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Abstracts

14th Meeting of the Irish Society of Human Genetics, Friday 2nd September 2011

Health Sciences Building, University College Dublin

PROGRAMME:

10.00 – 10.55	Registration / Tea and Coffee.
10.55 – 11.00	Welcome.
11.00 – 12.30	Spoken Presentations: Plenary I.
12.30 – 13.30	**Keynote address: '**From childhood encephalopathy to lupus: Mendelian type I interferon-opathies**.' Prof. Yannick Crow,** University of Manchester, UK.
13.30 – 14.30	Lunch and Poster viewing.
14.15 – 14.30	Council Meeting.
14.30 – 15.30	Spoken Presentations: Plenary II.
15.30 – 16.15	Tea and coffee / Poster viewing.
16.15 – 16.30	Business Meeting.
16.40 – 17.40	**Keynote address**: 'Whole-genome sequencing and the detection of disease-causing mutations.' **Prof. Lynn Jorde**, University of Utah, USA.
17.40 – 18.30	Wine reception / Presentation of Prizes / Meeting Close.

SPOKEN PAPERS:

S01. Ptosis, arched eyebrows, hypernasal speech, obesity and mild learning disability - a clinical & mapping study.

SA Lynch[1], M Akram[2], N Goggin[2] M Earley[3], S Ennis[4], J Conroy[4].

[1.] NCMG, OLCHC, Crumlin Dublin 12, [2.] Paediatric Department. Waterford Regional Hospital, [3.] Dept of Plastic Surgery, Temple Street Children's Hospital, Dublin 1, [4.] School of Medicine & Health Science, UCD, Belfield Dublin 4.

We report 15 members of a three generation pedigree with ptosis, velopharyngeal incompetence, dysmorphism and a learning disability. The index case presented with nasal regurgitation & a dysmorphic appearance (medical flaring & arching of the eyebrows). Ophthalmological examination revealed a congenital ptosis, hypermetropia & a right convergent squint. He had grommets inserted for otitis media.

His mother has similar features. She has 6 children, 4 of whom are affected. The three older children & the mother, are obese. The maternal grandfather had ptosis & cannot read or write. He had 8 children, 5 affected & 3 unaffected. Five of this sibship have been assessed of whom two are unaffected. Two aunts of the index case have ptosis, obesity & learning difficulties. One has a son with ptosis.

Linkage analysis was performed on 7 of the samples including 6 affected individuals and 1 unaffected individual. Samples were genotyped on an Illumina Human-1M array. Parametric linkage analysis was undertaken using MERLIN. Two loci, on chromosomes 2p16.3-2q14 (14.4Mb) and 10q25.1-10q26.1 (13.5Mb), with LOD scores of 1.79 and 1.59 respectively were identified. Ninety-seven genes are contained within these regions. Additional samples (n=8) have been collected. Future linkage studies and mutation screening are ongoing.

S02. Familial catecholaminergic polymorphic ventricular tachycardia in Ireland.

Liyen Ng, Nicola Harper, Andrew Green.

National Centre for Medical Genetics, Our Lady's Children's Hospital, Crumlin, Dublin 12.

Catecholamine polymorphic ventricular tachycardia (CPVT) is a rare inherited heart disease, which can predispose to ventricular arrhythmias and sudden death in young patients. Early detection of CPVT is crucial because opportune medical intervention prevents sudden cardiac death.

Mutations in the ryanodine receptor (RYR2) explain nearly 70% of the CPVT cases and cause the autosomal dominant form of the disease. Genetic analysis of RyR2 is available clinically and it has provided important insights into the mechanism underlying the disease.

Therefore in this retrospective study, we report the cases of CPVT families that have presented to the National Centre for Medical Genetics in OLCHC for genetic testing for CPVT. We discuss the phenotype of the CPVT carriers in our centre. Pathogenic variants in RyR2 have been identified in 2 families. 16 carriers have been identified and of these 8 (50%) have had symptoms prior to molecular analysis.

CPVT represents a clearly defined but still insufficiently recognised entity. The consequence of misdiagnosis is sudden death in children or young adults with an otherwise normal heart. There is wide range of phenotype for gene carriers from asymptomatic to syncope to sudden adult death syndrome (SADS). Better awareness may aid earlier diagnosis and appropriate medical treatments that can prevent sudden death.

S03. Incidence of I-cell disease (mucolipidosis type II) in the Irish population.

F McElligott[1], E Beatty[1], S O'Sullivan[1], J Hughes[2], D Lambert[3], A Cooper[4], E Crushell[1]

[1.] National Centre for Inherited Metabolic Disorders (NCIMD), Dublin, Ireland, [2.] Department of Metabolic Disorders, Royal Belfast Hospital for Sick Children, Northern Ireland, [3.] National Centre for Medical Genetics, Dublin, Ireland, [4.] Willink Biochemical Genetics Unit, Royal Manchester Children's Hospital, Manchester, England.

Cases of I-cell disease diagnosed in Ireland over 13 years (1/1/1998– 31/12/2010) were identified in collaboration with the clinical diagnostics laboratory. A database documenting details of diagnosis, and clinical course where available was compiled. Results were correlated with published birth rates, including that of Irish Travellers (available only for the Republic).

Twenty infants from 14 families were diagnosed with I-cell disease during the study period. 18 were born to Irish Traveller parents, one to non-Traveller Irish parents and one to parents from Southern Europe. Mutation analysis was available for 7 cases, of whom 6 (all Travellers) were homozygous for the c3503_3504delTC mutation. Median age of death in patients of the Traveller community was 232 days (range 3-936).

Overall incidence, calculated using population data for the Republic (ROI) and Northern Ireland, was 1.56 per 100,000 live births. The incidence amongst Travellers (based on ROI cases and population data) was 114 per 100,000 live births, suggesting a carrier frequency of the common mutation in this group of 1 in 15. The carrier rate amongst Irish non-Travellers remains rare at 1 in 512. This high incidence and carrier rate found in the Irish Traveller population is relevant for genetic counselling of this consanguineous community.

S04. The Irish Giants: when truth meets fiction.

Lisa Bradley, Patrick J Morrison.

Department of Genetic Medicine, Belfast HSC Trust, Belfast, BT9 7AB.

Most countries and civilizations have stories about giants in their culture. In Northern Ireland, we have the Giant's Causeway, columns of hexagonal and octagonal basalt, built by a group of engineering-conscious giants who ran a combined operation with the giants of Scotland to facilitate easier access between the two sister countries. Another 'Irish Giant', Charles Byrne, was born in Littlebridge in 1761. His father was native to the area but his mother was Scottish. He was supposedly related to the Knipe brothers, the tallest identical twins (at 7ft 2in), from nearby Magherafelt. He grew rapidly and in his late teens featured in street shows in Ireland and London. After death his skeleton was acquired by the surgeon John Hunter and was eventually deposited in the Hunterian Museum in London. His enlarged pituitary fossa implied that his gigantism was due to a pituitary adenoma.

DNA studies have confirmed that mutations within the Aryl Hydrocarbon Receptor Interacting Protein (AIP) gene cause pituitary tumourigenesis, and can cause familial pituitary adenomas displaying autosomal dominant inheritance and variable expression. We present two families (one of whom is a proven descendant of Charles Byrne) with AIP mutations and discuss current recommendations for screening and predictive testing.

S05. An Overview of patients with Li-Fraumeni Syndrome and Li-Fraumeni-like syndrome in Northern Ireland.

Deirdre E Donnelly, Patrick J Morrison.

Department of Genetic Medicine, Belfast HSC Trust, Belfast, BT9 7AB.

Li Fraumeni Syndrome is a rare, autosomal dominant, cancer predisposition syndrome. Tumours can present at any age and, as the tumour spectrum is so wide, surveillance of at-risk individuals is difficult. We carried out a case note analysis of all families in Northern Ireland with this syndrome, two out of five of whom were positive for a TP53 mutation. The data obtained allow delineation of the phenotype, tumour distribution and age of onset and prognosis of specific tumours within the families.

We present the range of tumours and discuss screening implications for family members. This important condition is under-diagnosed and this analysis will allow better recognition of this disorder.

S06. A new locus for Episodic Ataxia.

J Conroy[1], R Murphy[2], C McDonagh[2], D Webb[3], J Casey [4], R Regan [1], S Ennis[4], SA Lynch[5].

[1.] National Children's Research Centre, OLCHC, Crumlin, Dublin 12, [2.] AMNCH Tallaght Dublin 24, [3.] OLCHC, Crumlin Dublin 12. [4.] School of Medicine and Medical Sciences, UCD, [5.] NCMG, OLCHC, Crumlin Dublin 12.

Episodic Ataxias (EA) comprise a genetically heterogeneous group of neurological conditions characterised by spells of ataxia, nystagmus & slurring of speech. The duration of clinical attacks vary from minutes to days. The various subtypes (EA1 to EA6) are differentiated by clinical presentation. Mutations in KCNA1, CACNA1A, CACNB4 and SLC1A3 are responsible for the development of EA1, EA2, EA5 and EA6 respectively. A locus has also been mapped for EA4 (1q42).

A three-generation Irish family with autosomal dominant EA was identified. Presentation occurred in early childhood and symptoms are controlled by Clonazepam (a GABA agonist).

Genome-wide linkage analysis was performed on 8 members of the family (6 affected, 2 unaffected) using the Illumina Human CytoSNP12 array. Following pruning the data, linkage analysis was performed using MERLIN on ~30,000 SNPs. Parametric analysis revealed three linkage regions on chromosomes 1, 7 and 20 with LOD scores of 1.8. These regions represent novel loci for EA. These results in addition to some of the novel features of the phenotype suggest that this family represent a new subtype of Episodic Ataxia.

S07. Identification of a novel disease gene for paediatric mitochondrial disorder.

Jillian Casey[1], Judith Conroy[1], Regina Regan[1], Ellen Crushell[2], SallyAnn Lynch[3], Sean Ennis[1].

[1.] Health Sciences Centre, University College Dublin, Dublin 4, Ireland, [2.] National Centre for Inherited Metabolic Disorders, Children's University Hospital, Temple Street, Dublin 1, Ireland, [3.] National Centre for Medical Genetics, Our Lady's Hospital for Sick Children, Crumlin, Dublin 12, Ireland.

Mitochondrial disorders are amongst the most common inherited human diseases with a particularly high incidence in Ireland (1/9,000 births). We have studied a consanguineous Irish family that includes 3 children with clinical features consistent with a mitochondrial disorder. Molecular, biochemical and genetic analyses excluded all of the known causes of mitochondrial disease. It was concluded that the disease gene segregating in this family represented a novel cause of paediatric mitochondrial disorder.

We applied SNP homozygosity mapping (HM) and whole exome sequencing to investigate if a shared recessive mutation was common to this pedigree. The 3 patients and 5 unaffected relatives were genotyped for 1million SNPs (Illumina array). SNP HM identified

38 homozygous segments containing 134 genes that were shared by the patients.

On average, we detected 76,500 variants per patient exome. We identified 34 homozygous non-synonymous variants that segregated with the phenotype. Of these, only 2 were located within the 38 candidate regions of homozygosity, resulting in a 17-fold reduction in the number of putative disease variants for further investigation. Both candidate variants are located within the same gene, which belongs to a gene family previously implicated in mitochondrial disease. Limiting the search to the candidate homozygous intervals proved to be a powerful filtering strategy for the analysis of exome data and resulted in the successful isolation of the causative gene.

S08. The Role of Common Genetic Variation in Autism Spectrum Disorders.

Richard JL Anney on behalf of the Autism Genome Project and the Psychiatric GWA consortium. Institute molecular medicine, St. James' hospital, Dublin 8, Ireland.

Autism spectrum disorder has been established as a highly familial disorder with siblings of a proband showing at least 25-fold higher prevalence than that of the general population. Genome-wide association studies of ASD have highlighted signals on chromosome 5 intergenic to CDH9/CDH10 and SEMA5A/TAS2R1. As part of the Autism Genome Project (AGP) we have also previously identified strong association signals tagging the genes MACROD2, PLD5 and ST8SIA2. We present analysis from a follow-up of an additional 800 AGP families and preliminary data from the meta-analysis performed as part of Psychiatric GWA Consortia (PGC) Autism Study including data on approximately 4400 cases and 4400 pseudo-controls for greater that 1.25 million SNPs. The PGC Autism Study is comprised of GWA scans done by the AGP, The Autism Consortium in Boston, Johns Hopkins University, Children's Hospital of Philadelphia, and on samples collected by the Simons Simplex Collection and from Montreal and Finland. Using an additional 800 families from the AGP we do not observe significant validation of the previously GW-significant association signals from ours or others GWA of ASD.

However, we do see increased association signals in strong biological candidates for less noteworthy markers previously associated in the modest to strong range (p<10e-4). We observe a number of strong association signals for the PGC meta-analysis on chromosomes 5, 6, 7, 9, 16, 17 and 19 which are currently being followed up to validate their authenticity. We will present these data in the context of previous ASD findings and discuss the challenges faced and overcome in detecting loci that influence ASD susceptibility.

S09. Identification of a second dihydrofolate reductase activity in humans: the former annotated pseudogene *DHFRL1* is expressed and functional.

Grainne McEntee, Stefano Minguzzi, Kirsty K.O'Brien, Nadia Ben Larbi, Christine E. Loscher, Ciarán Ó'Fágáin, Anne Parle-McDermott.

School of Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland.

Dihydrofolate reductase (DHFR) is a folate enzyme which reduces dihydrofolate into tetrahydrofolate in the presence of NADPH, ensuring a constant supply of biologically active folate. DHFR was previously thought to be the only enzyme capable of this reaction however we show that humans have a second dihydrofolate reductase enzyme encoded by the former pseudogene *DHFRL1* (dihydrofolate reductase like – 1), located on chromosome 3. We demonstrate that the *DHFRL1* gene is expressed and shares some commonalities with DHFR. Recombinant DHFRL1 can complement a *DHFR* negative phenotype in both bacterial and mammalian cells. Enzyme kinetics shows that the K_m for NADPH is similar for both enzymes but DHFRL1 has a higher K_m for dihydrofolate when compared to DHFR, indicating a lower affinity for the substrate. Localization of DHFRL1, visualized using confocal microscopy, shows that DHFRL1 has a strong presence in the mitochondria, indicating that mitochondrial dihydrofolate reductase activity may be optimal with a lowered affinity for dihydrofolate. We also found that DHFRL1 has the ability to bind its own mRNA in the same translational auto-regulation method as DHFR; with both enzymes capable of replacing each other. The identification of a second dihydrofolate dihydrofolate reductase enzyme will have a major impact on previous research surrounding DHFR.

S10. A genome scan for vesicoureteric reflux reveals a new recessive locus on chromosome 10 with an HLOD score >6.

MG Dobson[1,2], JM Darlow[1,2], M Hunziker[2,3], CM Molony[4], P Puri[2,3], DE Barton[1].

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Vesicoureteric reflux (VUR), the retrograde flow of urine from the bladder towards the kidneys, results from a developmental anomaly of the vesicoureteric valve mechanism and has an incidence at birth of 1-2%. It often resolves with age, but ~8% of cases develop renal failure, accounting for ~25% of all renal failure. Several genome scans have been performed with conflicting results. We have performed a new scan on 530 cases and 435 other members of 246 families with 900,000 markers on the Affymetrix SNP Array 6.0. Linkage analyses have confirmed our previous non-parametric linkage peaks on 2q, 6q and 10q, all on a dominant model (highest with an HLOD of ~5 on 10q) and our previous peak on 7q is confirmed with HLOD >3 on a common recessive model, but also some linkage on a very rare dominant model. However, the most exciting finding is a very narrow recessive peak in a different position on 10q with an HLOD >6 that was previously missed because the markers were too far apart. There is also a dominant peak on 22q in one analysis. Numerous other smaller peaks remain to be examined. Our results did not replicate the findings of Briggs *et al.* (2009), Weng *et al.* (2009) or Conte *et al*. (2007). Analysis for association and copy-number variation is ongoing.

S11. HLA-A*3101 is a genetic marker for carbamazepine but not all anti-epileptic drug induced hypersensitivity reactions.

Mark McCormack[1], Ana Alfirevic[2], Stephane Bourgeois[3], John F. Farrell[4], Dalia Kasperaviđđč, Mary Carrington[6], Graeme J. Sills[2], Tony Marson[2,7], Xiaoming Jia[8], Paul I.W. de Bakker[9], Krishna Chinthapalli[5,10], EUDRAGENE collaborators, Mariam Molokhia[11], Michael R. Johnson[12], Gerard D. O'Connor[13], Elijah Chaila[13], Saud Alhusaini[1], Kevin V Shianna[14], Rodney A. Radtke[15], Erin L. Heinzen[14], Nicole Walley[14], Massimo Pandolfo[16], Werner Pichler[17], B. Kevin Park[2], Chantal Depondt[16], Sanjay M. Sisodiya[5,10], David B. Goldstein[14], Panos Deloukas[3], Munir Pirmohamed[2], Norman Delanty[1,13], Gianpiero L. Cavalleri[1]

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Carbamazepine, phenytoin and lamotrigine are amongst the most commonly prescribed anti-epileptic drugs. However, their use can cause various subcutaneous adverse reactions; maculopapular exanthema (MPE), Hypersensitivity Syndrome (HSS) and Stevens-Johnson syndrome (SJS). A strong association between carbamazepine and phenytoin-induced SJS and *HLA-B*1502* exists in Asian populations however as *HLA-B*1502* is largely absent in European populations, the test is not applicable here.Through genome-wide association studies, we investigated whether genetic variants play a role in susceptibility to drug-specific hypersensitivity reactions when compared to drug-tolerant controls. We used genotype data to impute HLA types for our cohorts. *HLA-A*3101* strongly associated with carbamazepine-induced hypersensitivity and we confirmed *HLA-A*3101* as a risk factor for MPE (OR 8.3; 95% CI 3.6-19.4), HSS (OR 12.4; 95% CI 1.3-120.4), and SJS (OR 25.9; 95% CI 4.9-116.2). Neither HLA-A*3101 nor any other genetic marker associated with MPE resulting from phenytoin or lamotrigine. Due to low case numbers for HSS and SJS, we could not perform similar drug-specific analyses for the more severe phenotypes.

Our research provides the foundation for genetic testing of *HLA-A*3101* for all prospective European carbamazepine users. However, *HLA-A*3101* does not appear to be a genetic marker for lamotrigine and phenytoin-induced MPE reactions in Europeans.

POSTER PRESENTATIONS:

P01. Börjeson-Forssman-Lehmann Syndrome.

C Murphy[1], AM Murphy[1], M Mavinkurve[3], S Smith[1], E Cullen[1], N Irving[1], M O'Sullivan[1], SS Lim[1], SA Lynch[2], E Roche[1,3].

Department of Paediatrics, Trinity College Dublin, Ireland[1], National Centre for Medical Genetics, Our Lady's Hospital for Sick Children, Dublin 12, Ireland[2], AMNCH National Children's Hospital Tallaght, Dublin 24, Ireland[3].

Introduction: Börjeson-Forssman-Lehmann syndrome is a rare x-linked condition associated with learning disability, short stature, obesity, gynaecomastia, small genitalia and dysmorphic features, long ears, tapering fingers and short toes. The disorder is due to mutation of the PHF6 gene on the X chromosome.

Aims: The purpose of this report is to describe the phenotype in adolescence and In addition we wish to highlight the features useful for diagnosis of this underrecognised condition.

Methods: Clinical history, detailed physical examination and clinical photography were carried out for a 15 year old male presenting with obesity and learning disability. The diagnosis was confirmed by molecular genetic testing.

Results: He was born to non-consanguineous healthy Irish parents. He had a raised BMI of 29, height 3rd to 10th centile; physical examination is remarkable for gynaecomastia, large ear lobes, tapering fingers and hypogonadism. Endocrinological investigation revealed growth hormone deficiency. He has a learning disability and dyspraxia and is attending main stream school with assistance.

Conclusion: The fundamental key to clinical diagnosis is recognition of combination of obesity, learning disability and subtle dysmorphic features such as large ears and tapering fingers. These patients can gain significant benefit from symptomatic supportive treatment and counseling of family.

P02. Incidental finding of a beaked vertebra.

F McElligott[1], V Donoghue[2], E Crushell[1].

[1.] National Centre for Inherited Metabolic Disorders, Children's University Hospital, Dublin, Ireland, [2.] Department of Radiology, Children's University Hospital, Dublin, Ireland.

Case: A 14 month old boy was referred for investigation of possible dysostosis multiplex associated with a storage disorder. Having presented with pectus carinatum, a lateral chest x-ray showed abnormal anterior "beaking" of the 2nd lumbar vertebral body (L2), confirmed on lateral spinal view (figure 1). He was the first child to non-consanguineous parents. He was otherwise well with normal growth and development. Examination showed pectus carinatum but no other abnormalities were found. There was no coarsening of features; skin, hair, joints, hands and spine appeared normal.

Skeletal survey did not reveal other skeletal abnormalities. Ophthalmology assessment was normal. Urinary mucopolysaccharides and oligosaccharides were normal, as were Leukocyte α-iduronidase, iduronate-2-sulphatase, galactose-6-sulphatase, α-mannosidase, and β-galactosidase.

Discussion: The finding of a beaked vertebra is rare and may be associated with lysosomal storage disorders (in particular the mucopolysaccharidoses), bone dysplasias, and neuromuscular conditions. Pectus carinatum, while occasionally the presenting feature of Morquio syndrome, is usually an incidental finding in healthy children. Increased prevalence in families suggests a hereditary origin.

Our case is unusual given the strong suggestion of initial radiographs, which self resolved. We hypothesise that delayed maturation of a growth centre may have lead to a temporary minor modelling abnormality in L2.

P03. Optimising the application of IHC in identifying germline MMR mutations in HNPCC.

Gillian Rea[1], Alex Magee[1], Maurice Loughrey[2].

[1.] Northern Ireland Regional Genetics Service, [2.] Department of Pathology, Royal Victoria Hospital, Belfast.

In the investigation of individuals with potential Hereditary Non-Polyposis Colon Cancer (HNPCC), immunohistochemistry (IHC) for mismatch repair (MMR) proteins may direct subsequent germline molecular genetic testing and need for screening endoscopy. Delays in the time taken to obtain IHC results will negatively impact the overall time taken to obtain mutation results.

The aims of this study were to assess current practice and streamline the use of IHC to enable an overall reduction in the time taken to obtain a molecular genetic result.

We carried out a retrospective case notes review of 32 cases with abnormal IHC results, examining the time at which IHC was requested. This audit of current practice revealed that IHC was requested at the time of surgery in 2/32 cases; at the time of referral to clinical genetics in 4/32 cases and after genetics clinic in 26/32 cases. To date 12 germline MMR mutations have been identified. The time taken to obtain IHC MMR results was variable but significant.

We have made a number of recommendations and actions to increase the frequency with which IHC is requested at the time of surgery or at the time of referral. We plan to re-audit our practice in 12-18 months.

P04 BRCA1/BRCA2 Mutation Negative Hereditary Breast Cancer in Ireland.

Fatima Al Oraifi, Trudi McDevitt, Nuala Cody, Marie Meanie, Cliona de Baroid, Rosemarie Kelly, James Geraghty, Andrew Green.

National Centre for Medical Genetics, University College Dublin.

The National Cancer Registry of Ireland statistics reveal that breast cancer is diagnosed in more than 2,000 women every year. Family history is an established risk factor for breast cancer. Studies on twins indicate that most of the excess familial risk is due to inherited predisposition.

The identification of the susceptibility genes BRCA1 and BRCA2 enhanced clinicians' ability to select high-risk individuals for aggressive surveillance, prevention, management, and led to the development of improved therapies. However, BRCA1, BRCA2 and several other identified susceptibility genes account for only 28% of hereditary breast cancer.

In Ireland, patients suspected to have familial breast cancer are referred to the National Centre for Medical Genetics (NCMG) for BRCA1 and BRCA2 mutation testing. Based on the Manchester scoring, we can predict the likelihood of familial breast cancer. As much as 84% are found to be negative to mutations in BRCA1/BRCA2 in Ireland. In this study, we aim to describe the clinical phenotype of affected breast cancer patients negative to mutations in BRCA1 and BRCA2 with a Manchester score of ≥16. We evaluate the proband's clinical status, including the age of onset, bilaterality, histological diagnosis, stage, receptor status, other affected relatives and presence of other cancers within the pedigree.

P05. Validation of Luminex xTAG™ Cystic Fibrosis 39 Kit v2 for Diagnostic Testing & Newborn Screening using Dried Blood Spots.

Melissa Rogers, Solvig Roring, Trudi McDevitt, David E Barton.

National Centre for Medical Genetics, Our Lady's Children's Hospital, Crumlin, Dublin 12.

With the National Newborn Screening Programme (NBS) for CF due to begin in July 2011, a method of detection is required which is amenable to analysis of dried blood spots (DBS), compatible with high sample through-put, and is highly sensitive in the Irish population.

Using the Luminex™ Liquid Bead Array Platform, we evaluated the xTAG™ Cystic Fibrosis 39 kit v2 which tests for 39 of the most common CF mutations found worldwide. The panel of mutations present in the xTAG CF 39 kit has an estimated detection rate in the Irish population of ~93.5%.

We have evaluated a panel of 130 DNA samples of known genotype from a range of sample material, including DBS. DNA was prepared from these samples using a variety of methods (Salting out, Qiagen EZ1 whole blood kit, Qiagen EZ1 tissue kit & phenol/chloroform extraction). DNA derived from DBS has proven difficult to amplify on multiplex assays in the past and is thus of particular interest for NBS.

All samples genotyped correctly indicating that the assay is both sensitive and specific. The assay performed equally well on all sample material including DNA extracted from DBS using the Qiagen EZ1. The system is now validated and in service ready for the CF NBS programme.

P06. Atypical "mild" Non-Ketotic Hyperglycinaemia in siblings.

Malikiwi Andra, Murphy Anne-Marie, Monavari Ahmad.

The National Centre for Inherited Metabolic Disorders, The Children University Hospital, Temple Street, Dublin 1, Ireland.

Nonketotic hyperglycinemia is a rare metabolic disorder that characteristically presents with hypotonia, refractory seizures and death in early infancy. Milder phenotypes presenting with developmental delay and hypotonia are occasionally encountered. Diagnosis is established by measuring CSF: plasma glycine ratio. Molecular characterisation is possible.

Natural history and clinical findings of 2 Irish sisters with atypical NKH are described.

A neonate presented with a history of poor head control and reduced feeding. Examination revealed truncal hypotonia and visual inattention. Brain imaging was normal. Plasma and urine glycine levels were noted to be elevated along with her CSF:plasma glycine ratio.

Her developmental milestones in the first 2 years of life were mildly delayed. Her older sister also previously had a history of poor feeding at birth and developmental delay.

Mutation analysis identified 2 missense mutations on exon 10 (L4221) and exon 23 (V905G) of the GLDC gene. They have learning difficulties with occasional behavioural disturbances and have required the care of a psychiatrist. Tremor and co-ordination difficulties are significant clinical features, seizure however is absent.

A mild form of NKH compatible with long-term survival exists. This diagnosis should be considered in children presenting with developmental delay and appropriate investigations should be implemented.

P07. Validation of the Asuragen Amplidex FMR1 kit for diagnostic Fragile X testing and further characterization of the WHO FRAX Reference Panel.

Michael Sweeney[1], Karen Meaney[1], David E Barton[1,2].

[1.]Division of Molecular Genetics, National Centre for Medical Genetics (NCMG), Our Lady's Children's Hospital, Crumlin, Dublin 12, [2.] School of Medicine & Medical Science, University College Dublin, Ireland.

Fragile X syndrome (FRAX) is the most common cause of inherited mental retardation and is caused by expansion of an unstable (CGG) n repeat in the FMR1 gene. In most diagnostic centres a PCR test is first performed and samples that fail to amplify (males) or show a single normal allele (females) proceed to Southern blot analysis, which is time-consuming and expensive. We have evaluated a fluorescent PCR assay using a panel of DNAs including the recently-certified WHO FRAX Reference Panel. The Asuragen Amplidex assay is based on gene-specific FMR1 PCR, CGG Repeat Primed PCR and employs 1/200th the amount of DNA required for Southern blot analysis, making the assay very amenable to robotic DNA extraction methods.

Following PCR optimization, the assay consistently identified all full mutations and could accurately size normal, intermediate and premutation alleles. All results were concordant with previous Southern blot and in-house PCR results. Overall, this assay is efficient, robust and greatly reduces laboratory workload and reporting times. The sensitivity of the assay will assist in detecting expanded alleles in prenatal samples and in cases with limited starting material. The results obtained have provided additional information on the sizes of normal and premutation alleles in the WHO Reference Panel, which will be valuable to labs calibrating their own assays.

P08. Familial occurrence of distal foregut atresia type I.

Li Yen Ng, Ian Robinson, Roisin Hayes, Harinder Gill.

National Centre of Medical Genetics; Radiology Department, Our Lady's Children Hospital Crumlin.

Aims: To investigate a family in which four members are affected with distal foregut atresia type I

To review cases reported in the literature and ascertain the pattern of inheritance and its pathogenesis.

Methods: 1. Information obtained from a variety of sources including the parents, medical records, radiological and histology reports, 2. Literature review pertaining to the pathogenesis of distal foregut atresia and previous familial cases was undertaken.

Results:

1. **Our Family:** Three female siblings, their maternal uncle and male second cousin were affected by distal foregut atresia. The age of presentation varied from 2 days to 43 months of age. The atresia ranged from complete to partial web, locating from the gastric antrum to preampullary duodenum.
2. **The Literature Review:** Cases reported from regions with high incidence of consanguinity suggesting an autosomal recessive pattern of inheritance. 2 pathogenic mechanisms have been proposed:
   i. failure of recanalisation of solid phase of duodenal development by Tandler in 1900
   ii. vascular theory by Louw and Bernard (Lancet 1995).

Conclusion: Familial distal foregut atresia is rare. It is inherited in autosomal dominant or recessive pattern. Improved knowledge and awareness may help in reaching an early diagnosis. Most children do well after corrected surgery.

P09. A three generation type 2 Stickler family with a multiexonic COL11A1 gene deletion.

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Stickler syndrome, with an estimated newborn prevalence of 1/7500-9000 has no consensus minimal clinical diagnostic criteria. It is a multisystem connective tissue disorder with ocular, auditory, craniofacial and skeletal features resulting in chronic morbidity.

10-20% of Stickler syndrome is attributed to mutations in COL11A1 gene. These individuals typically have more severe hearing deficit and type 2 congenital vitreous anomaly (beaded).The frequency of COL11A1 deletions is unknown. A multiexonic COL11A1 deletion was previously reported by Martin S *et al,* 1999.

The 14 year old female proband has a 9 year history of moderate myopia, joint hypermobility and chronic hearing loss and backache. Ophthalmic findings of congenital beaded vitreous anomaly suggested a type 2 vitreous phenotype. Radiology showed irregularity of end plates of several cervical, thoracic and lumbar vertebrae. A heterozygous COL11A1 deletion of exons 14-24 was detected which was consistent with Stickler type 2 phenotype.

Subsequent investigation identified at least three other affected relatives in 3 generations with variable phenotype. Further assessment of the family is ongoing.

This would appear to be the second but first 3 generation multiexonic COL11A1 Stickler syndrome type 2 family to be reported in the literature highlighting and further defining the phenotypic variability.

P10. 16p11.2-p12.2 microdeletion syndrome.

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Introduction: 16p11.2-p12.2 microdeletion syndrome is a rare condition associated with autism, developmental delay and obesity. This diagnosis is confirmed by array comparative genomic hybridization. The purpose of our report was to describe the adolescent phenotype and highlight diagnostic clues for rare undiagnosed causes of learning disability and obesity.

Methods: Clinical history, detailed physical examination and clinical photography were carried out for a now 16 year old female presenting to Paediatric services in childhood for developmental delay, speech delay and developmental dysplasia of the hip and in adolescence with obesity, learning disability and pubertal delay and

was diagnosed with a 16p11.2-p12.2 microdeletion syndrome by the Genetics service.

Results: The fourth child to non-consanguineous Irish Caucasian parents, physical examination is remarkable for short stature in 3rd to 9th centile and weight on 98th centile with stria on abdomen and webbing of neck. She has a history of headaches and benign intracranial hypertension. She is currently in mainstream school and has mild learning disability.

Conclusion: The early reports of this microdeletion syndrome describe children with autism. However more recently an association with obesity and primary amenorrhea has been identified which are fundamental clues in diagnosis. Further reports are required to delineate this condition.

P11. Next-generation sequencing of known and putative susceptibility genes for schizophrenia and autism spectrum disorders to detect rare high-penetrant risk variants.

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Schizophrenia (SZ) and autism spectrum disorders (ASD) are complex neurodevelopmental disorders that share certain phenotypes including cognitive deficits and some behavioural characteristics. Such similarities suggest that these disorders may share an underlying pathology and thus may share some genetic risk variants. This study involves next-generation sequencing of the exonic regions of 215 potential susceptibility genes in an Irish sample of 150 cases of ASD, 300 cases of SZ and 300 controls, in order to identify single nucleotide polymorphisms, indels and structural variants contributing to one or both disorders. A multiplex target enrichment method is used whereby DNA samples are multiplexed together using DNA indexes/barcodes and enriched for the exonic regions of these genes using the Agilent SureSelect target enrichment method. This is followed by 80bp paired-end sequencing in a single lane of an Illumina GAII. Gene selection comprised of five categories: 1) Interactors of NRXN1, 2) Interactors of DISC1, 3) Genes within the Glutamate Receptor Complexes; NMDA, mGluR5 and AMPA, 4) Cell adhesion molecules and 5) Functional and Positional Candidates. Analysis of the pilot set of samples indicates that the approach undertaken is successful with an even spread of sequence information for 24 indexed samples per lane, >8X coverage for 84% of target regions and overall SNP concordance with previous GWAS data (Affymetrix 6.0) of 99.3%. A preliminary SNP analysis of 219 SZ cases and 206 controls has identified an excess of rare (nonsense) mutations in the cases. We are currently validating these findings using capillary sequencing and details of these analyses will be presented.

P12. Genetic Determinants of Thromboxane and Prostacyclin – an Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) Sub-study.

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Introduction: The balance between two eicosanoids, prothrombotic thromboxane (TxA_2), and antithrombotic prostacyclin (PGI_2), regulates the formation of platelet plugs (thromboses). Thromboses prevent excessive bleeding when blood vessels are injured, but can also block blood vessels, causing heart attacks and strokes. This is the first study to investigate genetic determinants of TxA_2 and PGI_2 levels.

Methods: 544 participants in the HACVD substudy gave urine samples at two separate time-points. TxA_2 and PGI_2 were measured using LC/MS-MS, expressed as pg/mg creatinine to correct for urine concentration. Participants were genotyped on the cardiovascular-specific CVD50K chip, containing >50,000 SNPs. Linear regression analyses were performed assuming an additive model and adjusting for relevant covariates.

Results & Discussion: Nine loci were associated with either TxA_2 or PGI_2 at $P<1x10^{-05}$, two of which exceeded the Bonferroni threshold of $1.6x10^{-06}$. All nine loci were associated with effect sizes of ~0.5 standard deviations of the eicosanoid distributions per minor allele carried.

This study adds to the successes of the CVD50K chip in finding SNPs associated with cardiovascular phenotypes. SNPs associated with TxA_2 and PGI_2 in this study may be novel genetic biomarkers of thrombosis and bleeding risks, and/or provide a pharmacogenetic assay for therapies influencing thrombosis and bleeding.

P13. Characterisation of putative Autism Susceptibility Genes: Translating Genome Wide Analysis to Causation.

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Introduction: In a recent GWA of autism we identified an association within the gene MACROD2.[1] Establishing a functional consequence of association is required to determine a causal link between marker and disease. MacroD2 is a macro domain containing protein which may have a function in DNA silencing and sirtuin biology.[2]

Methods: Expression profiling using a human total RNA master panel. Exon-crossing primers were used to demonstrate the expression profile of MACROD2 across 21 tissues. Promoter mapping using luciferase reporter assays.Promoter mapping constructs were examined in SHSY5Y neuroblastoma cell lines. We observed differential expression of the various promoter lengths. From this data it would appear that an enhancer element of MACROD2 lies ~500bp upstream from exon 1.

Results: MACROD2 is expressed across mulitple human tissues, including brain, kidney, placenta, skeletal muscle, testis and thyroid gland. Promoter mapping constructs were examined in SHSY5Y neuroblastoma cell lines. We observed differential expression of the various promoter lengths.

Discussion: This project is structured to examine the biological

role of MACROD2 in humans, with the hypothesis that disruption of MACROD2 will impact on the structure and/or function of the neuron.

References: 1. Anney R, *et al*. A genomewide scan for common alleles affecting risk for autism. *Hum Mol Genet*. 2010;**19(20):**4072-4082. 2. Chen D, *et al*. Identification of Macro Domain Proteins as Novel *O*-Acetyl-ADP-Ribose Deacetylases. *J Biol Chem*. 2011;**286:**13261-13271.

P14. Heritability of subcortical brain structures in temporal lobe epilepsy.

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Introduction: Temporal lobe epilepsy (TLE) exhibits a complex inheritance pattern and is likely caused by interaction of multiple environmental and genetic factors. As part of an ongoing effort to identify brain structural endophenotypes, we aimed to i) identify TLE-related changes in brain structural volume and ii) compare the heritability of such structures in TLE patients to published values calculated in neurologically healthy control populations.

Methods: MRI-based volume measurements of a number of subcortical brain structures were calculated in TLE patients, their unaffected siblings and healthy controls. Structural volume measurements of TLE patients were compared to those of the healthy controls. The heritability of the structures that displayed volume changes was calculated in the patients and their unaffected siblings.

Results: Significant reduction in hippocampal, amygdalar, and thalamic volume was found in TLE patients. Similar trends of volume reduction across the same structures were also observed in the unaffected siblings of TLE patients. High heritability value was observed for thalamic volume and was comparable to those previously reported; however, the heritability values for the hippocampus and amygdala were reduced.

Conclusion: Although a role for genetic factors in the development of TLE is likely, environmental factors (such as early brain insults and repeated seizure activity) seem to play a significant role in causing the observed volume loss in the hippocampus and amygdala. The reduced heritability of the volume of these two structures may affect their suitability as TLE-related endophenotypes. Given the observed volume reduction and high heritability of thalamic volume in TLE patients, the thalamus fits the profile for a novel TLE-related endophenotype.

P15. The interaction of vesicle associated membrane protein and sterol regulatory element binding protein and the implications for amylotrophic lateral sclerosis.

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Vesicle associated membrane protein–associated proteins (VAPs) are endoplasmic reticulum membrane proteins implicated in diverse cellular functions. Recently missense mutations in the gene encoding the VAPB protein has been found in patients with familial neurodegenerative disorders such as amylotrophic lateral sclerosis. It has been shown recently that cholesterol and triglycerides levels could be a prognostic marker for ALS patients. However, data is limited with conflicting reports and conclusions as to whether hyperlipidemia or dyslipidemia can prolong the survival of ALS patients and whether a statin treatment regime would be beneficial. In order to determine the molecular mechanisms of this connection between VAPB and lipid metabolism, the interaction between VAPB and sterol regulatory element-binding protein (SREBP), a transcriptional regulator of lipid metabolism was investigated. This work identified a novel functional and physical interaction between SREBP and VAPB. VAPB knock-down and over-expression experiments indicated that VAPB may potentially be a negative regulator of SREBP. Whilst further analysis is required to determine the precise mechanisms involved in the interaction between SREBP and VAPB, our initial results suggest a potential role for deregulated lipid metabolism in neurodegenerative diseases.

P16. Galactosaemia, a systemic glycosylation defect? Biochemical and molecular aspects.

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Classical Galactosaemia (Gal) is a rare autosomal recessive disorder of carbohydrate metabolism caused by GALT deficiency. It is screened for as part of the Irish National Newborn Screening Programme. Long-term outcomes of treatment with dietary galactose restriction are extremely disappointing as the pathophysiology is poorly understood.

Methods: We developed biochemical and molecular methods to study systemic glycosylation **(**HILIC fluorescence of *N*-glycans enzymatically released from whole serum and IgG) and gene expression (Affymetrix U133a plus2.0arrays from T-cell RNA) in adult Gal patients (n=12, 5 female and 7 male) with differing neurological outcomes. We also examined these parameters in patients undergoing supervised dietary galactose modification.

Results: HILIC of *N*-glycans released from serum glycoproteins (in particular IgG) demonstrated specific peak differences in informative patients with improvements noted with slightly higher galactose intake. Microarray and KEGG analysis demonstrated multiple pathway disturbances related to systemic glycosylation abnormalities (up to 67 KEGG pathway glycosylation genes affected) with specific central pathway genes also affected such as *SCL5A3* (myo-inositol co-transporter).

Conclusion: Our studies indicate Galactosaemia functions as a systemic glycosylation defect. We propose serum *N*-glycan analysis in combination with T-lymphocyte microarray data will provide improved biomarkers to understanding the pathophysiology of this and related disorders with a view to improving therapeutic options.

P17. Investigation of High Resolution Melting analysis as a tool for mutation detection.

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High Resolution Melting (HRM) analysis using dsDNA-binding dyes and real-time PCR instrumentation is an attractive mutation detection technique due to its rapid, high-throughput and sensitive post-PCR analysis. HRM methodology is based on amplifying a region of interest using primer specific PCR, followed by gradual denaturing of the target region and generation of a melt curve, allowing successful detection of genetic variation in the sequence. In order to test the efficiency of HRM we applied it to exonic regions of the VIPR2 gene. Our two-stage approach to testing the HRM method was: (1) Analyse a proportion HapMap CEU samples of known mutation content based on online data (1,000 Genomes) to optimize performance of the method. (2) Blindly screen remaining HapMap CEU samples for mutations and compare results with online data. Stage 1: Melt curve analysis successfully identified a high proportion of expected mutations in tested HapMap samples. Stage 2: We will report on the specificity and sensitivity of the method when applied to remaining HapMap samples. If the method shows accurate performance, it can be applied to our large sample of schizophrenia cases and control samples. Along with subsequent capillary sequencing, it could potentially identify rare risk variants in patient samples.

