

Abstracts

9th Meeting of the Irish Society of Human Genetics, Thursday 7th September 2006

Trinity College, Dublin



PROGRAMME:

- 10.00 - 11.00 Registration / Tea and coffee
11.00 - 11.01 Welcome
11.01 - 12.00 Spoken presentations - Plenary I
12.00 - 13.00 **Keynote address:** Dr Alan Irvine - "Genetic skin barrier defects in atopy"
13.00 - 14.00 Lunch + poster viewing
14.00 - 15.15 Spoken presentations - Plenary II
15.30 - 16.00 Tea / coffee & poster viewing
16.00 - 16.15 Business meeting
16.15 - 17.15 **Plenary address:** Prof Tim Spector - "Use of twins for gene discovery"
17.15 - 18.00 Wine Reception / Presentations / Meeting Close

ABSTRACTS

SPOKEN PAPERS:

S1. An approach to therapy for retinitis pigmentosa: siRNA-mediated suppression of IMPDH1

Lawrence Tam, Anna-Sophia Kiang, Avril Kennan, Pete Humphries.

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Inosine monophosphate dehydrogenase I (IMPDH1) has been identified as the gene responsible for the RP10 form of autosomal dominant retinitis pigmentosa (adRP). Mutations within the IMPDH1 coding region have been associated with significant conformational changes in protein structure, resulting in the formation of aggregates with concomitant depletion of GTP. In contrast, IMPDH1^{-/-} mice have shown a slower and milder form of retinal degeneration than that observed in human RP10 patients. These observations suggest that the disease pathology is not caused by haploinsufficiency of normal IMPDH1, but by a dominant negative phenotypic effect exerted by mutant protein. As a result, RP10 is a potential target for therapeutic intervention where simultaneous ablation of wild type and mutant IMPDH1 alleles by siRNA molecules may decrease the rate of photoreceptor degeneration to a similar extent to that observed in the IMPDH1^{-/-} mouse model. In this study, we have identified two highly efficient siRNAs and

have demonstrated very successful suppression of IMPDH1 transcripts at the mRNA and protein level in mammalian cell cultures and murine retinal explants. Our results indicate the potential of these siRNAs to form the basis of a worldwide therapy for the many patients harbouring mutations in the IMPDH1 gene.

S2. Novel Retinal Expression of Beaded Filament Structural Protein 2 Observed in the C57BL/6JOLA^{Hsd} Mouse.

Alison Reynolds, Avril Kennan, Paul F. Kenna, G. Jane Farrar, Pete Humphries,

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The Bfsp2 gene (also called CP49) encodes an intermediate filament protein, whose expression to date has only been reported in the eye lens fibre cell. Some substrains of the 129 strain of inbred mouse carry a 6303bp deletion within the Bfsp2 gene, which includes the start of exon 2 and results in dramatically reduced expression levels, rendering these substrains functional knockouts. The only recorded phenotype of this functional knockout is a slow but progressive loss of optical clarity with age. During the analysis of a microarray study, performed to compare retinal gene expression levels in C57BL/6JOLA^{Hsd} and 129S2/SvHsd mice, it was noted that the expression of Bfsp2 mRNA was reduced in the 129S2/SvHsd strain. We have subsequently confirmed, by diagnostic PCR, that this substrain of 129 mice also carries the known 6303bp mutation in Bfsp2. Novel retinal expression of Bfsp2 mRNA was confirmed by real time rtPCR in the C57BL/6JOLA^{Hsd}. Histological analysis was carried out to verify the real time rtPCR findings and localise the retinal location of Bfsp2 mRNA expression. The results of *in situ* hybridisation in mouse retina localise Bfsp2 expression to the photoreceptor layer, possibly within the inner segments in the C57BL/6JOLA^{Hsd} retina. These results point to a previously unknown role for Bfsp2 within the retina.

S3. Hypoxia-inducible suicide gene therapy strategy for prostate cancer.

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Exploiting tumour hypoxia represents a novel gene therapy approach for cancer. We have cloned hypoxia response elements (HREs) from oxygen-responsive genes including vascular endothelial growth factor (VEGF) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) upstream of the cytosine deaminase (CD) gene. Constructs drive expression of this prodrug activation enzyme, converting inactive 5-fluorocytosine (5-FC) to active 5-fluorouracil (5-FU), allowing selective killing of vector containing cells. Constructs were transfected into 2 prostate cancer cell lines (DU145, 22Rv1) and exposed to oxygen concentrations of 0.5% (pO₂<2mmHg). Western blot analysis indicated detectable CD at 16h with a peak at 48 hours hypoxic exposure as well as 24h after a 3h-hypoxic exposure. No expression was observed in aerobic cells, confirming the specificity of the approach. Transfected cells exposed to hypoxia for a 48h period showed a decrease in cell number and associated cell death following a 5 days aerobic 5-FC treatment at the clinically relevant dose of 1mM, when compared to untransfected as well as transfected aerobic controls. These data suggest that targeting hypoxia using a gene therapy approach has demonstrated efficacy in selective killing of prostate cancer cells. Furthermore, cells exposed to 5-FC in hypoxia appear more sensitive to the treatment than aerobic cells.

S4. Association of the IL18 gene with celiac disease in the Irish population

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IL18 is a proinflammatory cytokine which promotes development of the Th1 lymphocyte response. IL18 is known to play an important role in inflammatory, autoimmune and infectious diseases. The aim of this study was to investigate any genetic association between IL18 and celiac disease in the Irish population.

We genotyped 395 celiac disease patients and 354 controls for 4 SNPs within IL18. SNPs were chosen to allow a haplotype tagging approach based on previously defined haplotypes consisting of 27 SNPs as characterised in a European population. SNPs were also chosen based on potential effects on protein structure or expression. Haplotypes were constructed computationally and compared between groups.

Three SNPs in IL18 (IL18_S2-137_rs187238, IL18_S3_rs5744241 and IL18_S4-607rs1946518) showed a significant association with disease either at allele frequency, genotype frequency or carrier status level prior to correction for multiple testing. IL18-137 was significantly associated with disease at the genotype level (p=0.0380). IL18_S3 was significantly associated with disease when allele frequencies (p=0.0385) and carrier status (G allele; p=0.0490) were

examined. IL18_S4-607 also showed a significant association at the allele frequency level (p=0.0100), the genotype level (p=0.0265), and at the allele carrier status level. (G allele; p=0.0159). Total gene haplotype analysis was carried out using all four tSNPs. A significant association was observed for haplotype rs1946518T, rs187238G, rs2043055A, rs5744241A (p=0.0379). Analysis of the IL18 promoter SNPs (S4-607 and S2-137), which have been associated with altered IL18 expression, revealed a significant association for two promoter haplotypes (haplotype -607 G, -137 G) (p=0.0153), (haplotype -607 T, -137 G) (p = 0.0053).

In this study, we have shown that IL18 is significantly associated with disease status at the level of haplotype and functional SNP allele and genotype distribution suggesting that IL18 is involved in the pathogenesis of celiac disease.

S5. An Interesting Fragile X Family

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We describe a Fragile X family manifesting an interesting FMR1 inheritance pattern. Patient A presented with global developmental delay, speech problems and dysmorphic features not typical of Fragile X. His cousin had classic Fragile X showing a full FRAXA FMR1 expansion mutation. Patient A was found to have a 38 (CGG)_n repeat FMR1 allele. Somatic mosaicism was excluded by Southern blot analysis. Patient A's mother, grandmother and maternal aunt all have expansions in the premutation range and an unusual X inactivation pattern was detected. The size of the CGG₃₈ repeat allele was confirmed by sequencing which also revealed a pure CGG tract with no AGG interruptions. This was suggestive of a regression mutation from a larger expansion. Alternative mutations around the promoter / CGG repeat region were also excluded by this analysis. Immunohistochemistry analysis showed FMRP was detectable in 94% of Patient A's lymphocytes, however the level of functional FMRP was not measured by this technique. Presented with these results we reported Patient A "as unlikely to be affected with Fragile X-A". Subtelomeric MLPA analysis is underway for Patient A. Microsatellite and (CGG)_n repeat analysis of the FRAXA region is ongoing to trace the inheritance of the unstable FMR1 allele.

S6. Update on the complex CF mutation spectrum in the Republic of Ireland

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The incidence of CF in Ireland is 1/1461.¹ The National Centre for Medical Genetics has provided a cystic fibrosis (CF) genetic testing service since its inception in 1995, using an ARMS method designed to detect the 11 most common mutations in the Irish Republic (sensitivity of 93%). A total of 3,243 patients with clinically diagnosed, classical CF or with symptoms suggestive of CF, have been tested to date. The ARMS test has provided a confirmatory genotype in

768 of these patients (approximately 24%). A cohort of 129 probable CF patients, with one or no mutation identified by the ARMS method, were selected for comprehensive mutation screening. Small gene alterations were screened for by DGGE and dHPLC and confirmed by sequencing. QMPSF was used to identify large genomic rearrangements. This additional analysis has so far resulted in the identification of a second CFTR gene alteration in 59/129 patients, and the identification of both CFTR gene alterations in 2/129 patients. A total of nine patients have not had any mutation identified. Four novel point mutations and one *de novo* (2623-2A>G) mutation have been identified as well as two multi-exon deletions involving exons 2-4 and exons 14-15. We present details of these findings and assess their contribution to the CF mutation spectrum of the population of the Republic of Ireland. ¹Cashman *et al.* The Irish Cystic Fibrosis Database. *J Med Genet* 1995;**32**:972-975.

S7. The MTHFR 1298CC and 677TT genotypes have opposite associations with red cell folate levels

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Individuals homozygous for the thermolabile variant (677TT) of methylenetetrahydrofolate reductase (MTHFR) exhibit reduced folate status as evidenced by a drop in the biomarker red cell folate (RCF) compared to those who carry at least one 677C allele. The 677TT genotype is reported to protect against colon cancer when folate intake is high. We now report that a different polymorphism in the same enzyme, namely 1298A>C is associated with increased RCF levels.

Many previous studies of the 1298A>C polymorphism have been confounded by the fact that it is in linkage disequilibrium with the 677C>T variant. Our study eliminated this problem by focusing on a large number of subjects with the 1298A>C polymorphism all of whom were 677CC (wildtype) homozygotes. We studied 508 subjects, determined their genotypes at MTHFR positions 677 and 1298 and measured levels of plasma and red cell folate and homocysteine. Consistent with previous studies, the 49 677TT individuals had reduced RCF levels ($P=0.0001$) compared to 677CC wildtype individuals ($n=231$). However, among the 231 677CC wildtype individuals, the 41 1298CC homozygotes had significantly higher RCF levels compared to 1298AA ($P=0.0016$). Thus, these two common polymorphisms change a

metabolic phenotype in opposite directions.

Our results suggest that future studies into the relationships between MTHFR and disease should take these phenotypes into account. The association of the 1298CC genotype on RCF indicates that the reported protective role of this variant in cancer risk is by a mechanism that is different to the 677TT genotype.

S8. Examining Extracellular Domains Identifies New Candidate Genes Subject to Positive Selection in Humans and Chimpanzees.

Lilian PL Lau, Daniel G Bradley, David J Lynn.

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Genome-wide analyses of positive selection in the human genome have recently become possible with the availability of the chimpanzee genome and offer the chance to identify those genes that were of most importance during our evolution. A number of studies have investigated signatures of adaptive evolution in our genome but have uncovered relatively few cases of positive selection acting on protein coding genes. By examining a particular domain, the extracellular region, where selection is likely very prevalent due to the functions of these regions in antigen and ligand binding and pathogen interactions, we aim to identify additional candidate genes that may have been missed in previous analyses. A mouse outgroup is used, where possible, in the comparisons of human and chimpanzee extracellular encoding regions, to provide directionality to the signal of selection. 592 genes were identified with $d_N/d_S > 1$ in the extracellular domain on the lineage leading to humans. In pairwise comparisons, we have identified a dataset of more than 1,100 genes with $d_N/d_S > 1$ in this region, approximately 65% more than have been previously identified. From both these datasets we identify a set of 28 genes with roles in immunity, olfaction, neuronal development and metabolism that have robust statistical support for positive selection and discuss the potential selective forces that may be driving adaptive evolution in them.

S9. Allele frequency spectra in disease populations

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The Ewens-Watterson (EW) test compares the within-population distribution of alleles at a locus to a theoretical distribution. If allele frequencies show a preponderance of one or a few alleles and a number of much rarer variants, directional natural selection may be inferred. This effect may be mimicked in disease population samples, where risk associated alleles may be over-represented. In order to evaluate this effect, we have analysed data from two association studies, one case-control sample with a known coeliac disease association, and a second sample of family trios with reading disability association, using a modified EW test, to account for the step-wise mutation model of microsatellite loci. Both datasets show evidence of significant allele frequency distortion at the disease associated locus in populations of affected individuals. Furthermore, there is some indication that the test may be sensitive to parent of

origin effects. The test may, therefore, provide additional information, particularly in Transmission Disequilibrium Test studies, which consider transmission by heterozygous parents, and thus disregard some of the available data. Furthermore, the test may provide insight in situations where more than one allele at a locus is associated with disease, as the entire allele frequency spectrum is considered.

S10. SuperAIMs: Detecting Intra-Continental Population Stratification

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Stratification occurs in human populations when individuals within a group have varying degrees of ancestry from different sources. Knowledge of this is important in case/control association studies where a mismatch in the ancestry of the two cohorts can cause spurious false positive association between a gene and a phenotype. It can also be useful for identification of genes through admixture mapping and exploring population origin and history. Stratification is detected using genome markers whose alleles show large frequency differences between populations and consequently are informative as to ancestry. These are termed Ancestry Informative Markers (AIMs) and are well described for the differentiation of continental level ancestry. However, as diversity is less within continents, intra-continental AIMs are more difficult to identify and are rarer. We have examined up to 3.45 million SNPs typed in three populations of the HapMap project (Europe, Africa, Asia) and in a Native American cohort for population specific F_{ST} branch lengths. Those showing exceptionally high divergence in one of these continental groups were then typed in 1052 individuals from 51 global populations in the Human Genome Diversity Panel to identify those that also show intra-continental variation. These may act as 'superAIMs' able to distinguish regional continental ancestry.

POSTER PRESENTATIONS:

P1. Towards Tissue Specific RNA interference – A Study of Novel RNAi Delivery Vectors Designed to Achieve Suppression Using a Polymerase II Transcribed Single Transcript

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RNA interference (RNAi) is a post-transcriptional, sequence-specific gene-silencing technology that utilises double stranded (dsRNA) molecules to degrade messenger RNA containing homologous sequence. A significant body of research has been undertaken in the field of suppression (utilising RNAi) *in cellulo* and *in vivo*, however the majority of these studies have utilised synthetic siRNAs or polymerase III promoter-driven shRNAs which are ubiquitously expressed. The aim of this study was to create and evaluate a polymerase II system that could be used to achieve long term expression of functional siRNAs. This approach combines PolII promoters with cis-acting hammerhead ribozymes. Potent siRNA molecules do not tolerate many over-hanging bases 5' or 3' of the double stranded region of the molecule, the cis-acting hammerhead

ribozymes in the designs act to remove the extra overhanging bases created by PolII promoters. In summary, the designs produced functional siRNA and suppressed the target reporter gene (eGFP) both *in cellulo* and *in vivo*. As the promoter used in this study (CMV) is a PolII promoter, it can potentially be substituted with any PolII promoter including a range of tissue specific promoters. The benefit of an RNAi system which can potentially use any promoter type is the ability to maintain a level of control superior to traditional methods. Additionally, an increased type-1 interferon response is a risk associated with any novel siRNA delivery method; however, these constructs did not elicit a significant type-1 interferon response compared to traditional H1 transcribed shRNA.

P2. Mutation analysis of KCNQ1, KCNH2, KCNE1 and SCN1B genes in genome of patients with LQT syndrome

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LQT syndrome is a cardiovascular disorder characterized by an abnormality in cardiac repolarization leading to a prolonged QT interval on the surface ECG. There is a significant risk of syncope, *torsade de pointes* and sudden death. Inherited long QT syndrome was first clearly described in 1957. There are two variants, the autosomal dominant Romano-Ward type and the autosomal recessive Jervell and Lange-Nielsen type. Genes mutated in patients with LQT syndrome encode ion channels. Long QT is associated with two cardiac muscle ion channels: voltage-gated K⁺ channels (KvLQT1-KCNQ1 gene, minK-KCNE1 gene, HERG-KCNH2 gene, MiRP1 subunit) and voltage-gated Na⁺ channels (SCN1B).

The objective of this study is to identify the underlying genetic basis of patients with LQT syndrome anamnesis. In this group of patients we have identified 19 nucleotide exchanges with probable pathological influence, 14 nucleotide polymorphisms and IVSs and two amino acids polymorphisms in genes KCNQ1 (11p15.5), KCNH2 (7q35-q36) and KCNE1 (21q22). This basic set of three LQT genes has been extended about next candidate gene-SCN1B (19q13.1) gene. The mutation analysis was performed using methods multiplex-PCR, multiplex-SSCP (single strand conformation polymorphism) as the screen method, and automated sequencing. This work was supported by grants IGA MZ R NA 7424-3 and NR/8063-3.

P3. Polymorphisms within the Regulatory Region of the Human MTHFD1 Gene

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The human *MTHFD1* gene encodes a trifunctional cytoplasmic enzyme involved in folate metabolism and plays an important role in the biosynthesis of DNA. A polymorphism, R653Q, within *MTHFD1* has previously been identified as a maternal risk factor for the development of neural tube defects (NTDs), abruptio placentae and unexplained mid-trimester miscarriage in the Irish population (Brody *et al.*, 2002; Parle-McDermott *et al.*, 2005(a); Parle-McDermott *et al.*, 2005(b)). This indicates that *MTHFD1* plays an important role in normal development and, therefore, expression and regulation of the *MTHFD1* gene will be critical for the developing embryo. We sought to identify polymorphisms within the *MTHFD1* gene that influence transcriptional regulation.

We previously determined that the transcriptional initiation window of *MTHFD1* mRNA occurs between -150 and -30. SNP rs1076991 occurs within this initiation window and, therefore, is a good candidate to influence gene expression. We performed luciferase reporter gene assays to investigate the possibility that this polymorphism influences transcriptional regulation of the *MTHFD1* gene. These assays were performed using cloned fragments of the *MTHFD1* promoter (from position -600 to -6) that contained either the 'C' or the 'T' allele. The results from three separate experiments (each experiment was performed in triplicate on two consecutive days) revealed that the mean transcriptional activity of the 'T' allele is $43.3 \pm 20.9\%$ that of the activity of the 'C' allele. This suggests that this polymorphism may have a direct functional affect on expression of the *MTHFD1* gene.

P4. Comparing Y chromosome haplogroup frequencies and surnames of Norse and Irish origins in men in Northern Ireland

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The paternally-inherited Y chromosome is widely used for studies of population diversity and human migrations. Studies have found that that nearly 90% of the Y chromosomes in Irish populations derive from haplogroup (HG) R1b3. By contrast, modern Norwegians have roughly equal proportions of HG R1b3, R1a1 and I1a. We have used this difference to examine the Viking contribution to the genetics of males in Northern Ireland (NI). Ten Single Nucleotide Polymorphisms (SNPs) were tested in 244 men from Northern Ireland. Additionally, a subset of 157 of these individuals were typed for 17 Short Tandem Repeats (STRs). Surnames were divided into four groups according to origin: Irish (n= 74), Norse (n=94), Scottish (n=34) and English/Norman (n=42). At the HG level, although the Scottish and English/Norman populations were distinct from the Norse and Irish names, the Norse and Irish showed no differentiation. Preliminary analysis of the STR variation shows little differentiation within samples in the

R1b HG. Of the 21 Northern Irish samples in HG I, seven were found to be in HG I1a. All seven of these were surnames of English, Scottish or Norman origin. It appears even the minimal Viking contribution to the modern NI population likely derives from Scotland or England, rather than directly from Norway.

P5. Does Noggin cause twinning?

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We report monozygotic twin boys of a spontaneously conceived triplet pregnancy with facio-audio-symphalangism (FAS) syndrome. Their non-identical triplet brother is unaffected. In addition, to the typical dysmorphic facial features of FAS they also had hypermetropia and conductive deafness due to congenital stapes ankylosis.

In 1994 Lynch *et al* reported monozygotic twin boys of a spontaneously conceived triplet pregnancy with caudal appendage, short terminal phalanges, conductive deafness, cryptorchidism and mental retardation¹. These boys had the typical facial appearance of facio-audio-symphalangism. Their unaffected triplet sister was not affected. It is highly probable that both these sets of monozygous twins have a Noggin mutation.

Spontaneously conceived triplet pregnancies occur in about 1 in 6500 births in the Republic of Ireland²⁻⁴ and 1 in 8000 in the UK⁵. Noggin mutations occur in approximately 1 in 10000 births. Given the unlikely probability of FAS recurring in monozygotic twin boys from a triplet pregnancy we propose that this suggests that a Noggin mutation may predispose to twinning.

There is embryological evidence for this. In 1903, Hans Spemann divided a cleaving amphibian embryo into two halves. The dorsal half developed into a perfectly well-proportioned half-sized embryo containing both dorsal and ventral tissues. The ventral half developed into a "bauchstück" (belly piece). It is now known that the dorsal centre or Spemann organizer is a source of secreted BMP antagonists (such as Noggin, Chordin, Follistatin and Cerberus)⁶. Mutations in Noggin could therefore theoretically predispose to twinning. Our cases add evidence to this theory. We have reviewed pedigrees of families with likely Noggin mutations and have not found an excess of twins. However, it may be that the presence of the non-identical triplet influenced the twinning process or that a certain mutation type is more prone to twinning than others. Mutational analysis on both families is pending.

References:

- 1: Caudal Appendage, Short Terminal Phalanges, Deafness, Cryptorchidism and Mental Retardation; a new syndrome? SA Lynch, SG Lee, VA Murday. *Clin Dysmorph* 1994;3:340-346.
- 2: Annual reports 1960-1990, Rotunda Hospital, Dublin.
- 3: Annual reports 1960-1990. Coombe Hospital, Dublin.
- 4: Annual reports 1960-1990, Holles Street Hospital, Dublin.
- 5: Multiple births association www.multiplebirths.org.uk

- 6: Regulation of ADMP and BMP2/4/7 at opposite Embryonic poles generates a self-regulating morphogenetic field. *Cell* 2005;123:1147-1160.

P6. Exploration Of Information Seeking Processes And Behaviours By The Parents Of Newly Referred Patients To The Northern Ireland Regional Genetics Service.

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The main aim of this study was to explore the information seeking behaviours of the parents of patients who were referred to the Northern Ireland Regional Genetics Services. This exploratory study analysed themes relating to the resources parents use for information and the motivations for information seeking. The analysis revealed that healthcare professionals are the primary source of information and further information seeking depends on this interaction. Uncertainty, reassurance and responsibility were identified as the main factors contributing to health information seeking during a genetics referral. It also emerged that the need for information and the motivation for seeking information was influenced by the how long the patient had had the diagnosis and how well they were adapting and accepting the diagnosis.

Several points reported in this study are relevant to clinical practice. It is important for genetic counsellors to remain aware of patient's expectations and address information needs at an appointment. The importance of informing patients about what the genetics service can offer prior to the clinic was highlighted. The value in writing to patients after the appointment with information that was given at the appointment was also demonstrated.

P7. An Audit of Genotypes and Borderline Sweat Tests in Irish CF Patients

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The sweat test (ST) is seen as the gold standard diagnostic test for cystic fibrosis (CF). Borderline STs (i.e. 40-60 mmol/L) present particular difficulties, not only in the clinical diagnosis of a patient, but also in terms of genetic counselling. Borderline results are seen in 4% of STs; 23% of these patients will subsequently be found to have two CFTR mutations, one of which is usually "mild." We investigated CFTR mutations in 105 patients with borderline STs referred to the NCMG from 1995 to 2005. If not originally provided, ST levels were sought retrospectively. ST levels were obtained for 77/105 (73%) of referrals. 43% (33/77) of referrals with "borderline" STs actually had normal ST levels according to accepted reference ranges. None of the referrals with normal STs had two CFTR mutations. 45% of referrals (35/77) were confirmed to have borderline ST levels. Of these, 77% (27/35) had no mutation, 14% (5/35) had one classic mutation and 9% (3/35) had 2 mutations (one classic + one mild). 12% (9/77) had ST levels in the diagnostic range. The high proportion of referred "borderline" STs that actually had normal levels indicates a problem with interpretation or use of ST guidelines

by clinicians. The implications for genetic testing services are: (1) data audit is required in order to maintain testing and reporting standards (2) ST levels should be sought if not provided with a query CF referral (3) a ST should be performed before comprehensive mutation screening.

P8. Association of Methylenetetrahydrofolate reductase (MTHFR) polymorphism and the risk of Squamous Cell Carcinoma in renal transplant patients.

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The relative risk of developing cutaneous squamous cell carcinoma (SCC) is significantly increased following organ transplantation.

Objective: We investigated genetic association with SCC in two pathways associated with cancer risks, with potential for modification by vitamin supplementation.

Measurements: 401 renal transplant recipients (117 with SCC and 250 without any skin cancer) were genotyped for key polymorphisms in the folate pathway (MTHFR:C677T; methylene tetrahydrofolate reductase), and the vitamin D pathway (VDR: Intron8G/T; vitamin D receptor).

Results: Individuals carrying the MTHFR 677T allele had a marked increase in risk of SCC (adjusted OR= 2.54, p=0.002, after adjustment for age, sex, skin type, sun exposure score and immunosuppression duration; lower 95% confidence boundary OR of 1.41). In contrast, VDR polymorphisms were not significantly associated.

Conclusion: Folate-sensitive pathways may play a critical role in the elevated rate of SCC in renal transplant recipients. Further studies are required to assess the impact of high levels of folate supplementation on the incidence of SCC in transplanted and nontransplanted populations.

P9. Dicentric Chromosome 15 Syndrome

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Introduction: Dic (15) syndrome is associated with developmental delay, learning difficulty, epilepsy, autism, and facial dysmorphism. Prader Willi/Angelman Critical Region (PWACR) was postulated to be responsible for the phenotype of dic (15) syndrome.

Methods: We reviewed records for 14 patients with extra structurally abnormal chromosome (ESAC) 15.

Results: 13 patients had 47,+der(15) karyotype; 1 patient with an unidentified heterochromatic segment was excluded. Six patients had involvement of the PWACR region; 4 of these had dic (15) syndrome; 1 result was on amniocentesis and the pregnancy terminated; 1 adult was diagnosed on cytogenetic investigation for recurrent miscarriages. 7 individuals were PWACR negative; 4 probands had cytogenetic analysis for concerns regarding their developmental and/or mild

dysmorphic features, however all 4 patients were later felt to be clinically normal on follow-up. All 7 patients did not have features of dic (15) syndrome.

Discussion: Some of the patients were seen in early 1990s, Involvement of PWACR had been assumed, based on G-banding. We believe that FISH using SNRPN/UBE3A probes for PWACR is today's gold standard. The PWACR negative group did not have features of dic (15) syndrome; therefore we conclude that involvement of the PWACR region is responsible for phenotype of dic (15) syndrome.

P10. Two Cases of AML with 8p11 rearrangements and different clinical presentations

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8p11 rearrangements involving the MOZ gene in AML are rare. The most common translocation being the t(8;16) but variant rearrangements have been reported. The majority of cases are associated with haemophagocytosis. Some smaller studies have suggested that these abnormalities are associated with a poor prognosis, however, it was grouped as intermediate prognosis in the UK AML 10 cytogenetic classification, 1998. We report 2 cases of AML with different 8p11 rearrangements with the characteristic haemophagocytosis, but with very different clinical presentations. The first case is a 51 year old man who presented with DLBCL but relapsed after one year with DLBCL and AML, cytogenetic analysis at relapse showed an apparently balanced t(8;16) translocation and an add(9p). The Second patient is a 61 year old man who presented with M5 AML and an unbalanced t(8;22) translocation.

P11. A third case of a t(1;21) translocation involving AML1 in treatment related leukaemia.

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AML1 gene rearrangements have been reported extensively in the literature. In particular the t(8;21) with ETO/AML1 Fusion in M2 AML, the t(12;21) with TEL/AML1 fusion in pre B cell ALL and the t(3;21) with EVU1/AML1 fusion in blast crisis CML and treatment related AML or MDS with prior therapy with a topoisomerase II inhibitor. However, the AML1 gene at 21q22 similar to the MLL gene at 11q23 is a promiscuous gene with at least 30 partner chromosomes identified in the literature. Rare and novel AML1 rearrangements have also been reported in a number of cases with prior exposure to radiation or topoisomerase II inhibitors. Two cases of a t(1;21)(p36;q21) translocation involving PRDM16/AML1 gene fusion have been previously reported, one with prior exposure to radiation and one after treatment with a topoisomerase II inhibitor. Here we report a third case of a t(1;21) translocation in a patient with therapy related AML following treatment for AML four years previously. None of the drugs used were topoisomerase II inhibitors.

P12. Northern Irish pedigrees with familial pheochromocytoma / paraganglioma syndrome, demonstrate clinical heterogeneity and variable penetrance in addition to a founder mutation

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Paragangliomas and pheochromocytomas are benign, highly vascularized, slow growing tumours derived from the neural crest. Inherited predisposition to the development of these tumours may occur as a result of several cancer predisposition syndromes, including von Hippel-Lindau syndrome, multiple endocrine neoplasia type 2 and the recently described familial pheochromocytoma paraganglioma syndrome. This rare condition is caused by mutations in genes encoding 3 of the subunits of mitochondrial complex II, succinate dehydrogenase, SDHB, SDHC and SDHD. SDHD mutations show maternal genetic imprinting, higher frequency of head and neck tumours and increased risk of tumour development at higher altitudes.

Previous reports of SDHD founder mutations have not involved UK populations. We now present data involving five apparently unrelated Northern Irish families with an identical mutation in SDHD (P81L). These families exhibit considerable clinical variability in terms of tumours present in the known heterozygotes. The families also exhibit variable penetrance, with an age range of 16-60 years in the probands and 30-87 years in non penetrant heterozygotes. This is further highlighted by an age penetrance range of 40-68 years in siblings of one pedigree. In light of this apparent founder effect, the implications for genetic testing in this rare condition will be discussed.

P13. Two patients with different deletions of 1q and a similar phenotype.

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We present two cases with deletions of chromosome 1q44. Case 1 was referred for genetic assessment at age 17m, with failure to thrive, developmental delay, seizures and normal karyotype. She was hypotonic, with brachycephaly, hypertelorism, clinodactyly, wide-spaced nipples and small puffy feet. At age 5y she was hypermetropic, had prominent lower lip pads, widely spaced uneven teeth and severe developmental delay. MLPA indicated a deletion of the 1q telomere region, confirmed by subtelomere FISH to be a terminal 1q deletion. Case 2 was referred at birth in 1993 with microcephaly, low set ears, pre-auricular tag, sloping forehead, prominent orbit, high arched palate, poor feeding, hypotonia and normal karyotype. At age 3y, brachycephaly, protuberant lower lip, wide-spaced teeth, puffy hands and feet, hypermetropia and cerebral palsy were noted. At age 12 he had severe developmental delay and repeat karyotyping identified 46,XY,del(1)(q43q44). MLPA and subtelomere FISH confirmed an interstitial deletion.

1q deletions are rare but have a recognised phenotype.

Subtelomeric “pure” terminal deletions are extremely rare as these are usually complicated by partial trisomy for another chromosome. Our subtelomeric deletion is the 4th reported pure terminal deletion of 1q. These cases also illustrate the value of repeat chromosome analysis in cases of developmental delay and dysmorphism.

P14. Validation of a Luminex-Based Multiplex Assay for 25 Cystic Fibrosis Mutations

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New technologies for multiplex testing for single-nucleotide polymorphisms are finding applications in the diagnosis of genetic disorders. Plans for newborn screening for cystic fibrosis (CF) in Ireland and issues of sensitivity and efficiency with our current “home-brew” ARMS assay led us to look for a multiplexed CF assay which could be adapted to the mutation spectrum of the Irish population. We have an excellent knowledge of the Irish mutation spectrum, as all patients with mutations not detected by our ARMS assay are screened by DHPLC of the entire CFTR gene at the laboratory of Professor Claude Ferec in Brest, France.

Using Luminex™ Liquid Bead Array Platform (Applied Cytometry), we evaluated the Signature™ CF 2.0 ASR from Asuragen (formerly Ambion Biosciences) which tests for the 24 CF mutations included in the ACMG/ACOG CF panel.

We evaluated the assay on a variety of sample types (blood spot, buccal, CVS, amniocyte) and also on a large cohort of DNA extracted from peripheral blood samples (n=468) of known CFTR genotype, to examine the sensitivity and specificity of the assay. A total of 385 samples (82%) were genotyped correctly on initial analysis, while 18% failed to yield a genotype. Assay failures could usually be resolved by dilution of the sample such that the true number of samples that failed to yield a genotype and therefore required a rebleed was 7 (1.5%). No samples were genotyped incorrectly, indicating that the Signature™ CF 2.0 ASR is a sensitive and robust assay for CF diagnostics.

P15. Lujan syndrome: report of a possible case and consideration of diagnostic difficulties

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Lujan syndrome (also known as Lujan-Fryns syndrome) is an X-linked mental retardation syndrome, first described by Lujan in 1984 in four affected males. The patients, sons of three normal sisters, showed Marfanoid habitus, narrow face, high palate and hypernasal speech, in addition to other features. Further patients have been described over the years, some more convincing than others. The patient described here is an 18 year-old male with significant learning difficulties and epilepsy. He is the only child of non-consanguineous parents, and although his father has a brother with learning difficulties, this is not thought to be connected. He is tall and thin, with a long narrow face, high-arched palate, and markedly hypernasal voice. The thorax is narrow, and the legs and arms long. Fingers and toes are slender and hypermobile. Chromosome and Fragile X analyses were normal. This patient

fits the proposed diagnostic criteria for Lujan syndrome, but it is readily apparent that there is a lack of “hard handles” to allow this diagnosis to be made with certainty, indeed to reliably differentiate Lujan syndrome from the many other causes of mental retardation in males.

P16. The angiotensin II type 2-receptor -1332G/A gene polymorphism and coronary artery disease in Ireland

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Introduction. Coronary heart disease (CHD) remains a leading cause of death worldwide. It is a complex disease resulting from interactions between multiple genetic and environmental factors. Recently, an X-linked angiotensin II type 2-receptor gene polymorphism (-1332G/A) was reported to be associated with premature CHD in a family-based study in the UK (Alfakih *et al*, *Eur Heart J*, 2005). We, therefore, undertook a similar approach to identify if there was an association between this polymorphism and premature CHD using an Irish family-based DNA resource.

Methods. A total of 1494 individuals from 580 families were included. Linkage disequilibrium between the polymorphism and disease status was tested using the X-linked reconstruction-combination transmission/disequilibrium test (XRC-TDT).

Results. Of the 580 families genotyped, 156 were informative. No significant association was found between this polymorphism and premature CHD in the Irish family-based collection, either in the whole study group (P=0.34) or when males (P=0.13) and females (P=0.45) were studied individually.

Conclusions. Using the XRC-TDT test, we have found no association between the X-linked angiotensin II type 2-receptor gene polymorphism (-1332G/A) and premature CHD in this Irish family-based study.

P17. Identification of tandem repeat variations using whole-genome shotgun sequences

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Tandem repeat sequence length polymorphisms have been implicated in a wide range of diseases and are also known to modulate the pathogenicity of a number of bacteria. This study describes a bioinformatics approach to detect repeat polymorphisms on a genome-wide scale. A set of 257,256 tandem repeats detected in the human genome sequence were searched against whole-genome shotgun sequences from the Ensembl trace archives. Results were validated using repeat polymorphisms from the CEPH genotype database, showing correlation between the heterozygosity measure of repeat

variability with that inferred from the trace archive search (Spearman 0.172, $p < 0.0005$). Statistics were gathered on the sequences of repeats and on sequences flanking these repeats. Statistical modelling confirmed the findings of previous reports on predictors of repeat variability but also revealed a number of novel predictors, such as a marked increase in variants with certain ranges of GC content. These findings give insight into the forces influencing repeat polymorphism and facilitate predictions of repeat polymorphisms from repeat and flanking sequence. They also enable the estimation of heterozygosity, which will be of use in genotyping studies, where a repeat can be excluded if the expected level of variability is likely to be too low to be detected.

P18. Candidate Gene Analysis of the 21q22 Bipolar Affective Disorder Susceptibility Locus

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A genome-wide scan for linkage in 60 bipolar disorder (BPAD) affected sib-pairs identified weak linkage of region 21q22 to BPAD (D21S1446, multipoint NPL=1.42, $P=0.08$, bipolar type I model). 21q22 is a highly replicated BPAD susceptibility locus. The location of the peak linkage marker, D21S1446, 12Kb upstream of S100B identified it as a candidate susceptibility gene. S100B is a calcium-binding, astrocytic protein that exhibits neurotrophic and neurodegenerative effects. Serum S100B protein levels are increased in schizophrenic and bipolar patients and a two marker haplotype is associated with susceptibility to schizophrenia.

We investigated whether variants within S100B are associated with BPAD in a collection of 125 Irish BPAD trios. SNPs located within the promoter (rs3788266: $P=0.03$) and 3' UTR

(rs2839350: $P=0.02$) of S100B were associated with BPAD. The association increased in significance when restricted to families with psychotic traits, suggesting that S100B is a susceptibility factor for psychosis. The location of the associated variants within the promoter and 3' UTR suggests that they may directly affect expression of S100B. The results of the single- and multi-marker association tests will be presented in addition to association analyses of TRPM2, a 21q22 candidate gene previously associated with BPAD.

P19. The Use of the Gene Dossier in the UK

Fiona J Stewart (on behalf of the UK Genetic Testing Network)

One of the remits of the UKGTN is to ensure that tests being offered through the network have clinical utility. Other countries are now actively looking at methods of assessing the clinical utility of genetic tests. The method used by the UK is the assessment of Gene Dossiers. These are based on the ACCE framework and can be downloaded from the UKGTN website www.UKGTN.org.

One of the important principles is the Test-Disease-Population triad. Providers are asked to give details of the technical methodology, sensitivity and specificity. Information about the disease and, most importantly, how the test result will influence management of the individual and their family must also be provided. Details of the target population, a clear referral pathway and testing criteria should also be supplied.

The gene dossiers are assessed by the Gene Dossier Committee which is a multidisciplinary group and a decision is made on whether the dossier can be accepted.

We feel that this model enables decisions about particular genetics tests to be made in a timely, robust and transparent manner without the enormous resource implications of full scale health technology assessments.