Poster abstract: Next generation sequencing to interrogate Vitamin D related genes in patients with new onset diabetes after transplantation (NODAT)

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Abstracts

17th Meeting of the Irish Society of Human Genetics, Friday 5th September 2014.

Trinity Centre for Health Sciences, St James's Hospital, Dublin.

PROGRAMME:

10.00 – 10.55	Registration / Tea and Coffee.
10.55 – 11.00	Welcome.
11.00 – 12.15	Oral Presentations. Plenary I: clinical research.
12.15 – 13.15	**Keynote address:** *"Marfan syndrome and related disorders: from gene to therapy"* Prof. Bart Loeys, University of Ghent, Belgium.
13.15 – 14.15	Lunch (Provided) and Poster viewing.
14.00 – 14.15	Council Meeting
14.15 – 15.30	Oral presentations. Plenary II: Basic research.
15.30 – 16.00	Tea and coffee / Poster viewing.
16.00 – 16.15	ISHG AGM.
16.15 – 17.15	**Keynote address**: *"Clinical Genomics: Merging Human Genetics and Genomics"* Prof. James R. Lupski, Neurogenetics, Baylor College of Medicine, USA
17.15 – 18.00	Wine reception / Presentation of Prizes / Meeting close.

SPOKEN PAPERS:

S01. Malformation risks of antiepileptic drug monotherapies in pregnancy

PJ Morrison[1], E Campbell[1], F Kennedy[1], A Russell[1], WH Smithson[3], L Parsons[3], B Liggan[2], B Irwin[1], N Delanty[2], SJ Hunt[1], J Craig[1], J Morrow[1]

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Antiepileptic drug (AED) exposure during pregnancy increases the risk of major congenital malformations (MCMs). The risk magnitude varies by AED exposure. We provide results from the UK and Ireland Epilepsy and Pregnancy Registers, of the risk of MCMs after monotherapy exposure to valproate, carbamazepine and lamotrigine.

Fifteen-year prospective observational study (1996 – 2012). The main outcome measure is the MCM rate. Informative outcomes were available for 5206 cases. 1290 women were exposed to valproate monotherapy, 1718 to carbamazepine monotherapy and 2198 to lamotrigine monotherapy. The MCM risk with valproate monotherapy exposure in utero was 6.7% (95% CI 5.5% to 8.3%) compared with 2.6% with carbamazepine (95% CI 1.9% to 3.5%) and 2.3% with lamotrigine (95% CI 1.8% to 3.1%). A significant dose effect was seen with valproate (p=0.0006) and carbamazepine (p=0.03) exposed pregnancies. A non-significant trend towards higher MCM rate with increasing dose was found with lamotrigine. MCM rate for high-dose lamotrigine (>400 mg daily) was lower than the MCM rate for pregnancies exposed to <600 mg daily of valproate, but this was not significant (3.4% vs. 5.0%, p=0.31).

In-utero exposure to valproate carries a significantly higher MCM risk than lamotrigine (p=0.0001) and carbamazepine (p=0.0001) monotherapy. High-dose lamotrigine was associated with fewer MCMs than all doses of valproate. While lamotrigine has a favourable profile compared with valproate for adverse pregnancy outcomes, the requirements for seizure control should not be overlooked. These risks should help in the genetic counselling of mothers with epilepsy considering a pregnancy.

S02. Pre-Implantation Genetic Diagnosis (PGD) in Ireland – from validation to introduction of a clinical service

T Dineen[1], X Zhang[1], J Flanagan[1], A Kovacs[1], R Mihart[1], J O'Callaghan[1], J Culligan[1], N Daly[1], J Waterstone[1]

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Pre-implantation Genetic Diagnosis PGD was first described in 1990. Until recently Irish couples opting for PGD had to travel abroad for treatment. With the first reported clinical pregnancy following embryo biopsy in an Irish fertility centre, pursuing IVF treatment with PGD in Ireland is now a reality. In 2012, the Irish Medicines Board (IMB) licensed two Irish fertility clinics to carry out embryo biopsy for PGD. To validate the PGD process, Cork Fertility Centre (CFC) biopsied non-viable oocytes and embryos and transferred the biopsied material to Reprogenetics, UK for genetic diagnosis. The gene amplification rate and the contamination rate were recorded to assess the biopsy and sampling procedures. Embryo integrity following biopsy, together with the proportion of biopsy cells remaining intact after removal were used to evaluate embryo biopsy skill. The results of all validation measures were evaluated according to internationally recognised guidelines. Having been licensed for embryo biopsy, the first clinical case of PGD at CFC involved a couple where the male was affected by Cystic Fibrosis (CF) and the female was a CF carrier. A number of Assisted Reproductive Technology (ART) techniques were involved for this couple, including testicular biopsy, IntraCytoplasmic Sperm Injection (ICSI), embryo biopsy and embryo vitrification. The couple are currently 33 weeks pregnant. Increasing awareness of genetic risk is inevitable and where Irish couples are burdened with difficult reproductive choices, the availability of PGD in Ireland is a welcome development.

S03. Cerebral Cavernous Malformations: four families illustrating phenotypic diversity with implications for counselling and management.

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Cavernous cerebral malformations (CCMs) are characterised by abnormally large and leaky capillaries arranged in mulberry-like structures with no clear flow pattern, occurring in 0.5% of the population. CCMs can remain neurologically silent, or predispose to seizures, neurological deficits and haemorrhage. Three CCM loci have been identified; the phenotype includes cutaneous vascular malformations (CVM). We present 5 families: 4 with mutations in KRIT1 (CCM1 locus) and 1 in MGC4607 (CCM2 locus). Including the probands, 16 gene carriers were identified. All carriers had either CCM (13), CVM (12) or both (9). Four had only CCMs, and another 3 only CVMs. Seven are asymptomatic (age range 3y-71y). All 7 have CVMs, 4 have both CCMs and CVMs. Nine gene carriers are symptomatic. Five presented with cerebral haemorrhage (age 6m-66years, 2 with spinal symptoms (age 33 and 45), and 2 with seizures (age 2and 43). One obligate gene carrier, asymptomatic at age 71,declined investigation. This series supports previous reports suggesting that around 50% of gene carriers remain symptom free. It also illustrates an extremely wide range of age at onset and phenotypic expression. These findings raise many issues for families, in particular the genetic testing of children who may be at risk. Surgical intervention for asymptomatic CCMs is rarely indicated. There is no agreed screening protocol for carriers. However, future therapeutic strategies including antioxidant compounds, statins, and antiangiogenesis agents will provide families and individuals with possible therapeutic options.

S04. A retrospective review of Hereditary Paraganglioma/ Phaeochromocytoma referrals to the Northern Ireland Regional Genetics Service

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Hereditary Paraganglioma (PGL)/ Phaeochromocytoma (PCC) Syndrome (HPPS), an autosomal dominant inherited condition falls within the differential diagnosis for paragangliomas and phaeochromocytomas which are currently estimated to have a genetic predisposition in up to one third of cases. There is no universally agreed consensus for diagnostic investigation of these individuals which has been compounded with the advent of multi-gene testing panels. A retrospective review of clinical details including age, gender, tumour site(s), malignancy, family history, gene mutation frequencies and characteristics and referral source were obtained from chart reviews of referrals for HPPS. Patients with three cancer predisposition syndromes associated with the development of Paraganglioma/ Phaeochromocytoma – Von Hippel-Lindau, multiple endocrine neoplasia type 2 (MEN2) and neurofibromatosis type 1 (NF1) were excluded. Endocrinologists and vascular surgeons had the highest referral rates. The majority of patients presented with carotid body tumours or phaeochromocytomas with an age range of 8-81 years for first tumour diagnosis. 74 individuals from 20 pedigrees tested positive for a familial SDH gene mutation of which 30 had an SDHD mutation and 44 an SDHB mutation. Two possible founder SDH gene mutations and 12 tumours were identified on screening. The importance of identifying the genetic aetiology of PGL/PCC which facilitates identification of gene mutation carriers and appropriate genetic counselling and screening recommendations is supported by this review. Education of health professionals and the use of multi-gene testing panels may result in a higher mutation detection rate, more complete genetic counselling and improved understanding of clinical phenotypes of these predisposition syndromes.

S05. Identification of MLL4-GPS2 Fusion as an oncogenic driver of Undifferentiated Sarcoma in a Child

D Stack[1], E O'Meara[1], S Phelan[1], N McDonagh[1], L Kelly[1], R Sciot[2], M Debiec-Rychter[3], T Morris[4], D Cochrane[5], P Sorensen[6] & MJ O'Sullivan[1,7]

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Undifferentiated sarcoma is a poorly understood, therapy-resistant cancer, lacking both diagnostic and prognostic markers. Our index case was a spindle cell undifferentiated sarcoma, which arose as an intracranial, extra-axial mass in a 12-year old girl. Karyotypic analysis revealed a balanced, non-constitutional chromosomal translocation t(17;19)(p13;q13) as the sole aberration. By FISH analysis using increasingly proximate BAC pairs, followed by paired fosmid probes, we narrowed the chromosomal breakpoint regions to loci containing the MLL4 and GPS2 genes. With 3'RACE, RT-PCR and Sanger sequencing, we confirmed an in-frame fusion of MLL4 and GPS2 containing the first 2 and partial 3rd exons of MLL4 fused to GPS2 partial 5'UTR and entire coding sequence. MLL4 belongs to the MLL family, with MLL1 frequently rearranged in infant leukaemia. GPS2 is a potent suppressor of Ras-Mapk signalling. The fusion sequence and wild-type GPS2 were PCR-amplified and inserted into vectors phrGFPIIN and pcDNA3HA. These were then transiently and stably transfected into HEK293 and NIH3T3 cells, facilitating study of the cell biologic dysregulation resulting from expression of the transcript. Transient transfection of the MLL4-GPS2 fusion in HEK293 and NIH3T3 cells caused re-localisation of the fusion protein to the nucleus, whereas wild-type GPS2 is predominantly cytoplasmic. Importantly, stable transfection of the MLL4-GPS2 fusion produced significantly greater anchorage-independent growth compared to controls, as assessed by colony formation in soft agar assays. This study has identified a novel t(17;19)(p13;q13) translocation in undifferentiated sarcoma generating the fusion gene MLL4-GPS2, the expression of which promotes anchorage-independent growth, a classic in-vitro feature of malignant behaviour.

S06. Incidence of fragile X syndrome in Ireland an all-Ireland study

JJ O'Byrne[1], M Sweeney[1], D Donnelly[2], D Lambert[1,3], D Beattie[2], C Gervin[2], CA Graham[2], DE Barton[1], SA Lynch[1]

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Although Fragile X syndrome (FXS) is one of the most common causes of inherited developmental delay/intellectual disability with an estimated of 1:4000 males and 1:8000 females worldwide, the observed incidence in Ireland is unknown. Ireland has a unique situation with two health systems testing for FXS from the same population base which allows interesting comparisons to be drawn between incidences in the Republic of Ireland (ROI) and Northern Ireland (NI). To determine the observed incidence of FXS in the ROI and NI separately and combined. To compare these observed incidences to estimated worldwide incidences of FXS. A retrospective clinical and lab database review of positive fragile X

cases, born between years 2000-2009 inclusive, in both ROI and in NI was carried out.

Inclusion criteria: i) Birth place: ROI/NI, ii) Birth year: 2000-2009, iii) FXS confirmed on clinical examination iv) Full mutation allele (>200 CGG repeats) v) molecular test performed 2000-2014. Note: patients with intermediate or premutation alleles were excluded. The observed incidence of FXS in all Ireland is approximately 2.5 - 3 times less than the estimated worldwide incidence. Our study, along with other recent published studies, suggests that either the world wide incidence is over estimated or there is large frequency variability between different populations. As both ROI and NI cohorts had similar incidences we are confident that these figures are due to population incidence rather than poor ascertainment, inadequate testing or inappropriate referrals.

S07. Investigating polygenic contributions of common hippocampal variants to epilepsy predisposition

CD Whelan[1], DP Hibar[2], JL Stein[2], D Speed[3], The ILAE Consortium on Genetics of Complex Epilepsies, S Sisodiya[4], M Ohnson[5], D Goldstein[6], N Delanty[1,7], SE Medland[8], B Ranke[9], PM Thompson[2], G Cavalleri[1]

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Hippocampal sclerosis (HS) is a common feature of localisation-related epilepsies (LREs), present in 50-75% of all surgical resections in the disorder. However, the underlying cause of HS is debated. Animal models and post-mortem cell counts suggest that HS can result from recurrent epileptogenesis, but some MRI investigations have highlighted a familial component to HS and concomitant neuronal loss within hippocampal regions. Recently, the Enhancing Neuro Imaging Genetics through Meta-Analysis (ENIGMA) consortium identified genome-wide significant signals correlating with hippocampal volume in a study of 29,037 individuals. We tested the hypothesis that variants predisposing en masse to changes in hippocampal volume may, in turn, contribute to epilepsy predisposition. To test this, we summarised variation across nominally-associated ENIGMA SNPs into quantitative 'risk' scores, weighted for local linkage disequilibrium and effect size, and related these scores to disease state in (i) a phenotypically mixed sample of epilepsy patients (n=2,502), (ii) four epilepsy 'subtypes', including LREs (n=1,801), lesional epilepsies (n=280), non lesional epilepsies (n=614) and idiopathic generalised epilepsies (IGEs; n=194) and (iii) an independent sample of healthy controls (n=5,191). Results did not reveal a significant association between disease state and risk score: observed scores only explained a small fraction (0-0.2%) of total variance in our risk model. Our findings suggest that being genetically predisposed to having smaller hippocampal volume may not be a risk factor for epilepsy. However, further analyses in additional epilepsy patients and healthy control cohorts are required to confirm or reject this position.

S08. Contribution of common polygenic variation captured by the Immunochip to coeliac disease heritability in an independent Irish population

C Coleman[1], EM Quinn[1], AW Ryan[1], RJL Anney[2], V Trimble[1], DW Morris[2], G Donohoe[2], J Conroy[3], G Trynka[4], C Wijmenga[5], S Ennis[3], R McManus[1]

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Coeliac disease (CD) is a chronic immune-mediated disease with a prevalence of ~1% in European populations. Following the large Immunochip study of Trynka *et al* (2011) the HLA and 39 other CD susceptiblity loci are known. In an independent Irish CD case-control study we examined whether reported risk alleles were similar in direction of effect and whether a weighted burden of risk alleles (polygenic risk score) could be used to distinguish case status.

Following stringent quality control we analysed 143,074 markers genotyped on Immunochip in 425 cases and 453 controls. To examine concordance in the observed direction of effect in our sample, we performed a binomial sign-test for LD independent markers identified as genome-wide significant by Trynka *et al*. Secondly, for LD independent markers we calculated the polygenic risk score for each individual in our study. Regression was performed for disease status adjusting for marker-count-per-score (missingness), gender and population covariates. Binomial sign test indicated there was significant concordance in direction of effect between studies. 83% (122/147) of genome-wide significant SNPs show effect in the same direction (Pr (K>=122) = 7.9×10^{-17}). When restricted to non-HLA markers 10/11 (91%) show effect in the same direction (Pr (K>=10) = 0.0059). The polygene analysis showed that polygenic risk scores were significantly associated with coeliac case-control status across a range of p threshold values. Including the HLA markers up to 36% of the variance was explained by the polygenic score (SNPs P< 0.0001; P = 7.86×10^{-67}). We have replicated the findings of a large CD association study in an independent Irish population. Polygenic scores explained a significant proportion of the variance in coeliac disease confirming the contribution of common SNPs to CD susceptibility.

S09. AncestryMapper: Displaying and Calculating Genetic Distance Between and Within Populations At a Large Scale

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In recent years next generation sequencing has made it feasible to analyse individuals and populations genetic relatedness on a whole genome basis, advancing on the work previously done with Y chromosome and mitochondrial analysis. This has made it possible to trace ancestral human migrations and admixture events. It has also allowed for investigation into genetic diseases and predispositions specific or acute amongst specific populations. These methods are also useful for analysing internal population stratification of particular disease states. Traditional methods of illustrating this have relied on 2 dimensional methods. In the case of PCA and Admixture analysis, being context-sensitive to the input data. PCA analysis in particular is undermined by the limitations of plotting only two or sometimes three components at once. Ancestry Mapper provides a suite of software to plot multiple principal components at once, as well as display Admixture results in a more accurate and graded manner. It also can calculate genetic distance directly between populations that gives each individual an index related to a set of comparison populations. Our method produces numbers that are biological meaningful and easy to interpret. Ancestry Mapper also displays Admixture results in a more accurate and graded manner. Our initial dataset used the HGDP and HapMap data as a reference comprising 56 populations. We have now expanded

our method to over 170 human populations which provides better coverage and detail. Here we present the method applied to several admixed populations and demonstrated that the results concur with previous investigations and anthropological assumptions.

S10. The Genetic Ancestry of the Sherpa

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The Sherpa are an indigenous ethnic group of the Himalayas that display a remarkable ability to function at high altitude. It is thought that the Sherpa have migrated from Eastern Tibet to the Solu-Khumbu region of Nepal approximately 500 years ago.

We set out to shed further light on the history of the Sherpa and other indigenous high altitude populations through genetics. We aim to determine the genetic ancestry of the Nepalese-Sherpa in the context of their neighbouring populations that represent the ancestral genepool of indigenous Himalayan populations. We had access to Genome-wide genotyping data on 189 samples of Tibetan and Nepalese-Sherpa ancestry. We complemented this dataset with further additional data from the HGDP for Pakistan and Chinese populations, and the 1000 Genomes for Indian populations. We are performing a number of analytical tests to quantify and visualise population structure including Fst and Principal Component Analysis (PCA) as well as maximum likelihood tests of individual ancestries (using ADMIXTURE). PCA identified population substructure between two Sherpa villages in Nepal, namely Khumjung and Thame. Using ADMIXTURE we identified the ancestral 'high altitude component in the Nepalese-Sherpa, this was also present in Tibetans, believed to be admixed descendants of the Sherpa. Information about the nature and distribution of genetic variation in indigenous populations of the Himalayas can illustrate gene flow that might account for adaptive genetic and physiological traits of high altitude natives.

S11. In the era of the exome, how deep should we dig?

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Our research aims to identify the genetic cause of recessive disorders in the consanguineous Irish Traveller population using exome sequencing. Through our studies, we have encountered two important issues with implications for both researchers and clinicians; (a) clinical variability and (b) incidental findings. We have noted inter- and intra-familial variability in patients with the same disease mutation. This may be due to simple clinical heterogeneity whereby the same mutation can give rise to slightly different phenotypes. However, in consanguineous families, there is the possibility of more than one recessive disorder, distorting the phenotype. When faced with phenotypic variability, we found it useful to analyse each patient exome individually and have identified patients with up to three recessive disorders using this approach. But how extensively do we need to analyse genomic data in search of a potential secondary disorder?

Recent studies have reported a rate of incidental findings of 1-5% when the entire exome is analysed. The risk of incidental findings is minimised in our studies by limiting the search for causative variants to linkage regions identified in a mapping study. However, despite this approach, we have made incidental findings in two families. In family A, we identified a previously reported homozygous variant causative of glycogen storage disease type 5. In family B, we identified a novel homozygous variant in a gene associated with catecholaminergic polymorphic ventricular tachycardia. Does the benefit of these incidental findings outweigh any potential harm? Our findings support the need for specific thought processes and customised population-tailored approaches to exome sequencing in families from consanguineous populations.

S12. Methylation in Chronic Kidney Disease and its association with microRNAs: Analysis via Microarray, Sanger and Next Generation Sequencing

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This study was designed to identify differential methylation affecting microRNAs (miRNAs) in patients with chronic kidney disease (CKD). Comparative analyses between traditional Sanger and next generation sequencing techniques were then conducted. DNA samples from 255 patients with CKD (cases) were compared to 152 individuals without renal disease (controls) for differences in quantitative DNA methylation levels. Following stringent quality control procedures and correction for multiple testing, data was analysed from the HumanMethylation450K BeadChip array (Illumina) which assessed 485,577 sites. Extracting miRNA genes revealed 2,249 methylation sites within miRNAs, and five miRNAs associated with CKD (MIR940, MIR34A, MIR429, MIR141, and MIR329-2) were prioritised for follow-up.These results were supportedby replication analysis of an independent cohort of CKD cases and controls (n=400) using the 450K platform. Bidirectional Sanger sequencing was completed using carefully designed fragments for both genomic and bisulphite treated DNA flanking each miRNA. This approach determined 36 methylated CpG sites and 13 known SNPs, in the 23 cases and 23 controls analysed, but did not provide validation of methylation levels. Next generation sequencing, using the Ion Chef, OneTouch2, and Torrent® Personal Genome Machine™ (Life Technologies®) was employed for fine-mapping of SNPs and methylated sites in the target region, providing qualitative validation of 450K results. Forty-six individuals with both blood-derived and cell-line derived DNA were resequenced for approximately 500bp surrounding each miRNA. We have developed an effective technical protocol for validation and replication of EWAS studies, additionally confirming blood and cell-line may be useful sources of derived DNA for epigenetic studies.

POSTER PRESENTATIONS

P01. Neurofibromatosis type 2 – Review of patients in Northern Ireland & Patient Satisfaction with Multidisciplinary Clinic

DE Donnelly, L Jeffers, PJ Morrison, S Hampton, N Baillie, S Cooke.

Belfast Health & Social Care Trust

There are 18 families with Neurofibromatosis type 2 currently

being followed up in Northern Ireland. These patients attend a variety of specialists including Genetics, ENT, Neurosurgery, and hearing support workers, but have not always attended well. We have found that 40% of our patients have mental health issues, including depression, anxiety and anorexia, which has negatively impacted on their medical care. In order to address this we began a multidisciplinary team clinic in Sept 2012. We have recently completed an audit of patient satisfaction with this clinic and now present the results.

P02. An infant with a novel Kir6.2 mutation causing neonatal diabetes and unexplained lack of response to sulphonylurea

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Neonatal diabetes (NDM) is defined as diabetes developing before 6 months of age, affecting 1 in 100,000 live births. Permanent NDM is diagnosed in the first six months of life with no remission. The majority have a mutation in the ATP-sensitive potassium (K_{ATP}) channel, the majority of whom respond to sulphonylureas. We describe the response to sulphonylurea in an infant with NDM, heterozygous for a novel Kir6.2 subunit KCNJ11 missense de novo mutation (W68G). A female born at 37 weeks by Caesarean section for intrauterine growth retardation (birth weight 1.95kg <0.4th centile) was hyperglycaemic from day one of life. Initially stabilised with intravenous insulin she was treated with subcutaneous basal insulin with erratic glucose control. Glibenclamide was commenced slowly (0.05mg/kg/day) from day 20 of life up to a maximum dose of 1mg/kg/day over two months according to the Exeter transfer protocol. At age 2 months insulin pump therapy was commenced resulting in tighter glycaemic control and weight gain. Transfer off insulin was unsuccessful. In vitro testing of the mutant channels indicates she should respond to glibenclamide. Low levels of glibenclamide on pharmacokinetic studies suggest this patient may be excreting more rapidly than normal. This infant with a novel Kir6.2 mutation failed to respond to glibenclamide despite a sustained period on a recognised effective dose and clear in vitro response. As an unusually rapid rate of sulphonylurea metabolism is suggested by ongoing pharmacokinetic studies, a higher dose of glibenclamide has been commenced.

P03. The population incidence of childhood Gonadoblastoma over fifteen years in the Republic of Ireland

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Gonadoblastoma is a rare tumour of the gonads presenting in childhood or adolescence. It is associated with disorders of sex development (DSD), most commonly Turner mosaic syndrome with Y chromosome material (TMSY), and 46XY gonadal dysgenesis (GD). Little is known about the incidence of this rare tumour. A retrospective review of children and adolescents with a diagnosis of gonadoblastoma presenting before age 16 years in the Irish Republic (RoI) from 1999-2013 inclusive was undertaken using the records of the National Cancer Registry Ireland (NCRI) and the Departments of Endocrinology, Pathology and Surgery at the main children's hospitals. Clinical notes and histopathological findings were reviewed. Eight cases of gonadoblastoma were identified over the period. All were phenotypically female. Five cases had TMSY (age range gonadoblastoma diagnosis 6 months – 14 years), bilateral in two cases. Three cases of 46 XY GD were aged 4 months, 8 and 9 years at diagnosis of gonadoblastoma (unilateral). In one case of 46 XY GD with SRY deletion, clinical symptoms (age 8) prompted gonadectomy. Histology showed unilateral dysgerminoma and contralateral gonadoblastoma. In all other cases gonadoblastoma was diagnosed on elective gonadectomy. The incidence of gonadoblastoma in RoI over the past 15 years is 8, giving a population incidence of 0.08 per 10,000 births. To our knowledge this is the first population incidence rate of GB in children reported. Due to the low age of gonadoblastoma cases observed in this cases series, the recommendation for elective gonadectomy in high risk conditions is supported.

P04. Early Occurrence of Gonadoblastoma found at Elective Gonadectomy in Turner Syndrome mosaic for Y Chromosome

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Turner Syndrome (TS)is a common genetic disorder and occurs in phenotypic females who are missing all or part of one sex chromosome. The most common mosaic forms are 45X/46XXand 45X/46Xiq, however mosaicism for cells containing Y chromosome material (TSMY) is well documented. This is associated with increased risk of gonadoblastoma (GB) and elective gonadectomy is recommended following diagnosis. A review of TS patients attending the Paediatric Endocrinology clinic (n=9) identified three cases with TSMY. All underwent elective gonadectomy. Case 1 was diagnosed at 2 years. Peripheral blood karyotype showed mosaicism for 45X (25 cells) and an isodicentric Y chromosome made of Yp and proximal Yq material (25 cells). Gonadectomy at 6 years revealed extensive unilateral GB. Interphase FISH of the tissue showed isodicentric Y chromosome in 43% of GB cells. Case 2 presented with dysmorphic features at birth. G banded karyotype and interphase FISH of blood showed 45X in 95% and 47XY+18 (Edwards syndrome) in 5% of cells analysed. Interphase FISH of buccal cells showed 45X only. Gonadectomy at 13 months revealed bilateral GB, interphase FISH was similar to blood: 45X(86%), 47XY,+18(14%). Case 3 presented with severe neonatal aortic stenosis. Peripheral blood karyotype showed 45X (29 cells) and a pseudoisocentric Y chromosome with breakpoint at Yq11.23 (6 cells), confirmed on buccal and skin karyotyping. Gonadectomy revealed unilateral GB, karyotype pending. This case series highlights early age of occurrence of GB despite low mosaicism for SRY cell lines and supports a recommendation for early surgery in such cases.

P05. A Child with Clinical and Cytogenetic Features of Male Edwards Syndrome and Turner Syndrome with Bilateral Gonadoblastoma in Infancy

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Mosaic Turner Syndrome (TSM) commonly occurs in the form of 45X/46XX and 45X/46Xiq, although mosaicism including the presence of a Y chromosome is well documented. It is associated

with increased risk of gonadoblastoma (GB). To date, there are only 6 reported cases of TSM with a trisomy 18 karyotype (Edwards Syndrome), of which only 2 were phenotypically female with 45X, 47XY+18 karyotype. We present the case of an infant born with dysmorphic features (webbed neck, low set ears and broad chest). G banded karyotype and interphase FISH of blood showed 45X in 95% and 47XY+18 in 5% of cells analysed. However, interphase FISH of buccal cells showed only 45X. Antenatal ultrasound at 13 weeks gestation showed increased nuchal fluid, suggestive of Edwards Syndrome, but had resolved on follow-up scan at 15 weeks. Due to presence of SRY, an elective gonadectomy was performed at 13 months, which showed bilateral streak ovaries with early evidence of GB bilaterally, rudimentary uterus and bilateral fallopian tubes with unilateral ectopic adrenal tissue. Interphase FISH of the gonadal tissue was similar to the blood findings with 45X in 86% of cells and 47XY+18 in 14% of cells analysed. This case highlights a rare karyotype of TSM and Edwards Syndrome in the same patient. Current investigations are ongoing into the possible causes for this unusual finding. This case was also associated with a finding of bilateral gonadoblastoma. To the authors' knowledge this is the only reported case with the above karyotype and finding of gonadoblastoma.

P06. One SHOX after another: Two interesting patients with vice versa derivative X chromosomes identified by array comparative genomic hybridisation (aCGH)

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Two independent patients with derivative X chromosomes were identified using aCGH. Case 1: A 16 year old female was referred with suspected Marfan syndrome due to a history of tall stature, mild scoliosis and hyperflexibility. Development and intellectual ability were normal. She had a past history of anxiety and shyness and was noted to have very long legs. Karyotype was requested for suspected Triple X syndrome. However, G-banded analysis identified additional material of unknown origin on the long arm of one X chromosome in all cells. Further investigation by FISH and aCGH identified this derivative X chromosome to be der(X)(pter→q28::p22.12→pter), confirming this patient has functional trisomy of PAR1 (pseudoautosomal region), including SHOX, and functional monosomy of PAR2. Parental studies suggested that this rearrangement arose de novo. Case 2: A 32 year old male referred with primary infertility, short stature and mild to moderate learning disabilities. The proband's brother has epilepsy and severe learning disabilities. Both the proband's brother and mother are of short stature. Array CGH and FISH investigations identified an X chromosome rearrangement: der(X)(qter→p22.33::q28→qter), confirming this patient has functional monosomy of PAR1, including SHOX, and functional trisomy of PAR2. Inheritance studies identified this derivative X chromosome in both the proband's mother and brother

Genetic mechanisms for these two cases with conversely rearranged X chromosomes are suggested and genotypic and phenotypic features are explored.

P07. Watch out – mosaicism about! - Prenatal diagnosis of mosaic partial duplication of 3q

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Array-Comparative Genomic Hybridisation (a-CGH) is becoming the first line investigation for prenatal samples where an underlying genetic pathology is suspected. Here we describe an instructive case where older techniques would have missed an important genetic abnormality. A genetic opinion was sought on a 14 week old female infant admitted with apnoeic episodes secondary to gastroesophageal reflux. She was the second child of non-consanguineous parents. She had previously presented elsewhere for routine second trimester anomaly scan and was found to be small for gestational age with short (<3rd centile) long bones. Third trimester scanning found bilateral renal hydronephrosis and an oedematous extra postaxial left digit. She was born at 38 weeks in good condition.

On examination at 14 weeks, length and weight were <0.4th centile and head circumference was between the 0.4th – 2nd centile. Dysmorphic features included prominent forehead, hypertelorism, long bushy eyebrows, a broad nasal bridge, long fingers with bilateral clinodactyly and short limbs. Comparison with reported cases is presented. An aCGH performed on amniotic fluid indicated mosaicism for partial duplication of the long arm of chromosome 3q (93,630,075-197,766,791) which was confirmed by MLPA analysis. Karyotype analysis of cultured cells from the same sample did not detect any abnormalities. Notably, aCGH on a postnatal blood sample did not reveal any areas of copy number variation. This case illustrates the superiority of new molecular cytogenetic technologies being applied to prenatal diagnosis over more traditional approaches, particularly with reference to the diagnosis of mosaicism.

P08. BRCA1/2 testing in individuals who do not meet the Manchester Score

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With demand for BRCA tests increasing genetic departments need to ensure testing is offered to appropriate patients to avoid blockages and delays in their systems. While a significant family history of breast and/or ovarian cancer and certain pathologies may warrant BRCA testing there are other risk factors for genetic cancers that lead to referrals and requests for testing. We carried out an audit to ascertain prevalence of pathological BRCA1/2 mutations in particular groups of affected individuals without significant family history of relevant cancers. An audit of three groups of patients who do not meet the Manchester score (>10/10 or combined score >15) was carried out; 1. Women diagnosed with breast cancer under the age of 40 years without a family history, 2. Women with bilateral breast cancer without a family history, 3. male breast cancer without a family history. 294 chart searches yielded 10 individuals who met audit criteria: 4 women diagnosed under age of 40 years, 2 women diagnosed with bilateral breast cancer, 4 male breast cancers. No pathogenic mutations were identified in BRCA1/2 in any of the 3 groups. Prevalence of pathogenic mutations is too low in specific groups not meeting Manchester score to offer high cost testing on NHS. Although our numbers are small, Manchester score of >10/10 or combined score >15 seems to be a good criterion to offer BRCA testing to breast cancer families for better yield and resources utilization.

P09. TP53 mutation analysis in breast cancer: What is the evidence?

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Hereditary breast cancers account for around 5-10% cases and are predominantly due to BRCA1/2 genes and less commonly due to other high penetrant (TP53, STK11,PTEN) and less penetrant

(CHEK2, ATM, PALB2, BRIP1) genes, Premenopausal breast cancer is part of Li-Fraumeni syndrome (LFS) related cancer spectrum. TP53 mutation is found in 0-8% young breast cancers (<30 years) without family history of LFS while BRCA1/2 mutations are found in 8-12% unselected young breast cancer cases. Unlike TP53 gene testing BRCA1/2 mutation analysis is not routinely offered in young breast cancer cases alone unless the testing criterion is met (Manchester score, triple negative receptor status etc.) at our centre. We analysed our data of past six years (2008-2013) of TP53 gene testing in women with breast cancer. Information was obtained regarding family history of cancers, age of onset, receptor pathology and BRCA1/2 testing. No TP53 mutation was found in our young breast cancer alone cohort (0%). The solitary mutation was found in a woman with family history suggestive of LFS (10 %). TP53 gene mutation detection in breast cancer is likely to be better when supported by family history of LFS tumours and should be considered along with receptor pathology for better resources utilization. Our observation is similar to previously published studies and support revised Chompret criteria for better TP53 mutation detection rate. NGS panel testing for breast cancer genes would be cost effective and useful for understanding the genetic epidemiology of breast cancer genes.

P10. Epidemiology, clinical features and genetics of Multiple Endocrine Neoplasia type 2B (MEN 2B) in a complete population

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Multiple endocrine neoplasia (MEN2B) or the mucosal neuroma syndrome is an autosomal dominant hamartoneoplastic syndrome. Features include multiple mucosal neuromas, phaeochromocytoma, medullary thyroid carcinoma, and Marfanoid body habitus. MEN2B is thought to be relatively rare, but the prevalence has not previously been reported.

Objective: To assess the prevalence of MEN2B in a complete population, and delineate the clinical features. Methods: Prospective study of all cases of MEN2B since 1988. Results: The prevalence of MEN2B ranges from 0.178 – 0.219x10^{-5}. Conclusions: The minimum prevalence of MEN 2B in a complete population (Northern Ireland) is low. MEN2B is a rare disorder.

P11. Array CGH demonstrates multiple loss events in a de novo t(2;7) translocation in child with craniosynostosis

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Chromosomal abnormalities are a frequently underlying reason for congenital malformations. We report a child that presented with craniosynostosis, bilateral epicanthic folds and small fingers and toes. A karyotype was performed on an amniocentesis that had indicated an apparently balanced de novo t(2;7)(q32.1;p15.3) translocation. At 9 months of age peripheral blood from the child was referred for array CGH analysis with the question of whether there were any imbalances associated with the translocation. Array CGH demonstrated multiple loss events around the apparent breakpoints in the form of a 2.3Mb loss within 2q32.1, and separate 493kb and 280kb losses within 7p21.3. These small loss events would also explain the interpretation discrepancy between the indicated 7p breakpoint by G-band that indicated by array. Investigations of online databases demonstrated a previous case report in a child with craniosynostosis and with a de novo translocation involving 7p21.3, potentially suggesting that the chromosome 7 events are related to this aspect of the proband's phenotype. To date there is no defined phenotypic association with the 2q32.1 region of loss. This case illustrates a number of aspects; the importance of array analysis in instances of an individual with an apparently balanced translocation but with phenotypic abnormalities, and the challenges faced in determining the consequence of rare chromosomal abnormalities that are not directly associated with a well-defined syndrome.

P12. Implementation of a Hereditary Breast/Ovarian Cancer Predictive Testing Service

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We describe the introduction of a new service for the predictive testing of hereditary breast/ovarian cancer genes: BRCA1 and BRCA2. When a BRCA 1 or BRCA2 mutation is identified in family, following mutation screening of both genes in an individual with breast/ovarian cancer and a family history of the same; predictive testing can then be offered to unaffected members of the family. Prior to implementation in September 2012, predictive testing for a known familial BRCA1 or BRCA2 pathogenic mutation had only been available to family members via an external laboratory in the UK. Approximately 90-95% of pathogenic mutations in BRCA1 and BRCA2 are detectable by Sanger sequencing analysis; therefore this was the method of choice for the new predictive service, together with Mutation Surveyor (SoftGenetics®) data analysis. For the verification, we re-analysed 86 samples that had been previously tested and reported by an independent external laboratory over a 6 month period. Following this analysis, we were able to show concordance with previous results and to obtain data on sensitivity and specificity. In addition, we describe the systems employed to ensure an efficient workflow system and the policies and procedures devised to minimise the risk of a false normal result. Following subsequent implementation in 2012, a hereditary breast/ovarian cancer predictive testing service is now available through family cancer clinics for those individuals at risk of inheriting a BRCA1 or BRCA2 mutation.

P13. Parental gonadal mosaicism for a BRAF mutation in Cardiofaciocutaneous syndrome

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Cardiofaciocutaneous syndrome (CFCS) is a rare autosomal dominant (AD) condition characterized by cardiac abnormalities, a distinctive craniofacial appearance and short stature. CFCS is part of the RASopathy group including Noonan, LEOPARD and Costello syndromes. The four associated genes are BRAF (~75%), MAP2K1 and MAP2K2 (~25%), and KRAS (<2%). Most individuals represent new sporadic mutations. Two brothers presented for paediatric management of failure to thrive (FTT) and developmental delay. The parents are healthy, unrelated with one unaffected daughter. The first boy was born at term with a normal birth weight (50th centile). There was polyhydramnios, intrauterine growth restriction and right sided hydronephrosis noted on antenatal scans. The neonatal period was complicated by FTT and gastro-oesophageal reflux disease. A phenotype suggestive of Noonan syndrome with short stature, pulmonary stenosis, global developmental delay, and sensorineural hearing loss became apparent. His brother was born at term with a normal birth weight (50th centile). He has a similar phenotype. Mutation analysis of the PTPN11, MEK1 and MEK2 genes were normal. Mutation analysis of the BRAF gene showed heterozygosity for a pathogenic mutation

in BRAF c.770A>G (p.Gln257Arg) in both brothers. Neither healthy parent had the BRAF mutation in their blood DNA. The likely explanation for these findings is that one or other parent has mosaicism for the BRAF mutation at least in their gonadal tissue. There could be up to a 50% chance of the parents having another child affected by CFC. We describe the first reported family with cardiofaciocutaneous syndrome due to gonadal mosaicism for a pathogenic BRAF mutation

P14. Increasing testicular size due to bilateral Large Cell Calcifying Sertoli Cell tumours (LCCSTs) in a peri-pubertal child with Carney Complex.

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Carney Complex (CNC) is a rare multi endocrine neoplasia syndrome associated with endocrine and non-endocrine tumours. Three types of testicular tumour have been described; Large cell calcifying Sertoli Tumours (LCCST), Leydig cell tumours and testicular tumours of adrenal origin. LCCST is a rare benign stromal tumour, which has been observed in 41% of males affected with Carney Complex, It is generally benign although malignant transformation has been described. In pre-pubertal patients conservative management is preferred, with anti sex steroid therapy as needed, to manage secondary sexual characteristics. LCCST can cause replacement obstruction of seminiferous tubules leading to reduce fertility. CNC patients have morphologically reduced sperm and abnormal sperm number. An 11 year old boy diagnosed with Carney complex one year previously with multiple lentigines and blue naevi was referred for endocrine management. He was heterozygous for a known nonsense mutation of the PPKAR1A gene (p.R42). Height was < 2nd centile. Bone age was normal. Testicular volume was 4mls bilaterally. Six months later testicular volume had increased and appeared bulky. Height velocity was 5.6cm/yr (+0.8 SDS). Biochemical work up was consistent with a pre-pubertal boy. Ultrasound showed bilateral multiple small echogenic foci, not typical for microlithiasis, irregularly spread throughout testes. Histology confirmed LCCSTs. Assessment of boys with CNC in the peri-pubertal age group can be complex. The clinical evaluation of growth and puberty must be balanced with known complications of this multi-system condition, with a high index of suspicion for the associated endocrine features.

P15. Hyperferritinaemia with or without cataract

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Hyperferritinaemia Cataract syndrome is an autosomal dominant condition characterised by high serum ferritin and an increased risk of early cataract formation. There is no iron overload and the transferrin saturation is normal which distinguishes it from haemochromatosis type I and IV. It is caused by mutations in the L-ferritin gene (FTL). It is generally regarded as being extremely rare as opposed to haemochromatosis which is very common in the Irish population.

In the last 2 years we have tested 11 individuals for this condition. They appear to come from 4 families. 8 were mutation positive. The other 3 were predictive tests in at risk relatives .All have had the same c.-167C>T mutation. The age of onset of cataracts is very variable within families ranging from early childhood to old age and is not inevitable.

The raised ferritin appears only to cause cataracts in sharp contrast with haemochromatosis which can involve the major organs. One patient had been mistakenly diagnosed with haemochromatosis and had serial venepunctures leading to a haemoglobin of 4 g/dl. The diagnosis should be considered in all cases of raised ferritin with normal transferrin saturation or early onset of cataracts +/- a family history.

P16. Study of an extended 4 generation family with A143T Fabry mutation. Presentation of variable phenotypes including very mildly affected individuals

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Fabry disease results from deficient activity of the enzyme α-galactosidase (α-Gal A) and progressive lysosomal deposition of globotriaosylceramide (GL-3) in cells throughout the body. The classic form, occurring in males with less than 1% α-Gal A enzyme activity, usually has its onset in childhood or adolescence with periodic crises of severe pain in the extremities (acroparesthesias), the appearance of vascular cutaneous lesions (angiokeratomas), sweating abnormalities (anhydrosis, hypohydosis, and rarely hyperhidrosis), characteristic corneal and lenticular opacities, and proteinuria.

Variant phenotypes with later onset presentation have now been recognised and are caused by mutation with some residual enzyme activity. One such mutation is Alanine to Threonine substitution at position 143 (A143T). The disease causing potential of the A143T mutation is currently debated. Studies linking the mutation to phenotypic presentation have varied conclusions. Some reports conclude that the A143T is non-pathogenic mutation, while others conclude it is a late onset variant taking primate manifestation in cardiac, renal or central nervous system without associated classic manifestations. Studies of 9 individuals with confirmed A143T mutation. We compared their phenotypes and researched their enzyme levels with regards to the typical clinical features. In this family, the A143T mutation is proven to be pathogenic, but majority of the affected individuals appear to be remarkably asymptomatic with only on relative requiring enzyme replacement therapy (ERT). We will continue regular and thorough follow-up and review the need for ERT in the affected individuals.

P17. Familial Hypercholesterolaemia cascade screening service in Northern Ireland

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Genetic diagnostic testing for familial hypercholesterolaemia (FH) has been available at the Belfast Trust Regional Genetics Laboratory since 1989.As NI has a population of 1.8m, and FH has a prevalence of 1 in 500, it is estimated that there are 3,680 people with the condition. Over 26% of the expected total has been identified. The Health Commissioning Board has set year-on-year targets increasing to 40% by March 2016. Currently there are 222 identified index FH patients, of these 171 families have had cascade screening, an average of eight relatives per family have been counselled and tested. Of the 1366 relatives screened 53% have the family mutation and are confirmed with FH. This demonstrates that a further 4.2 affected FH patients are identified per family from

the index case. The appointment of the first FH specialist nurse in Northern Ireland (NI) was in 2009, to commence cascade screening in index patients attending the Belfast Trust lipid clinic. This service was extended in 2013 to enable each Trust to have an FH Nurse and a Lead FH Nurse was appointed to co-ordinate the team. The FH Nurses have completed training and are now based in their own Trust arranging to see FH patients at Lipid Clinics, drawing family pedigrees and identifying relatives who could be contacted to offer genetic testing for the known family DNA mutation and cholesterol. We hope to establish a regional FH database within the next year.

P18. 17p13.2p13.1 microduplication – two new cases provide evidence that the reciprocal duplication of the 17p13.1 microdeletion syndrome critical region is the cause of intellectual disability

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Although the 17p13.1 microdeletion syndrome is well defined, very few duplications of this region have been described. We present two new cases with a de novo duplication of 17p13.2p13.1 and compare their genotype and phenotype with two previously reported cases. All four patients exhibit intellectual disability and various dysmorphic features. There is a 260 kb minimal region of overlap (MRO) between the four duplications which encompasses the 148 kb minimal critical region described for the 17p13.1 microdeletion syndrome. The six candidate genes for intellectual disability (DLG4, GABARAP, CTDNEP1 (DULLARD), GPS2, NEURL4 and KCTD11) within the microdeletion syndrome critical region are, therefore, within the duplication MRO. Other genes involved in brain development (NLGN2, FXR2 and EFNB3), located outside of the MRO, are duplicated in some of the cases, which may be contributing to their intellectual disability. Several regions exist in the genome where deletion or duplication of the same dosage sensitive gene/s causes a specific phenotype; for example, the nearby 17p13.3 region. We propose that duplication of the 148 kb 17p13.1 microdeletion syndrome critical region causes a reciprocal microduplication syndrome with a phenotype that includes cognitive impairment and Dysmorphism.

P19. A prospective analysis of the diagnostic yield & cost benefit of array comparative genome hybridization (aCGH) in previously undiagnosed patient cohort

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Array CGH is now recognised to be the most appropriate front line test for children with developmental delay, dysmorphism or learning disability. Up until Nov 2011 The Northern Ireland Regional Genetics Centre had no revenue funding to provide a local array CGH service. In late 2011 funding was authorised for 500 send away aCGH tests during our local tender process. We carried out an audit to determine the diagnostic yield by array CGH within this cohort of 500 undiagnosed patients and to assess the cost benefit of array CGH and its possible impact on current practice of genetic investigations. The audit determined that the pick-up rate for new abnormalities by array CGH to be 18%, which compares very favourably with published data (8-17%). To assess the cost benefit of providing aCGH as a frontline test, 90 of the 500 patients analysed were reviewed. The estimated total prior test cost for these 90 patients was £42,676. The cost of the 90 array CGH tests was £29,250, indicating that ~£13,426 could have been saved for these 90 patients if a frontline array test had been available, thus confirming the cost benefit of this strategy. The results of this audit support this strategy to be good practice, as a significant proportion of patients showed a new abnormality, not detected by previous investigations. Array CGH is now offered as a frontline test in the NI Regional Genetics Centre for this patient cohort in line with other UK Genetics Centres.

P20. Integrating tertiary genetics practice with mainstream medicine

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Governmental bodies have recognised the need to educate Health care professionals (HCPs) on developments in genetic health as genetic testing becomes mainstream. A lack of funding and infrastructure with front line HCPs is limiting the awareness of genetic services in the Republic of Ireland. Following our recent KEDs/HRB grant, where we commenced engagement with HCPs to enhance education of genetics in health care, we have now successfully secured additional funding from UCD to develop e-learning modules for both rare and non-rare genetic disorders. e-Learning modules are becoming a popular method by offering distance learning to busy HCPs who need to keep on top of their CPD requirements. e-learning offers a cheap alternative as CPD is now obligatory & conference attendance expensive, especially for GPs, who are self-employed and may need to pay locums to cover absences. Our plan is to develop our microsite (http://www.ucd.ie/medicine/rarediseases/), further by development of e-learning modules on cancer genetics, cardiac genetics, haemochromatosis, investigation of developmental delay and fetal medicine. Our microsite, which went live in Sept 2013 has already had 700 hits and our animation videos > 1000 views. Whilst there are e-learning genetic alternatives in other countries, there are specific disorders and infrastructure issues (referral guidelines etc.) which require local knowledge. HCPs will receive a CPD certificate upon completion of a module, with1 credit per 1 hour of online learning. The e-learning will be open to all HCPs and we would anticipate it will continue to be developed over time. Our proposal is timely with the recent EU recommendation of rare diseases.

P21. Development of a targeted Next Generation Sequencing gene panel for Heritable Connective Tissue Disorders

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Connective Tissue Disorders (CTDs) are a heterogeneous range of disorders that affect the structural integrity of various tissues and organs. Heritable CTDs include Marfan, thoracic aortic aneurysm and dissection (TAAD), Loeys-Deitz and Ehlers-Danlos syndromes and although associated with definitive phenotypes these syndromes may have non-specific or atypical presentations. Patients may present with life-threatening complications due to defects in the heart and vascular system. Causative mutations have been identified in genes that are involved in the structure, production or processing of collagens as well as proteins that interact with collagens. A targeted next generation sequencing gene panel has been developed and validated for routine clinical diagnostic use. A Haloplex (Agilent) custom design kit and Ion Torrent PGM (Life Tech) were used to sequence the coding regions (+/- 20 bp) of 7 of the major genes associated with heritable CTDs (ACTA2, FBN1,

TGFBR1, TGFBR2, MYH11, SMAD3 and COL3A1) and the data analysed using the NextGENe Package (SoftGenetics). This method enables the detection of single nucleotide changes as well as small duplications and deletions in these genes. An initial validation of the heritable CTD panel was completed on 12 patients who carried mutations previously identified in the FBN1, ACTA2, MYH11 and COL3A1 genes. A further 53 patients have been analysed and novel likely-pathogenic variants identified and confirmed by Sanger sequencing in 9 additional patients. This method enables a more efficient and comprehensive diagnostic strategy for the analysis of heritable CTDs than the traditional single gene Sanger sequencing approach.

P22. Policy development for carrier testing in autosomal recessive genetic conditions

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Novel laboratory methods have led to an exponential increase in the number of genes being discovered for genetic disorders with mutation screening identifying causative genetic alterations in affected individuals. Many autosomal recessive conditions are rare but the arrival of an affected child inevitably leads to heightened anxiety among family members especially when considering a pregnancy. Increasingly relatives are requesting carrier testing for the family mutation in cases where the population carrier frequency is small and therefore the risk to a pregnancy is low. Often there is no straightforward carrier test for their unrelated partner. We were mindful that offering testing to relatives may unnecessarily raise anxiety. The clinical utility of this testing is questionable, in a climate where budgets are restricted and demand is great. We aim to offer our service in an equitable manner focusing our limited resources where need is greatest. Our current practice was reviewed, guidance was sought from other genetics units about their procedures and a policy document was developed. It was decided that if the prior risk to a couple of having an affected pregnancy is less than 1 in 400 carrier testing should be discussed with the clinical team. Criteria used to decide if cascade testing is indicated include seriousness of the condition, population carrier frequency and the presence of an effective carrier test due to common mutations. As no international guidelines addressing this issue exist we believe this represents a positive shift in our practice which could inform that of other genetic services.

P23. A case of atypical Angelman Syndrome

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An 8 year old boy presented with speech delay, tongue protrusion and an abnormal gait. He had major separation anxiety issues and was prone to sudden outbursts and disruptive behaviour. Although physically active he had excessive weight gain. As over-eating was part of his initial diagnosis, he was investigated for Prader Willi Syndrome. Methylation and copy number analysis of the Prader Willi/Angelman critical region using MS-MLPA showed normal copy number and 13% methylation. This result is compatible with a diagnosis of Angelman syndrome. There was no microdeletion or rearrangement observed by FISH analysis with the D15S10 probe (15q11.2-13). An imprinting centre deletion also seemed unlikely as there was normal MPLA copy number within the AS-SRO (smallest region of overlap). The possibility of paternal UPD was then investigated using chromosome 15 microsatellite markers which showed bi-parental inheritance. Therefore, AS in this patient is most likely caused by an imprinting centre defect. The recurrence risk for this defect in future pregnancies is low. Our patient belongs to a group of patients with a mosaic imprinting defect and an atypical phenotype who present with muscular hypotonia at birth and obesity.

P24. Overgrowth Syndromes - Ingeniously Heterogeneous

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Background: Overgrowth Syndromes can present in a myriad of ways and represent a set of disorders that pose categorisation, diagnostic, prognostic and management challenges to physicians. Aims: To highlight the heterogeneous presentations of overgrowth syndromes and difficulties in managing the conditions. Methods: Four distinct cases of overgrowth syndrome are presented. Results: Case 1 - Developmentally appropriate two year old boy with mild hypertrophy of left lower leg, macrodactyly of 4th and 5th toes and capillary malformation. Case 2 - Developmentally appropriate three month boy with right index finger macrodactyly present from birth. Case 3 - Mildly learning disabled sixteen year old girl with general overgrowth, hemihypertrophy and capillary malformation. Case 4 – Developmentally appropriate six week old boy with isolated overgrowth of right foot.

Conclusions: Disorders resulting in overgrowth are a rare heterogeneous group of conditions and represent a diagnostic and therapeutic challenge as an extensive list of differential diagnoses exists. Management often involves multiple specialities including plastic surgery, orthopaedic surgery, dermatology, oncology and clinical genetics. Attempts to develop an accurate molecular diagnosis for each condition are important as they guide management and may influence screening protocols for embryonal tumours which currently differ between and within countries. Married with detailed phenotyping, this genotyping will aid the development of future management protocols and tailored therapies. To this end, working with colleagues in the UK and USA, we hope to genotype the presented cases.

P25. Human DHFR and DHFRL1 both localise to the mitochondria of HEK293 cells.

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Dihydrofolate reductase is an essential enzyme in folate metabolism and considered to be exclusively cytoplasmic. In 2011 the formerly annotated pseudogene DHFRL1 was reported to be expressed and functional[1,2] as well as participating in de novo dTMP synthesis in mammalian mitochondria[2]. DHFRL1 has no mitochondrial targeting sequence so its localisation mechanism is unknown. Here we have used site directed mutagenesis to examine if altering the starting sequence of either protein affects their localisation.

Hek 293 cells cultured under standard conditions were transfected with pCMV6-ac-GFP vectors containing DHFR, DHFRL1, and mutated versions of both genes. Transfected cells were fixed and stained with Mitotracker™ red and localisation studies were performed by confocal microscopy and Western blotting of extracted mitochondrial proteins.

The first three amino acids of DHFR and DHFRL1 were successfully mutated, DHFR to DHFRL1 and DHFRL1 to DHFR. Localisation

of the wild type and mutated proteins was examined by Western blot and confocal microscopy. In both experiments all four proteins localised to the mitochondria. GFP from the transfected empty vector has a mitochondrial band on the Western blot but showed minimal localisation on confocal microscopy. Western blot analysis on mitochondrial extracts from untransfected Hek 293 cells showed the presence of an endogenous active reductase, specific activity 14.88 nmol/min/mg protein.

In conclusion, we have demonstrated that when coupled to GFP both DHFR and DHFRL1 localise to the mitochondria of HEK293 cells and that altering the first 3 amino acids of either protein does not affect localisation. We have also shown there is an endogenous active reductase present in the mitochondria of this non-cancerous cell line.

1. McEntee G, Minguzzi S, O'Brien K, Ben Larbi N, Loscher C, O'Fágáin C, Parle-McDermott A. The former annotated human pseudogene dihydrofolate reductase-like 1 (DHFRL1) is expressed and functional. *PNAS* 2011;**108(37):**15157-62. 2.Anderson DD, Quintero CM, Stover PJ. (2011) Identification of a de novo thymidylate biosynthesis pathway in mammalian mitochondria. *PNAS* 2011;**108(37):**15163-8.

P26. Exploration of Lacosamide Response

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Approximately 65 million people in the world have epilepsy. In up to 70% of patients, seizures are effectively controlled by anti-epileptic drugs (AEDs). Lacosamide (LCM) is an AED that was first approved in 2008 for the treatment of focal-onset seizures. We aimed to determine the clinical relevance of genetic variation in predicting LCM responsive and non-responsive patients. We also aimed to determine the clinical relevance of genetic variation in predicting optimal LCM dose and predicting adverse drug reactions to LCM. A total of 484 patients were recruited from four tertiary epilepsy referral centres: Dublin, Ireland; London, UK; Brussels, Belgium; North Carolina, USA. Response to LCM was determined into four categories; (i) seizure freedom, (ii) ≥75% reduction in seizure frequency, (iii) no response and (iv) seizures worsening. Overall, 3% of patients experienced seizure freedom, while 8% experienced an increase in seizures while on LCM. These figures are in line with expectation given that we ascertained for refractory patients. Seizure freedom was observed at lower doses compared to the other response categories. LCM response also varied depending on epilepsy diagnosis with the Genetic Generalised Epilepsies emerging as a potential target group for LCM treatment. Up to 40% of patients reported an adverse drug reaction (ADR), with variability across the four sites. Results will be presented from ongoing genetic analysis of this cohort.

P27. Investigation of Parent-of-Origin Effects in Autism Spectrum Disorders

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The detection of parent-of-origin effects aims to identify whether or not the functionality of alleles, and in turn associated phenotypic traits, depends on the parental origin of the alleles. Genome-Wide Association Studies (GWAS) have had limited success in explaining the heritability of many complex disorders and traits but successful identification of parent-of-origin effects using trio (mother, father, offspring) GWAS may help shed light on this missing heritability. Autism Spectrum Disorders (ASDs) are considered to be heritable neurodevelopmental disorders and a number of trio GWAS datasets exist for examining this heritability. Here, we have investigated parent-of-origin effects in large trio GWAS datasets that have previously been analysed for parent-of-origin effects using statistical approaches that did not have the capacity to detect epigenetic effects such as maternal-offspring genetic effects and all assumptions of the approaches may not have been satisfied. Here the approach of Estimation of Maternal, Imprinting and Interaction Effects Using Multinomial Modelling (EMIM) is used to identify SNPs associated with ASD through a parent-of-origin mechanism which has the potential to aid in understanding more fully the genetic underpinnings of ASD.

P28. Examination of Pathways possibly involved in the Pathogenesis of Neovascular AMD

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Age-related macular degeneration (AMD) is a major cause of blindness in the elderly, affecting 10% of those aged over 65 years and 25% of those aged over 75 years. The neovascular subtype of AMD is less common than the dry form, but is ultimately more likely to lead to blindness. Our research examined loci associated with neovascular AMD to inform molecular pathways that may contribute to disease causation. Data from the large US MMAP study were examined and participants split into groups dependent on their genotypes carried at the two most strongly associated loci: CFH(rs10801555) or ARMS2(rs932275).Each of the groups created was analysed using PLINK, and for each SNP the odds ratios (OR) and p-values were compared between groups. SNPs were ranked according to the change in OR between the most harmful and most protective genotypes. The top 200 SNPs were loaded into the DAPPLE online programme which creates networks between genes where SNPs have been published as having associations with each other.

There were two interesting findings. Stratifying on CFH, GDF6 closely associated with ARMS2, which together are thought to lead to angiogenesis. Stratifying onARMS2, four of the associated SNPs were found in genes that form part of a glutaminergic pathway which is involved in neuronal apoptotic death and therefore may possibly affect the death of reticulocytes in the retina. Confirmation of our results will be required in additional genotyping datasets that should become available shortly.

P29. Genetic susceptibility to reflux nephropathy in vesicoureteric reflux patients

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Vesicoureteric reflux (VUR), the reverse flow of urine from bladder to kidneys, due to a developmental defect of the vesicoureteric valve mechanism, occurs in about 3% of infants, but in many it resolves as the child grows. The principal cause of morbidity in VUR cases is reflux nephropathy (RN), an overall term for congenital

renal dysplasia and acquired renal damage due to hydronephrosis and pyelonephritis, and is the commonest cause of renal failure in children. VUR appears to be very genetically heterogeneous. One approach to the discovery of the factors that determine which children with VUR will have RN and which will not, is to perform a genome scan for VUR and then, when the genes and mutations responsible for the linkage and association peaks have been identified, to look for relationships between genotypes and phenotypes. Another approach is to perform a genome scan using RN as the phenotype in VUR patients to look for additional genes that affect whether RN will occur in the presence of a mutation that causes VUR. The SNP genotyping of individuals can be used for both types of analysis. We genotyped 500 VUR patients and 400 other family members and published a linkage and association genome scan for VUR (using genotypes of 850 controls from the IBTS-TCD BioBank) in January 2014, and are now preparing a scan for RN, for which we hope to present results. As of early June, we have classified 391 VUR cases as without RN and 129 with RN from 230 families.

P30. Investigation of cell signalling events induced by expression of YWHAE-NUTM2, a fusion protein resulting from t(10;17)(q22;p13) in Clear Cell Sarcoma of Kidney.

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Clear cell sarcoma of kidney [CCSK], the second commonest childhood renal cancer is diagnostically challenging, aggressive and therapy-resistant. Individual case reports of CCSKs with t(10;17)(q22;p13) prompted our characterization of the chromosomal translocation. This translocation results in rearrangement of YWHAE (encoding protein 14-3-3ε) on chromosome 17 and NUTM2 on chromosome 10, producing an in-frame fusion transcript YWHAE-NUTM2. To investigate the cell biological effects of the fusion protein, we developed cell lines allowing inducible expression of Ywhae-Nutm2 in HEK293 and NIH3T3 cells. We show that HA-Ywhae-Nutm2 is present in both nuclear and cytoplasmic compartments, while 14-3-3ε is localised primarily in the cytoplasm. Since 14-3-3ε can mediate signal transduction by phosphoprotein interactions, we reasoned that Ywhae-Nutm2, with its ability to enter the nucleus, could alter cell signalling events. Unfractionated, nuclear and cytoplasmic HEK293 cell lysates, induced to express HA-Ywhae-Nutm2 or mock-treated were applied to an antibody microarray encompassing 850 total and phospho-specific antibodies. Altered protein expression and phosphorylation events in response to HA-Ywhae-Nutm2 expression were identified by this screen. Of these, the levels of the anti-apoptotic proteins Mcl-1, Bcl-2 and Bcl-xL were shown to be increased in cytoplasmic fractions (Mcl-1), or decreased in nuclear fractions (Bcl-2 and Bcl-xL). Hsp60 has been identified by western blot as being increased following induction of HA-Ywhae-Nutm2 in unfractionated and both the nuclear and cytoplasmic fractions. Further validation of, and investigation into, the altered cell signalling events in response to Ywhae-Nutm2 expression will assist in identifying novel therapeutic targets for this treatment-resistant cancer.

P31. Next Generation Sequencing to interrogate Vitamin D related genes in patients with New Onset Diabetes after Transplantation (NODAT).

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New Onset Diabetes after Transplantation (NODAT) is a serious complication for 5-40% of organ transplant recipients. NODAT increases the risk of cardiovascular complications, graft rejection, infection and premature death. Low vitamin D (VitD) levels, and SNPs in the VDR gene, have been associated with the development of NODAT. Individuals (n=96) with an oral glucose tolerance test performed at baseline, 7 days, 3 months and 12 months after kidney transplantation were recruited, alongside kidney transplant recipients with established NODAT (cases) and transplant recipients with no evidence of NODAT (controls) matched for age, gender and follow-up time (n=173). 25-OH VitD levels were available for 90 individuals.

Targeted next generation sequencing was used to investigate 43.8 kb across 38 high value SNPs associated with VitD levels, exons, splice sites, and untranslated regions for CYP2R1, CYP12B1, CYP24A1, CUBN, GC, NADSYN1, and VDR genes. This method permits a cost effective, accurate and efficient examination of customised genetic regions. AmpliSeq amplicons were designed in two pools (n=163, n=166) and sequenced on an Ion Torrent PGM. Libraries were prepared using Ampliseq kit 2.0 and processed on an Ion Chef, or OneTouch2+ES machine. Reads were aligned using TorrentSuite v4.0.3 and SNP genotypes called using Partek Genomics Suite v6.6. Maximum reads per sample were 120,000 and 317,000 for 318 and 316 chips respectively. SNPs were compared with 25-OH VitD levels and development of impaired glucose tolerance or NODAT. We have developed a novel screening assay and reveal important findings for SNPs associated with vitamin D levels and NODAT.

P32. Next generation sequencing of the mitochondrial genome to evaluate association with end stage renal disease

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Introduction: Kidneys are highly aerobic organs, which are heavily dependent on the normal functioning of mitochondria - the cellular "power-plants". Mitochondria possess discrete DNA from the nuclear genome, mtDNA,which encodes important respiratory chain components. The uraemic state associated with end-stage renal disease (ESRD) may increase the risk of oxidative damage to mtDNA. Whole genome resequencingof mtDNA in a cohort of White kidney transplant donors and recipients was employed to identify mtDNA variants associated with ESRD. Methods: The mtDNA was amplified in two overlapping fragments with long range PCR, and size confirmed using 0.7% agarose gel electrophoresis. Both fragments were pooled for each sample, enzymatically fragmented, and each individual's DNA was barcoded before being pooled for library preparationand next-generation sequencing using the Ion Torrent Personal Genome Machine. Data was analysed using Partek Genomics Suite, HaploView, and PLINK. Results: DNA from 64 individuals (39 recipients, 25 donors) has been sequenced across the entire 16,569 bp (37 genes) mtDNA. This revealed 361 mtDNA variants, of which 89 had a minor allele frequency greater than five percent. Successful call rate exceeded 98% for all SNPs and samples. Seventeen SNPs were nominally associated with ESRD with $P<0.05$. The most significant result was 2706A>G, $P=0.0016$. Analysis of a further 127 individuals for replication is ongoing. Conclusion: Mitochondrial DNA variants are significantly associated with ESRD, though the importance of the association is still to be elucidated, and requires further analysis.

P33. A Northern Irish population study of renal disease in Tuberous Sclerosis Complex

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Tuberous sclerosis (TSC) is an autosomal dominant disorder caused by mutations in TSC1 or TSC2. It is characterized by benign tumours particularly in the kidneys and brain. Renal angiomyolipomas (AML) affect ~80% of patients causing morbidity and mortality through haemorrhage and renal insufficiency. Current treatments include embolization, nephrectomy and mTOR inhibitors. There is a risk of renal carcinoma. Methods: We have a database of all TSC patients in Northern Ireland, and investigated the incidence of renal complications in all patients. Results: 98 patients were recorded on the database, giving an incidence of 1 in 18,000 (age range 1-78 years). 36 had no evidence of renal disease on USS.

Two patients had no AMLs but had renal carcinoma (RCC). 55%of patients had renal AMLs; One patient also showed polycystic kidney disease and one patient had RCC. Most showed stable growth in AMLs but at the time of the study 6 (10%) had large AML which were actively growing - three of these have had embolisation, 1 was started on an mTOR inhibitor, two are being monitored to determine if intervention is needed.5 others patients had a history of embolization. Conclusion: Renal AML's are common in the TS population and careful monitoring to allow treatment and timely intervention is crucial. Northern Ireland has a total of 4 patients currently on an mTOR inhibitor for treatment of their AML's. To our knowledge this is the first total population study of renal complications in TSC patients.

P34. Investigation of the proliferative and invasive potential of cells expressing Ywhae-Nutm2, the fusion protein resulting from t(10;17)(q22;p13) in Clear Cell Sarcoma of Kidney

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Clear Cell Sarcoma of Kidney [CCSK], the second commonest pediatric renal cancer, is aggressive, therapy-resistant, and has poor outcomes. The oncogenic mechanisms underpinning CCSK are poorly understood. Specific diagnostic/prognostic markers and effective therapies are lacking. Prompted by three independent published case reports of a chromosomal translocation, t(10;17)(q22;p13) in CCSK, we characterised this chromosomal translocation to show that it results in rearrangement of YWHAE on chromosome 17 and NUTM2 on chromosome 10, producing a fusion transcript comprising exons 1-5 of YWHAE fused in-frame to exons 2-7 of NUTM2. To investigate the biological effect of Ywhae-Nutm2, we have generated stably transfected HEK293 and NIH3T3 cell lines with inducible HA-tagged YWHAE-NUTM2. To ascertain whether expression of Ywhae-Nutm2 confers oncogenic potential, cell-based assays were conducted using the xCELLigence system. This system provides a platform for real-time and label-free analysis of cell proliferation, migration and invasion by utilising electrical impedance signals generated by growing cells within a user-defined time-frame. Cell migration of HEK293 cells induced to express HA-Ywhae-Nutm2 was significantly higher than of the mock-treated cells (which express no fusion protein). We are currently investigating the ability of cells expressing, and not expressing, Ywhae-Nutm2 to invade and migrate through a matrigel layer. Furthermore, molecular changes within cells expressing Ywhae-Nutm2 will be investigated by gene expression profiling at a chosen time point derived from the xCELLigence analysis. This will contribute to our understanding of the molecular events underpinning CCSK, and, in conjunction with ongoing signalling studies in the laboratory, will help to elucidate novel therapeutic targets.

P35. Functional Investigation of Celiac Susceptibility Gene LPP in T cells

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CD4 T cells are known to play an important role in celiac disease etiology as they initiate an immune response to gluten displayed by antigen presenting cells. A large case control study using the Immunochip identified Lipoma-preferred partner (LPP) as the most significantly associated non-HLA risk locus.(Trynkaet al, 2011).mRNA sequence data of CD4 T cells from our lab (data not shown) shows that LPP is expressed at higher levels in celiac samples compared to controls. We aimed to investigate the role of LPP in T cells. In this study we wanted to confirm the expression of LPP in CD4 T cells and examine the effect LPP may have on cell migration through siRNA knockdown. In addition, using qPCR we tested a number of potential LPP targets that demonstrated dysregulation in our sequencing study for differences in expression when LPP is silenced. We confirmed LPP expression in peripheral blood T lymphocytes. T cells knocked down for LPP showed defects in transwell migration in response to chemotactic signals. Furthermore, preliminary data shows that when stimulated with the chemokine, CXCL12, knockdown of LPP is associated with alterations in the mRNA levels of the potential LPP interactors or transcriptional targets MMP25, TIMP1 and CXCR4 suggesting a possible mechanism by which LPP contributes to disease pathophysiology. Ongoing investigations aim to further delineate the role of LPP in T cells.

P36. Allelic expression imbalance at IL18 and CXCL16 in heterozygous clinical samples and cell lines.

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Gene expression may exhibit allelic expression imbalance (AEI), whereby one allele produces more mRNA than the other in heterozygous individuals. This variation may be detected by performing 5'exonuclease genotyping on cDNA, thereby measuring the levels of allelic mRNAs. This provides a powerful method to detect cis-acting genetic variants, and may uncover a functional role for non-coding SNPs in GWAS analyses. The CXCL16 gene encodes a chemokine involved in the pathology of multiple diseases. It is induced by interferon gamma, which is in turn induced by interleukin 18 (IL18).We performed 5'exonuclease assays on cDNA from heterozygous Acute Coronary Syndrome (ACS) patients and heterozygous immortalized lymphoblastoid cell lines (CEPH), with or without stimulation by TNFalpha, DMSO and Sulphoraphane (SFN), a Histone Deacetylase inhibitor. A number of clinical samples showed evidence of allelic expression imbalance at both IL18 and CXCL16. Some cell lines showed AEI at IL18, but not at CXCL16 unless the cells were stimulated by TNFalpha. Among those cell lines that showed no CXCL16 imbalance, treatment with DMSO or a combination of DMSO and SFN induced imbalance. In conclusion, AEI can be observed in clinical samples and can be enhanced in cell lines by stimulation with TNFalpha, thus reproducing the pro-inflammatory environment of ACS. This effect can be further enhanced by treatment with DMSO and SFN, suggesting that at least some of the observed AEI has an epigenetic component.

P37. A genome-wide meta-analysis of aromatic anti-epileptic drug induced maculopapular exanthema

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Carbamazepine is one of the world's leading anti-epileptic drugs (AEDs) for treating partial epilepsy. However, it is estimated that 5-10% of patients exposed to the drug develop a cutaneous idiosyncratic adverse drug reaction (ADR) characterized by a generalized maculopapular exanthem rash (MPE). Similar hypersensitivity reactions also occur with other aromatic AEDs including lamotrigine, phenytoin and oxcarbazepine. An allele in the human leukocyte antigen (HLA) locus, HLA-A*3101, has been shown to have diagnostic value in predicting carbamazepine-hypersensitivity (OR=8.58, CI=5.55-13.28) in Europeans, Japanese and Korean populations. However there are no known genetic markers of MPE to other AEDs. In this study we have genotyped 178 AED-specific MPE cases and 806 drug-tolerant controls from epilepsy patient cohorts of European, Asian and African-American ancestry. We imputed over 6 million genetic variants from the 1000 Genomes Project across each cohort and performed logistic regression of all cases versus controls per ancestral cohort, controlling for within-population differences by principal components analysis. Within the European cohort we further stratified cases according to the specific causal drug. Finally, we performed a meta-analysis of the results to identify enrichment for global markers of hypersensitivity to AEDs. Such markers will aid in reducing overall rates of AED discontinuation due to ADRs and will serve to advance genetic testing in clinic.

P38. Comparison of whole genome and whole exome next generation sequencing techniques on the Ion Torrent Proton platform for coverage of key genetic regions in chronic kidney disease.

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Whole genome sequencing (WGS) was compared with whole exome sequencing (WES) and microarray data for the investigation of genetic regions associated with chronic kidney disease (CKD). Using a two parent-offspring trio design. WGS was compared with TargetSeq WES (hybridisation method) and AmpliSeq (multiplex PCR method) WES on the Ion Torrent Proton™. Sequences were aligned to GRCh37.p13 using Torrent Suite version 4. Partek® Genomics Suite™ version 6.6 was employed for variation detection, familial trio validation and pathway analysis. WGS yielded the most comprehensive analysis of genetic variation with an average 4x coverage on P1 chip with 1-60,000x at SNPs; however the large datasets generated creates challenges for routine bioinformatic interpretation. WES reduces the size of generated data for each sample. TargetSeq covers ~50 Mb, including >29,500 protein and RNA genes (including >1470 miRNAs), >44,000 predicted miRNA binding sites, and untranslated regions with four probes per exon. Ampliseq covers primarily protein coding regions of the genome with ~90% reads on target. Literature review revealed 355 CKD associated genes. Variation was detected in 93% of these genes by WES and 84% by low pass WGS. We have demonstrated that Ion Torrent is an efficient and cost effective technology for use in research of the genetics of complex disease states such as CKD. The transition of NGS into the renal clinic may help to "personalise" patient care.

P39. Investigating the role of the KRAS variant in breast cancer phenotype and bilaterality

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The KRAS-variant rs61764370 is a functional variant in the Let7a binding site in the 3' UTR of KRAS oncogene that has been associated with increased risk of cancers of the lung, breast and ovary. The aim of this study was to investigate prevalence of KRAS-variant among Irish patients with breast cancer, and to examine the association between the KRAS-variant and bilateral breast cancer. An observational cohort study was undertaken. The study group included patients with breast cancer managed in a single tertiary referral centre. Patients with known high-risk single gene mutations were excluded from analyses. Cancer-free controls over the age of sixty were recruited from the community. DNA was extracted from whole blood, saliva and buccal swabs and genotyped for the variant using a Taqman-based platform. All data was analysed using SPSS. 531 controls, 1138 patients with unilateral and 74 patients with bilateral breast cancers were genotyped for the KRAS-variant. 79/531 (15%) controls and 162/1212 (13%) cases carried the variant. Differential expression of the variant was noted across subtypes, being identified most commonly in patients with TNBC (16%). Of patients carrying the variant, 12(7.4%) had bilateral disease compared to 62 (5.9%) patients with a wild type allele. The median age of diagnosis of patients with the wild type allele was 53years (24-96) compared to 51 years (27-86) in patients with KRAS-variant. The KRAS-variant carries an increased risk of triple negative breast cancer compared to other subtypes, and is associated with bilaterality of disease.

