sensitivity and throughput in a cost-effective manner. This progression



has made multi-gene testing a practical option when looking to identify inherited mutation(s) in a clinical setting. However, current clinically available multi-gene panels generate many variants of unknown significance in genes that are presently not considered clinically useful. The aim of our study was to design a multi-gene panel to enable the detection of rare, probably pathogenic variants contributing to the susceptibility of breast cancer in an Irish population. An extensive literature review was conducted in order to generate a list of 282 genes with potential association to breast cancer. Targeted DNA enrichment and multiplexed next-generation sequencing was performed on a cohort of 167 samples from the west of Ireland. 90 breast cancer patients and 77 geographically-matched controls were included in this study. Bioinformatic analysis was performed following GATK best practices workflow. Variant data for our 282 selected genes will be presented and discussed.

S07. BENEFICIAL EFFECTS ON PSYCHOSOCIAL AND COGNITIVE DEVELOPMENT OBSERVED IN CHILDREN FOLLOWING IN UTERO FOLIC ACID SUPPLEMENTATION TRACK WITH CHANGES IN THEIR DNA METHYLATION

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Increasingly accurate surveys of human health throughout the life course has led experts to propose that stresses on the developing child whilst in the mother's womb can affect the individual's health later in life. Such long-term effects on health are thought to be mediated by a semi-permanent trace on the genes called an epigenetic mark, mediated by processes such as DNA methylation. DNA methylation patterns may be altered by the mother's diet, particularly folate – a key component in the DNA methylation cycle. Currently, mothers are recommended to supplement their diet with 400µg folic acid/day as a preventative measure against neural tube defects prior to/during the first trimester. However, there remains no clinical recommendation as to whether mothers should continue supplementation during the latter two trimesters and the potentially heritable effects. Thus, we analysed cord blood samples ($n=93$) from the Folic Acid Supplementation in the Second and Third Trimesters (FASSTT) randomised control trial for genome-wide DNA methylation. Offspring exposed to folic acid in later pregnancy had fewer highly methylated genomic regions and more intermediately methylated sites. Upon further interrogation, gene ontology analysis revealed these sites are enriched for genes associated with cognition and neurological system processes, and tissue analysis revealed enrichment of affected genes associated with the brain. Cognitive and psychosocial testing of the children at age 7 years, using standardised tests (WPPSI, TEIQe-CSF, RASP), showed that the children supplemented during pregnancy scored significantly higher for emotional intelligence, resilience and verbal IQ. Thus, this study offers a potential biological mechanism linking maternal folate levels with childhood cognition.

S08. THE IMPACT OF MTHFD1L EXPRESSION ON FORMATE LEVELS AND THE CELLULAR PROTEOME IN A CELL LINE MODEL.

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Introduction: We previously identified the mitochondrial 10-formyltetrahydrofolate synthase enzyme, MTHFD1L, as a risk factor for human Neural Tube Defects (NTD). This association was further supported by a mouse model of mutant *mthfd1l*, that exhibited an NTD and was rescued with maternal formate supplementation. The abundance of MTHFD1L is also increased in a range of cancers. MTHFD1L performs the last step in mitochondrial one carbon metabolism to produce formate for transport into the cytoplasm.

Aim: Given the pivotal role of MTHFD1L in human disease, we sought to decipher the cellular response to the expression level of MTHFD1L in HEK293 cells.

Methods: Human MTHFD1L was overexpressed in a stably transfected line using a pcDNA3.2 vector and knocked down using two inducible shRNA constructs that were clonally selected. Cells were grown and sampled over a five-day period. Expression level was confirmed by RT-qPCR. Intracellular and media formate levels were measured using GC-MS. Proteomics analysis was performed on whole cell lysates using LC-MS/MS on an Ultimate 3000 nano LC system coupled to a LTQ Orbitrap XL.

Results: Intracellular and media formate levels directly correlated with expression level of MTHFD1L compared to controls within an approximately 1.5 to 3 fold range. Our proteomics analysis showed that MTHFD1L expression level had an effect on proteins involved in DNA synthesis, replication and repair.

Discussion: We have demonstrated that MTHFD1L expression level has a direct impact on both intra- and extra-cellular levels of formate and may act as a signal for uncontrolled cell proliferation.

S09. THE IRISH DNA ATLAS; REVEALING FINE SCALE POPULATION STRUCTURE AND HISTORY WITHIN IRELAND.

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Ireland has remained relatively isolated from mainland Europe, notwithstanding historical migrations including the Norse-Vikings, Anglo-Normans, and the British Plantations. Although previous studies have shown the Irish to have elevated levels of homozygosity compared to mainland Europe, the extent of genetic structure within Ireland, and the genomic impact of historical migrations, is largely unknown. Here we illustrate fine-scale genetic structure across Ireland that follows sociological boundaries and present evidence of admixture events into Ireland. Utilising the 'Irish DNA Atlas', a DNA cohort ($n = 194$) of genealogically described Irish individuals with four generations of ancestry linked to specific regions in



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Ireland, we analysed in combination with 2,039 individuals of regional British ancestry (the PoBI dataset) and show that the Irish population subdivides into 10 distinct geographically-stratified genetic clusters; three of shared British/Irish ancestry, and seven of predominantly 'Gaelic' Irish ancestry. This structure is remarkably homogenous, and is associated with very little gene flow barriers within Ireland. Additionally, using a reference of 6,760 European individuals and two ancient Irish genomes, we quantified the ancestry of these Irish clusters within the context of Europe as well as ancient Ireland. We show high levels of north-west French-like and Norwegian-like ancestry within Ireland, and homogenous levels of ancient Irish ancestry in our 'Gaelic' Irish clusters. Finally we detect admixture events into Ireland, coinciding with the Plantations of Ulster, as well as Norse-Viking activity within Ireland. Our work informs both on Irish history, as well as the study of Mendelian and complex disease genetics involving populations of Irish ancestry.

S10. A MOLECULAR ANALYSIS OF SDCCAG8, A SCHIZOPHRENIA RISK GENE THAT FUNCTIONS IN THE CENTROSOME

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Schizophrenia affects 1% of adults and is a major global health problem. I am interested in the potential role of the centrosome in schizophrenia. The centrosome, an organelle within cells, plays a crucial role in brain development where it directs cell shape, polarity and motility. The centrosome also seeds the growth of antenna-like signalling structures called primary cilia. Rare mutations in centrosome genes cause disorders that present with severe cognitive deficits and variable neuropsychiatric phenotypes.

GWAS data has implicated many genes in schizophrenia. We have shown that seven schizophrenia risk genes encode proteins with centrosomal functions. Of these, SDCCAG8 is also associated with educational attainment in GWAS and the genome-wide significant SNPs for the two phenotypes are in high linkage disequilibrium indicating a pleiotropic effect. We have found that a schizophrenia risk SNP in SDCCAG8 is significantly associated with poorer performance in a social cognition task, in a large Irish dataset of schizophrenia patients and controls ($p=0.001$).

To analyse the molecular function of SDCCAG8 we have used genome editing to knock it out in neuronal and retinal cells. Preliminary data shows that loss of SDCCAG8 impairs cells' ability to make primary cilia and that their capacity to repair genome damage is reduced. Current work is addressing whether SDCCAG8 affects activities that may contribute to schizophrenia, including cell migration and cell signalling. This could identify molecular mechanisms by which SDCCAG8 mutations contribute to schizophrenia risk and cognition, and help uncover the processes that implicate centrosome genes in neurodevelopmental phenotypes.

S11. POLYGENIC RISK SCORE AS A DETERMINANT OF RISK OF NON-MELANOMA SKIN CANCER POST-TRANSPLANTATION

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Multiple genetic loci have been identified for non-melanoma skin cancer (NMSC) in the general population. Polygenic risk score (PRS) was defined as the sum of all alleles associated with a trait weighted by the effect size of that allele as determined by a previous genome-wide association study (GWAS). We tested whether PRS, calculated using a GWAS of NMSC in a non-transplant population, can be used to determine risk of developing and time to NMSC post-transplant.

Post-kidney transplant NMSC cases ($n=155$) and controls ($n=442$) were collected from Tennessee, Ireland and Scotland. Genetic variants that reached pre-defined levels of significance were chosen from a squamous cell carcinoma (SCC), and basal cell carcinoma (BCC) GWAS, both conducted in non-transplant populations. Using these GWAS results, BCC and SCC PRSs were calculated at each p-value threshold (pT) for each sample. PRSs were tested as a predictor of case:control status using logistic regression and time to NMSC post-transplant in a survival model.

SCC PRS calculated at pT 1×10^{-6} was the most significant predictor of case: control status of NMSC post-transplant (OR per 1 stdev increase in PRS=2.3; corrected P (P_c)=0.04). When NMSC was subdivided into SCC and BCC, SCC PRS pT 1×10^{-6} significantly predicted case:control SCC (OR=2.5, $P_c=0.02$) and BCC status (OR=7.6, $P_c=0.02$). SCC PRS pT 1×10^{-5} also significantly predicted time to BCC ($P_c=0.007$, HR=1.8) and SCC ($P_c=0.05$, HR=1.4).

PRS of non-transplant NMSC can be used to predict case:control status of post-transplant NMSC, SCC and BCC as well as time to developing BCC and SCC post-transplant.

POSTER PRESENTATIONS:

P01. ESTIMATING THE NUMBER OF RARE DISEASE PAEDIATRIC PATIENTS SEEN BY A SINGLE NATIONAL GENETICS CENTRE BORN IN THE YEAR 2000.

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Introduction: Rare diseases are diseases, which affect a small number of people compared to the general population. In Europe, a disease is considered rare when it affects no more than 5 per 10,000 individuals. A disease can be rare in one region but common in another. The objective of this study was to derive a proxy estimate the number of childhood onset rare diseases through referrals to the country's only Genetics center, as the Republic of Ireland does not have a centralized rare disease registry.

Methods: A retrospective review of referrals to cytogenetics and clinical genetics for the years 2000-2016 for patients born in the year 2000 was undertaken. Anonymized data was catalogued into rare, common, normal, likely rare & unclassifiable by review of records, and assigned Orphacodes based on diagnosis. Census live birth data was used as the denominator.

Results: 54,7891 live births were recorded by the census in 2000. 1872 referrals to Genetics (representing 1749 individuals born in



2000) were retrieved for review. 1007 had cytogenetic testing only, of which 51 had rare chromosomal anomalies. Review of 742 referrals to clinical genetics yielded 581 with a rare disease (78%), 7 with a likely rare disease, 56 with a common disorder, 83 who were normal (at risk relative) & 15 unclassified (hadn't yet been seen). Of the 53/1749 who had died (3%), 51 had a rare disease with congenital malformations (24) the most common cause.

P02. A POPULATION STUDY OF TUMOURS IN NEUROFIBROMATOSIS TYPE 1 PATIENTS IN NORTHERN IRELAND

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Neurofibromatosis type 1 (NF1) is a relatively common autosomal dominant genetic condition, with an incidence of around 1 in 3000. All NF1 patients attend our regional NF1 clinic intermittently and our departmental database records clinical details. Currently, we have 468 living patients affected with NF1 in Northern Ireland. NF1 is caused by mutations, or occasionally deletions, of the neurofibromin tumour suppressor gene, which leads to over-activation of the RAS-MAPK pathway, and tumour formation. These vary from benign lesions, such as neurofibromas, through to malignant peripheral nerve sheath tumours (MPNSTs) and tumours in other sites, particularly the central nervous system, that can be associated with significant morbidity and mortality. MEK inhibitors have recently been shown to be an effective treatment modality in the tumours associated with NF1. We have studied our population to determine the number of patients with plexiform neurofibromas, who are at risk of MPNSTs, and the proportions of patients with tumours elsewhere. This will allow us to identify which patients could benefit from MEK inhibitors in the future.

P03. A COMPLETE POPULATION SURVEY OF EPILEPSY IN TUBEROUS SCLEROSIS PATIENTS IN NORTHERN IRELAND

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Tuberous Sclerosis complex (TSC) is an autosomal dominant genetic condition which results, in the majority of patients, from a mutation in the TSC1 or TSC2 genes. Many of the patients are affected by angiomyolipomas and sub-ependymal giant cell astrocytomas. There is evidence that mTOR inhibitors, particularly Everolimus, shrink such tumours. In addition, the recent EXIST-3 study showed that Everolimus led to a significant reduction in seizure frequency in TSC patients whose seizures had previously proved resistant to anti-epileptic drug treatment. Consequently, a European licence has been granted to prescribe Everolimus for this indication.

In order to determine the potential number of patients who may be eligible for consideration of this treatment, we undertook a complete population survey of epilepsy in our TSC patients. Information was extracted from our database and descriptive statistics were carried out. We were particularly interested in obtaining numbers of those whose seizures were poorly-controlled, defined as requiring 3 or more anti-epileptic drugs to manage their seizures, or requiring neurosurgical intervention. Many of the TSC patients with a diagnosis of epilepsy were also diagnosed with learning difficulties. The possibility of an association between degree of seizure control and severity of learning difficulties was explored. Finally, the annual

cost of prescribing Everolimus to Northern Ireland's TSC patients with poorly-controlled seizures was estimated.

P04. ZYGODACTYLY (SYNDACTYLY TYPE A1) IS ASSOCIATED WITH CHARCOT NEUROPATHY AND DIABETES

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Charcot neuroarthropathy is associated with neurological deficit and is often seen in patients with a history of diabetes. Zygodactyly is a common congenital malformation with cutaneous webbing of the second and third toes.

To determine the frequency of Zygodactyly in midfoot (tarsometatarsal) Charcot neuropathy due to diabetes, we analysed a prospective series of twenty-five patients with Charcot neuropathy referred to podiatry clinics from diabetes and vascular departments. Twenty-nine patients with diabetes (but no Charcot neuropathy) were used as controls. Nineteen of the twenty-five patients with type 2 diabetes, peripheral neuropathy, and midfoot Charcot neuroarthropathy, exhibited Zygodactyly as did one of the twenty-nine controls. There was a significant difference between the two groups (Chi squared test $p < 0.001$). None of the cases or controls had any dysmorphic features or other limb malformations.

Zygodactyly occurred in association with midfoot Charcot neuroarthropathy (diabetic neuropathy) in 76% of cases. No association between Zygodactyly, diabetes and Charcot neuropathy has previously been recognised. Genes such as OPG and RANKL affect foot and bone development and MSX1 and PLA2G6 affect spinal and distal nerve development. The possibility of a genetic contribution in patients who develop type 2 diabetes, peripheral neuropathy and Charcot neuroarthropathy must be considered. Zygodactyly may act as a predictive marker for Charcot neuropathy and further identification of regulatory genes may be possible. Until then, recognition of Zygodactyly may allow early intervention and a reduction of complications in patients with Charcot neuropathy.

P05. USING NEXT-GENERATION SEQUENCING STRATEGIES TO GUIDE PRECISION ONCOLOGY IN CASES WITH ATYPICAL CLINICAL PRESENTATION

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Development of an unusual clinical phenotype across both common and rare cancer types presents a significant challenge from a diagnostic and therapeutic perspective. We describe



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two distinct cases involving an Ovarian adenocarcinoma and a Medullary Thyroid cancer (MTC) patient and wherein both patients presented with metastases at highly unusual locations, followed by development of an aggressive disease. In first case involving a patient diagnosed with ovarian adenocarcinoma presented with a rare solitary extracranial brain metastases with no other associated metastases after 2 years post-hysterectomy and chemotherapy. Despite surgical removal of the metastatic lesion and stereotactic radiotherapy, the patient showed a further relapse at the initial as well as two additional extracranial regions. Our current analysis of whole-genome sequencing of primary tumour and extracranial lesion, reveal a remarkable difference in the genomic aberration landscape between the primary tumour and the metastases. In addition, we also identify several structural variants including novel gene fusions as well as gross chromosomal abnormalities, which could be potentially utilized as targets for treating this patient further. In the second case, whole-exome sequencing of primary tumour and bone-marrow metastases in the MTC patient identified three germline single nucleotide polymorphisms (SNPs) within the *RET* proto-oncogene that remained undetected using routine hospital genetic testing procedures. More importantly, we report for the first time in thyroid cancer on the occurrence of a “chromothripsis-like pattern”, which involved shattering of chromosome 4 leading to complete abrogation of normal chromosomal function, along with dramatic widespread copy number aberrations across both primary tumour and bone marrow samples. These results provide a rationale for the application of comprehensive genomic analysis of cancers presenting with unusual and aggressive phenotypes to facilitate more appropriate therapeutic options and diagnoses.

P06. CO-EXISTING TRANSIENT NEONATAL DIABETES MELLITUS TYPE 1 WITH CONGENITAL CHOLEDOCHAL CYST – COINCIDENCE OR CONNECTED?

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Transient Neonatal Diabetes (TNDM) is characterised by diabetes that develops in the first 6 weeks of life and resolves by 18 months. Approximately 70% of cases are classified as TNDM Type-1 (TNDM1), caused by methylation defects on chromosome 6q24.

It is associated with some congenital anomalies, however associated hepatobiliary abnormalities are not described. Choledochal cysts are congenital dilations of part or all of the bile duct, occurring in 100,000-150,000 live births. The 5 major types are classified according to the extent of hepatobiliary involvement. Surgical excision of the cyst is indicated to prevent complications such as stone formation, malignancy, cyst rupture and pancreatitis.

We describe a case of TNDM1 due to whole chromosome paternal uniparental disomy 6, with co-existence of a type 1a choledochal cyst in a female born following intrauterine growth retardation. Hyperglycaemia soon after birth led to insulin treatment and a diagnosis of TNDM1, with resolution of the diabetes by 4 months of life. Follow up of antenatal findings of a cystic anomaly demonstrated the presence of a type 1a choledochal cyst on ultrasound and magnetic resonance cholangiopancreatography. Successful surgical excision of the cyst and a roux-en-Y hepaticojejunostomy was

undertaken at 6 months of age.

To our knowledge the co-existence of these disorders has not previously been reported. Further genetic analysis by whole exome sequencing is now in progress to determine if a mutation in the *PKHD1* gene, unmasked by the paternal UPD of the entire chromosome 6, explains the associated choledochal cyst in this case.

P07. PARENTAL MOSAICISM FOR A PATHOGENIC FBN1 GENE MUTATION IN 3 SIBLINGS AFFECTED WITH MARFAN SYNDROME : IMPLICATIONS FOR GENETIC COUNSELLING

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Mosaic mutations can go unnoticed, underlie genetic disease or normal human variation, and may be transmitted as constitutional variants to future generations. Marfan syndrome (MFS) is a clinically variable systemic connective tissue disorder involving ocular, skeletal, and cardiovascular systems. The risk to siblings of an identified *de novo* variant in a proband remains above population risk but less than the 50% risk attributed probands (~75%) who have an affected parent. This is due to somatic and germline mutations reported in rare cases.

We describe the phenotypic variability in three siblings with a confirmed heterozygous pathogenic exon 52 fibrillin1 (*FBN1*) gene variant with clinically unaffected parents Parental leucocyte DNA was tested and did not identify the *FBN1* gene variant. Paternity has been unequivocally confirmed and subsequent testing of parental buccal samples failed to detect the variant.

One brother had aortic valve replacement and aortic aneurysm repair at 35 while another brother had surgery of aortic dilatation at the sinuses of Valsalva at 32. The brothers had variable joint hypermobility, patellar dislocations and ophthalmic presentations involving subluxed lenses, myopia and amblyopia. Early onset of varicose veins as a teenager in one and thoracolumbar scoliosis in another brother were present. Their 42 year old sister has apparently normal aortic and cardiac imaging and ophthalmology but has mild Marfanoid facial features.

To our knowledge this is the first reported family in the literature of 3 siblings as a result of parental mosaicism for a *FBN1* gene variant and highlights the impact for genetic counselling.

P08. TARGET 5000: A GENETIC CHARACTERISATION STUDY OF INHERITED RETINAL DEGENERATION (IRD) PATIENTS IN IRELAND.

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The inherited retinal degeneration (IRD) patient cohort used in the study has been obtained via a collaborative network of ophthalmologists whereby if an IRD is suspected given consent, a



DNA sample is taken and provided to a central laboratory for genetic analysis. The study seeks to detect previously identified, together with as yet undiscovered, pathological mutations in a panel of known retinal degeneration genes utilizing target capture next generation sequencing (NGS) for 264 IRD genes. The study to date includes over 700 IRD patients from more than 500 pedigrees.

While clinical trials are in progress for patients with IRDs, many such trials require patients to have a known causative mutation to participate in these trials. The Target 5000 research project aims to genetically characterise the estimated 5,000 people in Ireland with IRDs. To date, as part of Target 5000, over 10% of the Irish IRD population has been sequenced providing real insights into the genetic architecture of IRDs in Ireland. Target 5000 offers not only a chance to discover new relevant and pathogenic mutations, but is vital to providing patients with information regarding the underlying genetic pathogenesis of their disease.

Thus far, during the course of the study, genetic analysis of IRD patients has helped to resolve ambiguous phenotypes and to identify causative mutations in approximately 60% of IRD cases. The growing body of data from NGS studies of IRDs globally should facilitate better correlations between genotype and phenotype and refine methods for diagnoses and prognoses.

P09. UTILIZING DETAILED PHENOTYPING FOR INTERPRETING VARIANTS FROM WHOLE EXOME SEQUENCING IN PATIENTS WITH RARE OVERGROWTH SYNDROMES

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Overgrowth syndromes are characterized by tall stature, macrocephaly and other congenital features. These disorders typically arise sporadically through *de novo* dominant mutations in a growing list of genes. Although whole-exome sequencing (WES) allows us to examine all genes at once in a cost effective manner, we are left with a very large number of possible disease-causing variants to sift through. In addition, we must identify at least two patients with mutations in the same novel gene for the finding to be significant. To address this, we utilized detailed phenotyping of patients with undiagnosed overgrowth to group patients with significant phenotypic overlap and to help us interpret and prioritize the variants identified via WES.

We performed WES for 12 undiagnosed patients from our overgrowth cohort. For most patients, there were no obvious causative variants in genes that were previously associated with human overgrowth. Therefore we analysed the participants' clinical records to look for phenotypic traits that may lead us to new candidate genes. After further mining of the WES data, we prioritized possible disease causing variants based on a number of factors including biological function of the gene, predicted effect on protein function and a minor allele frequency <1%. High-priority autosomal heterozygous candidate variants were identified. These variants are being validated via Sanger sequencing and tested in parental samples to assess inheritance.

We have found that detailed phenotyping is a useful tool for narrowing down the number of candidate variants for rare overgrowth syndromes.

P10. WHOLE GENOME SEQUENCING OF NATIVE HIGH

ALTITUDE QUECHUA INDIVIDUALS FROM CERRO DE PASCO PERU, IDENTIFIES CLEAR SIGNALS OF POSITIVE SELECTION

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Living the 'high life' presents challenging conditions of extreme cold, hypobaric hypoxia and a restrictive diet that forces populations to adapt to survive.

The Quechua are an indigenous high altitude population of Peru and Bolivia. They have resided at altitudes greater than 2500 meters above sea level (m.a.s.l) for the past 10,000 years, following their arrival in South America. Previous studies have characterised their adaptive physiology and identified genes under natural selection (ref). However our understanding of their genetic adaptation to hypoxia is incomplete, as previous studies focused on common genetic variation and applied a limited number of selection tests.

To shed further light on genetic adaptation in the Quechua, we established a cohort of 43 Quechua individuals from Cerro de Pasco, Peru (4330 m.a.s.l). We performed whole genome sequencing to a mean depth of 34X. We detailed the demographic history of Quechua using principal components analysis, Admixture and Treemix. We performed five tests of selection, (iHS, XP-EHH, ΔiHH, F_{ST} and ΔDAF) on real, and simulated Quechua data incorporating details of the demographic history of the population. We performed a composite of multiple signals (CMS), which aggregates information from the five tests of selection, and identified robust signals of positive selection in high altitude Quechua individuals.

The Quechua appear as a relatively homogenous population, with 10% European ancestry. We report the top 1% of genes under selection identified by CMS. We identify putative hypoxia associated genes under selection as well as the previously reported well-characterised hypoxia gene *EGLN1*.

P11. THE CANCER TESTIS ANTIGEN AND REPLICATION-DEPENDENT HISTONE GENE CLASSES ARE HYPOMETHYLATED IN UHRF1 KNOCKDOWN CELLS, RESULTING IN INCREASED TRANSCRIPTIONAL ACTIVITY.

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DNA methylation is an important epigenetic mechanism of regulating gene expression that is affected in certain human diseases including imprinting disorders and cancer. In mouse, UHRF1 is an essential cofactor of DNMT1, the enzyme responsible for maintaining methylation patterns. To investigate the effects of loss of UHRF1 on methylation patterns in human cells, *UHRF1* levels were decreased in immortalized hTERT fibroblast cell lines using short hairpin RNA. Genome-wide effects on methylation were investigated by the Illumina Infinium HumanMethylation450 BeadChip array. Online bioinformatics software tools were used to identify FDR-significant hypomethylated gene classes, which were then verified by pyrosequencing. Transcriptional effects on these gene classes were investigated by the genome-wide Illumina



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HumanHT-12 v4 Expression BeadChip array, and verified by RT-qPCR. While *UHRF1* depletion caused widespread demethylation, the replication-dependent histone gene cluster and the cancer testis antigen genes were identified as most significantly hypomethylated in *UHRF1* knockdown cells. Pyrosequencing confirmed hypomethylation in promoter regions of cancer testis antigen genes *TSPY2*, *MAGEC1*, *MAGEC2* and *MAGEA12*, and histone gene *HIST2H2AA4* in knockdown cell lines. Hypomethylation in these gene classes correlated with an increase in expression in the knockdown cell line. In addition, cells were rescued using *UHRF1* cDNA and showed a return to wild type transcription levels in the rescue cell line. We have shown that these genes are regulated by promoter DNA methylation, confirming the sensitivity of cancer-testis genes to demethylation, supporting possible use of methyltransferase inhibitors to boost antigen presentation in cancers, and the crucial role of UHRF1 in cell cycle regulation.

P12. AAV-MEDIATED GENE THERAPY IN A PATIENT-DERIVED FIBROBLAST MODEL OF RETINITIS PIGMENTOSA 2

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X-linked Retinitis Pigmentosa (XLRP) is a severe, early-onset form of inherited retinal degeneration (IRD). It is estimated that approximately 15% of XLRP cases are due to mutations in *RP2* (*Retinitis Pigmentosa 2*). The ubiquitously expressed RP2 protein is involved in ciliary trafficking of lipid-modified proteins – a process vital for photoreceptor function and survival. Most pathogenic *RP2* mutations are suggested to result in truncation or complete loss of the protein. The most common stop mutation, R120X, appears to trigger nonsense-mediated decay of the transcript. RP2 is therefore an excellent candidate for gene augmentation therapy.

In recent years, personalised cell models have emerged as invaluable tools for the elucidation of disease pathogenesis and have greatly enhanced pre-clinical proof of concept studies. Through the Target 5000 programme, a project focused on genetic characterisation of the 5,000 IRD patients in Ireland, a male patient harbouring the R120X *RP2* mutation was identified. A patient-derived dermal fibroblast cell model of the disease was thus generated and characterised. The transduction efficiencies of AAV vectors of various serotypes in fibroblasts were tested and compared, after which it was decided to proceed with an AAV2/2.CAG.RP2 vector to explore RP2 delivery in this patient-derived cell model. In addition, the effects of RP2 overexpression *in vivo* in murine photoreceptors and retinal pigment epithelium cells were analysed.

P13. DEVELOPMENT OF ASSAYS FOR EVALUATION OF MITOCHONDRIAL FUNCTION AND CANDIDATE THERAPIES

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Mitochondrial dysfunction leads to a lack of energy production and ultimately the death of the cell. Recently a number of disorders have been shown to have mitochondrial dysfunction including but not limited to; Multiple Sclerosis, Parkinson's and Leber's Hereditary

Optic Neuropathy (LHON). In LHON, Complex I of the Electron Transport Chain (ETC) is affected which leads to a severe shortage of energy in the cell and eventually cell death. In particular retinal ganglion cells (RGCs) are affected, leading to retinal dysfunction and blindness. These observations have prompted interest in exploring innovative therapeutics to modulate mitochondrial disorders involving complex I deficiency.

The team has explored candidate gene therapies for complex I deficiency, which could classically be delivered via Adeno Associated Viruses (AAV) such as AAV serotype 2 (AAV2), among other vectors. As such the team has developed novel *in vitro* methods for the analysis of complex I deficiency and the evaluation of novel candidate therapies, allowing us to monitor the efficacy of these therapeutics. Assays include a suite of methods to enable evaluation of Complex I activity and oxidative phosphorylation efficiency among other mitochondrial biomarkers. Such assays in principle would be of value for future *in vitro* and or *in vivo* studies involving therapies directed towards targeting complex I deficiencies.

P14. WIDESPREAD RECOVERY OF METHYLATION AT GAMETIC IMPRINTS IN HYPOMETHYLATED MOUSE STEM CELLS FOLLOWING RESCUE WITH DNMT3A2

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Background: Imprinted loci are paradigms of epigenetic regulation and are associated with a number of genetic disorders in human. A key characteristic of imprints is the presence of a gametic differentially methylated region (gDMR). Previous studies have indicated that DNA methylation lost from gDMRs could not be restored by DNMT1, or the de novo enzymes DNMT3A or 3B in stem cells, indicating that imprinted regions must instead undergo passage through the germline for reprogramming. However new putative gDMR have recently been described, along with an improved delineation of the existing gDMR locations. We therefore aimed to re-examine the dependence of methylation at gDMRs on the activities of the methyltransferases in mouse embryonic stem cells (ESCs).

Method: We examined the most complete current set of imprinted gDMRs that could be assessed using quantitative pyrosequencing assays in two types of ESCs: those lacking DNMT1 (1KO) and cells lacking a combination of DNMT3A and DNMT3B (3abKO).

Results: Loss of methylation was approximately equivalent in both cell types. 1KO cells rescued with a cDNA-expressing DNMT1 could not restore methylation at the imprinted gDMRs, confirming previous observations. However, nearly all gDMRs were remethylated in 3abKO cells rescued with a DNMT3A2 expression construct (3abKO + 3a2). Transcriptional activity at the *H19/Igf2* locus also tracked with the methylation pattern, confirming functional reprogramming in the latter.

Conclusions: DNMT3A/B plays a vital role in methylation maintenance at imprints as the rescue with DNMT3A2 can restore imprints in these cells. This provides a useful system to explore factors influencing imprint reprogramming.



P15. GENE-SET ANALYSIS OF GWAS DATA IDENTIFIES A ROLE FOR SATB2 AND THE NURD COMPLEX IN SCHIZOPHRENIA AND EDUCATIONAL ATTAINMENT

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SATB2, *BCL11B* and *GATAD2A* map to regions containing genome-wide significant SNPs for schizophrenia and regulate key stages of neurodevelopment via epigenetic mechanisms. *SATB2* mediates the projection of neurons across the cerebral hemispheres by regulating the activity of *BCL11B* via the NuRD nucleosome remodelling complex, which contains *GATAD2A*. We hypothesized that genes within the NuRD complex and genes regulated by *SATB2* in the pre- and post-natal brain may contribute to schizophrenia etiology. To test, we developed three gene-sets. 1.) Genes reported in mouse knockout studies of *SATB2* during cortical development (*SATB2_Cortical*). 2.) Genes mapping to *SATB2* ChIP-seq peaks generated from mouse cortices at E15.5 (*SATB2_Pre-natal*). 3.) Genes mapping to *SATB2* ChIP-seq peaks generated from mouse P0 hippocampal neurons (*SATB2_Post-natal*). We performed competitive gene set analysis (GSA) using MAGMA to test if genes within a gene-set were more strongly associated with schizophrenia than other genes in the genome. We applied GSA to schizophrenia GWAS (n=150,064). We also investigated these gene-sets for a genetic contribution to educational attainment (EA; proxy for cognition) using GWAS (n=405,072). After multiple test correction, we observed significant associations for (1) *SATB2_Cortical* with schizophrenia (P=8.65x10⁻⁰⁵) and EA (P=0.00049), (2) *SATB2_Pre-natal* with EA (P=0.0068) and (3) *SATB2_Post-natal* with schizophrenia (P=0.0069) and EA (P=2.03x10⁻⁰⁶). Further GSA established that effect sizes are stronger for these gene-sets when analysis is limited to genes that are highly expressed in neurons or at different key timepoints during neurodevelopment of the cortex or hippocampus. These data support a role for the NuRD complex and genes regulated by *SATB2* in schizophrenia and EA

P16. COMPARISON OF DNMT1 INHIBITORS BY METHYLOME PROFILING IDENTIFIES UNIQUE EPIGENETIC SIGNATURE OF DACOGEN

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Background: Dacogen (5-aza-2'-deoxycytidine) is currently used to treat Acute Myeloid Leukaemia (AML) and is in trials for myeloid dysplastic syndrome and some solid cancers. As a hypomethylating agent it is thought to act by inhibiting the enzymes which add methyl groups to DNA, chief among them DNMT1. Improved targeting has been hindered by a lack of understanding with respect to the exact mechanism of action on DNMT1 and of the gene targets affected by altered methylation following treatment.

Methods: We performed a comparative treatment of the same normosomic, non-transformed fibroblast cell line hTERT1604 over three days with either pharmacological 5-aza-2'-deoxycytidine (Dacogen) or with SMARTpool siRNA directly targeting DNMT1. DNA was collected for analysis of methylation levels using Illumina 450k BeadChip methylation arrays. Data was analysed in R using the tailored RnBeads pipeline and in-house scripts.

Results: Both Dacogen and DNMT1 siRNA caused overall hypomethylation in the treated cells, with the latter proving more efficient at demethylation at genes in particular. Amongst the targets experiencing demethylation, some hypomethylated promoters were unique to Dacogen treatment and therefore off-target with respect to the reduction in DNMT1. However an unexpected phenomenon almost exclusively caused by 5-Aza-2'-deoxycytidine treatment was gain in methylation. Therefore we also compared our findings to an independent published 450k dataset of Dacogen treated AML cells (KG1a). Our results suggest Dacogen is also having an important effect on methylation unrelated to the inhibition of DNMT1 thus suggesting further avenues for therapeutic improvements.

P17. COMMON AND RARE RISK VARIANTS MAP TO GENES WITH SIMILAR CHARACTERISTICS IN BOTH SCHIZOPHRENIA AND EDUCATIONAL ATTAINMENT

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Disruptive, damaging ultra-rare variants (dURVs) are more abundant in schizophrenia (SZ) patients than controls and are more concentrated in neuronally-expressed genes with synaptic functions. dURVs in highly constrained genes influence educational attainment (EA; a proxy for cognition) in the general population. We used MAGMA to perform gene set analysis of the largest available GWAS datasets to investigate if association signals for SZ and EA similarly mapped to highly constrained genes and to neuronally-expressed genes with synaptic functions. We investigated if SZ and EA associations were enriched in brain regions at different timepoints from early development through to adulthood. Highly constrained genes (probability of being loss-of-function intolerant; pLI>0.9; n=3,230) are strongly enriched for association with SZ (p=3.14E-08) and EA (p=1.27E-09) in comparison to genes under less constraint (0.1<pLI<0.9; n=4,621; p=0.40 for SZ, p=0.34 for EA) or weak constraint (pLI<0.1; n=10,374; p=0.99 for both SZ and EA). Neuron-specific genes are strongly enriched in SZ (p=3.24E-09) and EA (p=1.33E-08) in comparison to oligodendrocyte- or astrocyte-specific genes. For neuronally-expressed genes, there is strong enrichment in the potentially synaptic gene set (p=4.53E-09 for SZ and p=2.74E-09 for EA) but no enrichment in non-synaptic genes (p=0.24 for SZ, p=0.17 for EA). The strongest enrichment for SZ and EA is in genes that are highly expressed during trimester 2 and this was consistent across all brain regions. Common and rare risk variants are mapping to genes with similar characteristics in SZ and EA but how they combine to influence an individual's risk of SZ or their cognitive function remains to be elucidated.

P18. IDENTIFICATION OF GENETIC MARKERS ASSOCIATED WITH SEVERITY OF TISSUE DAMAGE IN RHEUMATOID ARTHRITIS: AN APPROACH FOR PERSONALIZED MEDICINE

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Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease affecting 45,000 people in Ireland. Prolonged joint inflammation results in tissue damage with consequent reduced functional capacity and quality of life. Damage to the joints of hands and feet, assessed by x-ray, is an important outcome measure that has



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genetic input of around 60%. Recent studies have identified single nucleotide polymorphisms (SNPs) in immune-related genes that are associated with severity of tissue damage in RA. One of our studies identified an association with C5orf30, a previously uncharacterized regulator of tissue damage and inflammation (1, 2). However a more comprehensive genome wide analysis is required to more fully characterize the genetic basis of RA severity. This project will identify genetic variants, and their synergistic combinations, that are associated with severity of RA. We will analyse genome-wide SNP data in 1,007 RA patients using state-of-the-art genetic epidemiology and computational techniques, including negative binomial modelling, to identify variants linked with joint damage severity. The study population is uniquely large and detailed clinical and genetic datasets will be used for validation studies using five European early RA cohorts. Simulations for statistical power indicate excellent power will be achieved for moderately frequent alleles, for effect sizes (IRR) over 1.4. The aim is to develop both a genetic prognostic score for RA, and to identify novel mediators of tissue destruction. The earlier identification of RA patients at risk of poorer outcome would facilitate patient stratification and inform therapeutic targeting with more aggressive regimes whilst avoiding such treatment in patients likely to have a better outcome

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2. Teare MD, et al. *Arthritis Rheum*. 2013;**65**(10):2555-61.

P19. A MIR-330 MEDIATED GENOMIC SHIFT IN THE ENDOTHELIUM, TRIGGERING ENDOTHELIAL DYSFUNCTION FOLLOWING S. AUREUS INFECTION OF THE BLOODSTREAM

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Bloodstream infection and sepsis are often instigated by the bacterium *Staphylococcus aureus*. Upon accessing the bloodstream, *S. aureus* binds to the endothelium triggering vascular leakage, inflammation and oedema. These characteristics are difficult to treat pharmacologically as the nature of signalling guiding this host response remains unclear. microRNAs (miRNAs) regulate ~60% of the human genome through post-transcriptional silencing/ degradation of target genes. Previously, bacteria were shown to profoundly affect miRNA expression via up-regulation of dendritic miR-99b elicited by *M. tuberculosis* infection.

This study investigates contributions of *S. aureus* induced endothelial miRNA dysregulation to sustained and excessive host responses in sepsis.

Sheared (10dynes/cm²) human endothelial cells were treated with plasma and TNF α to mimic sepsis conditions. Infection induced miRNA alterations were uncovered using Taqman cards to generate miRNA profiles of uninfected and infected cells (RQ = 2- $\Delta\Delta$ Ct). Potential mRNA targets were established bioinformatically and confirmed by RNAseq, western blots and qPCR.

Following infection, 58 endothelial miRNA were significantly down- and 35 significantly up-regulated, including miR-330 (p<0.05). Bioinformatic analysis of RNAseq data identified 102 potential miR-330 targets that were down-regulated following both infection and miR-330 overexpression (p<0.005). Of interest were genes required for endothelial barrier integrity, including ADAM19 and ZO-1. Both *S. aureus* infection (p<0.05) and transfecting a miR-330 mimetic

into uninfected cells caused increased permeability (p<0.005). Consistently, western blot analysis demonstrated down-regulated of these proteins following infection.

We propose that *S. aureus* infection leads to rapid dysregulation of endothelial miRNAs, contributing to degradation of the endothelial barrier potentially through down-regulation of junction proteins.

P20. DEPLETION OF DNMT1 IN DIFFERENTIATED HUMAN CELLS HIGHLIGHTS KEY CLASSES OF DEPENDENT GENES

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DNA methylation is a critical mechanism for regulating gene expression and ensuring genomic stability. However, loss of function mutations of methyltransferase enzymes such as DNMT1 in normal differentiated cells result in a lethal phenotype. Consequently, existing investigations have only assessed DNMT1 knockdowns in embryonic stem cells or cancer cell lines. Here, isogenic lines of hypomorphic, normal, immortalised fibroblasts have instead been generated via stable integration with short hairpin RNA. Enrichment analysis of epigenome-wide methylation arrays indicated widespread demethylation within promoter and gene body regions. In addition, four specific gene categories were highlighted as most affected; protocadherins, genes regulating body mass, olfactory receptors and cancer/testis antigens. Comparison of short-term siRNA and long-term shRNA-mediated depletion of DNMT1 indicated that many regions recover methylation as shRNA-containing cell lines adapt to lowered levels of DNMT1. Interestingly, polycomb-regulated genes are refractory to de novo DNA methylation in these cells following recovery, reinforcing the concept of mutually-exclusive domains that are regulated by these two major epigenetic mechanisms.

P21. EPIGENETIC EFFECTS OF RIBOFLAVIN SUPPLEMENTATION ON HYPERTENSION IN ADULTS SCREENED FOR THE MTHFR C677T POLYMORPHISM

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Background: The *MTHFR* C677T is a common polymorphism of the folate metabolising enzyme methylene tetrahydrofolate reductase (MTHFR) associated with hypertension. Riboflavin is a cofactor to MTHFR in the one-carbon cycle for generating methyl groups important for biological reactions such as DNA methylation. Supplementation with riboflavin has previously been shown to reduce blood pressure specifically in individuals with the homozygous *MTHFR* 677TT genotype. The mechanisms underlying the blood pressure lowering effect of riboflavin are currently unknown however aberrant DNA methylation has been implicated in the development of hypertension. The aims of this study were to



examine global DNA methylation on hypertension in adults stratified by MTHFR genotype and in response to intervention with 1.6mg/day of riboflavin in individuals with the MTHFR 677TT genotype.

Methods: Stored peripheral blood leukocyte samples from participants who had consented and participated in targeted RCTs at Ulster University's Nutrition Innovation Centre for food and Health (NICHE) and previously screened for the MTHFR C677T polymorphism were accessed for this study. Bisulphite conversion and pyrosequencing was used to analyse global and gene-specific DNA methylation.

Results: Preliminary results show that methylation at the repeat element, LINE-1, and imprinted gene, IGF2 was not significantly different between the MTHFR C677T genotypes at baseline. However, subsequent supplementation with riboflavin resulted in a decrease in global methylation and an increase in IGF2 methylation in MTHFR 677TT participants.

Conclusion: This is the largest study to date examining the interaction between the MTHFR C677T genotypes, riboflavin supplementation and DNA methylation. Riboflavin supplementation influenced repeat element and imprinted gene methylation in MTHFR 677TT genotype individuals. Further work will provide insights into the mechanism of riboflavin action in lowering blood pressure in these genetically at risk adults.

P22. MIR-199A-5P IS A MARKER OF BLOOD PRESSURE IN PREMATURE CARDIOVASCULAR DISEASE PATIENTS HOMOZYGOUS FOR THE MTHFR C677T POLYMORPHISM.

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Background: microRNAs are small, non-coding RNAs which are potentially valuable markers of cardiovascular disease (CVD) risk, including hypertension. This novel investigation aims to profile circulating serum concentrations of microRNAs in premature CVD patients to identify microRNAs that correlate best with hypertension.

Methods: Serum samples from an existing cohort of 75 premature CVD patients were analysed for expression of 68 CVD-related microRNAs. Patients had been screened for the methylenetetrahydrofolate reductase (MTHFR) gene polymorphism C677T, a risk factor for hypertension. Samples had been collected at baseline and following intervention with riboflavin, co-factor for the MTHFR enzyme, as part of a placebo-controlled double-blind, randomized trial. The associations between miRNA expression and blood pressure at baseline and post-intervention were investigated. Comparisons of data between CC and TT MTHFR genotype groups, and in response to intervention, were assessed using ANOVA,

Pearson's correlation and corrected t-test statistical analyses.

Results: microRNA expression was successfully detected and quantified in all samples. At baseline miR-199a-5p expression was inversely correlated ($r=-0.51$; $p<0.001$) with blood pressure in patients with the MTHFR TT genotype only. The decrease in blood pressure in those TT genotype patients who responded to riboflavin intervention was inversely correlated with miR-199a-5p expression ($r=-0.55$; $p<0.05$). In vitro and in silico analysis of miR-199a-5p function was also performed.

Conclusions: This is the first study to identify miR-199a-5p as a potential serum biomarker of blood pressure in a cohort of at-risk CVD patients. We propose that serum profiling of microRNAs could aid early prediction of CVD and may lead to improved treatment regimes

P23. INVESTIGATING THE LINK BETWEEN MIR-210 AND HYPOXIA IN PROSTATE CANCER

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Background: Hypoxia in prostate tumours has been associated with disease progression and metastasis. MicroRNAs are short non-coding RNA molecules which are important in several cell processes, but their role in hypoxic signalling is still poorly understood. miR-210 has been linked with hypoxic mechanisms, but this relationship has not been extensively studied in a prostate cancer setting. Therefore, in this study, we investigate the link between hypoxia and miR-210 in prostate cancer cells.

Methods: In this study we have used prostate cancer models of hypoxia to investigate the functionality of miR-210. Expression levels of miR-210 have been measured by qPCR in in vitro and in vivo samples. Functional bioassays were used to examine its effect on prostate cancer cell behaviour. Target genes have been identified and bioinformatic analysis has been employed to investigate a clinical significance for miR-210 in prostate cancer.

Results: miR-210 is induced by hypoxia in prostate cancer cells. Over-expression of miR-210 impacts upon target genes which in turn may affect cell proliferation. Data-mining of online repositories of clinical prostate sample data shows that miR-210 is significantly correlated with Gleason grade and other clinical markers of prostate cancer progression. Further in silico analysis of miR-210 cellular networks reveal that miR-210 plays a key role in a number of important cell processes, the dysregulation of which can promote the development of prostate cancer.

Conclusions: We propose that miR-210 is an important regulator of cell response to hypoxic stress and may play an important role in the pathogenesis of prostate cancer. Further study will focus on determining its function in prostate cancer and its potential as a biomarker in this disease.

