Spoken: Evaluation of a modified Genomics England (GEL) Tiering System for NGS Variant Analysis.

Simon Thomas (Salisbury)

To overcome the bottleneck of variant interpretation, we evaluated the GEL 100KGP Tiering system in the analysis of 140 patients tested by Clinical Exome Sequencing (CES). Loss of function mutations and pathogenic variants in HGMDPro or ClinVar were designated as Tier 1; all other coding variants or within +/- 10 bp were designated as Tier 2. Filtering using MAF >2%, left a total of 92 Tier 1 and 368 Tier 2 variants.Patients in this cohort had previously been tested using one of 36 different gene panels: 57 different variants considered pathogenic or likely pathogenic were identified in 51 patients. Two variants were not covered by CES. 52 of the remaining 55 variants were in Tier 1 giving a diagnostic yield of 52/92 (57%) compared to 3/368 (0.8%) for Tier 2. In 39 patients where a molecular diagnosis had been made, 36 involved only Tier 1 variants.In a second cohort of 102 patients, without a previous diagnosis, the proportion of Tier 1 variants identified by CES in the gene panels applied was 16%.In summary, Tier 1 variants represent around 1/5 of all variants. Restricting analysis to Tier 1 variants dramatically reduces interpretation time without significantly affecting diagnostic yield.

Spoken: 'Dynamic Panelisation' as an approach to clinical exome analysis.

David Gokhale (Central Manchester University Hospital)

The introduction of clinical exome analysis provides the opportunity to improve diagnostic yield compared to traditional genetic testing approaches and panel-based genomic analyses. One way of streamlining analysis is to focus on genes known to be associated with the patient's phenotypes. This is possible by using tools built around the Human Phenotype Ontology (HPO), which link genes to their associated phenotypes in a hierarchical relationship.We have developed a web-based system for the referral of patients for clinical exome analysis that incorporates a 'dynamic' gene panel selection algorithm. Genes selected using this algorithm can be augmented by the manual selection of genes, either individually or by selection of genes from an existing 'fixed' panel.A review of 62 referrals for focused clinical exome analysis that have been received since this referral method was introduced showed the following:1.A very high proportion of the referrals received had multiple phenotypic terms submitted compared to paper-based referrals.2.The attitude of clinicians to the automated selection of a gene panel varied.3.The automated selection of genes for downstream variant analysis was effective but especially if used in combination with manual selection of genes.

Spoken: A targeted exome testing strategy for inherited Intellectual Disability.

Stephanie Barton (Central Manchester University Hospital)

The Deciphering Developmental Disorders (DDD) project* has demonstrated the clinical utility of Next Generation Sequencing (NGS) for patients with undiagnosed intellectual disability (ID) within a research environment. Now that recruitment to DDD has finished there is an urgent need to transfer this experience into the diagnostic service.We set out to provide a pragmatic solution, which balances cost against clinical utility and provides an immediate and beneficial impact to families referred for routine Clinical Genetics assessment in a National Health Service setting.Following discussion with local Clinical Geneticists we agreed on a set of six clinical presentation categories that guided the design of corresponding gene panels: (i) syndromic ID; (ii) X-linked ID; (iii) syndromic seizures; (iv) non-syndromic epilepsy; (v) RAS-MAPK disorders and (vi) brain/ cortical dysplasia. We sequenced singletons using the Agilent SureSelect Focused Exome enrichment and bioinformatically targeted genes within the panels requested by the referring clinician.

Testing of the first 170 patients demonstrates an overall mutation pick-up of ~20%. We are working to improve the assay coverage and optimise panel content in order to increase test sensitivity.We will present details of our results with a focus on specific cases and the challenges of targeted exome analysis.*https://www.ddduk.org/

Spoken: RNA-Sequencing to detect gene fusions in acute leukaemia: A proof-of-principle study.

Melissa Connolly (Birmingham Women's & Children's)

Leukaemia results from an accumulation of genetic mutations in haematopoietic stem cells. Accurate, specific and rapid identification of such mutations is important for patient management contributing to disease classification, prognosis, therapeutic choice and disease monitoring. In acute leukaemia, gene fusions are of particular relevance and are used in disease classification (WHO 2016). Current strategies to detect gene fusions (karyotyping, PCR and FISH) are limited in their ability to detect a wide range of fusions in a single assay and often involve sequential testing at significant cost. In this proof-of-principle study, Next Generation Sequencing of RNA, (RNA-Seq) was used to detect gene fusions in a series of acute leukaemias with known genetic rearrangements. RNA from 14 ALLs and 5 AMLs was enriched using the Archer® FusionPlex® PanHeme assay and sequenced using Illumina MiSeq and HiSeq platforms. Archer® analysis was used for alignment, fusion and variant calling. Fusion transcripts were successfully detected in all 10 patients with known gene fusions. In addition, two previously unidentified gene fusions of potential clinical relevance were detected. Full results will be presented, together with a discussion of how RNA-Seq technologies may be incorporated into genetic testing pathways for leukaemia in clinical laboratories.

Spoken: Nanopore long read sequencing for detection of point mutations and structural variants.

Kezia Brown (Viapath)

Long read sequencing has the potential to identify large variations, going beyond the limitations of “whole genome” sequencing to accurately identify structural variants and point mutations. We describe the multiplex analysis of barcoded BRCA1, BRCA2, SMN1, HLA and LDLR amplicons (3.6 to 16kb) on the Oxford Nanopore Technology MinION. Demultiplexed Nanopore reads were aligned using BWA MEM with SAMtools mpileup used for consensus variant calling. This was found to accurately call Genome in a Bottle truth set variants at 500x read depth with 2D (template + complement) reads.All 10 BRCA1/2 variants were identified although false positives were detected due to systematic (non-random) errors in the 1D data. With the new 1D2 technology and improving base calling software this accuracy should increase. Two LDLR deletions (3.3kb and 500bp) were characterised at the base pair level. Sanger analysis identified Alu repeats flanking the 500bp deletion. Haplotype phasing allowed 16kb SMN1 reads to be filtered enabling the identification of the pathogenic variant. Data analysis is ongoing, including an investigation of the HLA regions. Random error rates are tractable by consensus alignment and over-sequencing. Providing systematic errors are avoided, Nanopore sequencing can deliver unique tools for clinical use and point of care testing.

Spoken: Rapid clinical NGS response to containing infectious disease outbreaks.

Kim Brugger (Addenbrookes)

During hospital stays patients are routinely screened for Methicillin-resistant Staphylococcus aureus (MRSA) infection. Positive swabs are used by infection control teams to identify infected patients, potential outbreaks and wards requiring deep cleaning. A limitation of current laboratory methodology is that it cannot distinguish between different strains of MRSA in high resolution and thus identify the original carrier.During a recent MRSA outbreak at Addenbrooke's Hospital, Cambridge, 11 MRSA isolates from swabs were subjected to Next Generation whole genome sequencing to identify different MRSA strains at high resolution. The results were married up with the tracking of patient movements and staff rosters in the wards, to enable rapid identification of an individual as a potential source of the infection within 7 days of the outbreak, and halt further infections. This demonstrates how a Regional Genetics Laboratory can benefit patients throughout a hospital through collaborations with PHE and Infection Control.

Spoken: Non-invasive prenatal testing (NIPT) for fetal aneuploidy – Challenging cases and reporting conundrums!

Kirsten McKay Bounford (Birmingham Women's & Children's)

The UK National Screening Committee has recommended that non-invasive prenatal testing (NIPT) for common aneuploidies is made available to women at high risk following first or second trimester screening. The West Midlands Regional Genetics Laboratory (WMRGL) began offering a privately funded NIPT service in September 2015, using Illumina's Verifi testing method based on whole genome sequencing. We routinely screen for trisomy 13, 18 and 21, and Turner syndrome if clinically indicated. So far we have tested over 200 samples, with no technical fails, and reportable results obtained for 199 patients. We will present four cases which illustrate some of the technical and reporting challenges we have faced - a result with a borderline NCV, detection of likely trisomy of a chromosome other than 13, 18 or 21, and a false negative and a false positive result, both with follow up. The experience of provision of a private NIPT service has enabled WMRGL to develop technical expertise, and our referring centres to develop clinical protocols in advance of the expected roll-out of NIPT in the NHS in 2018.

Spoken: Challenges in interpretation of relative haplotype dosage analysis (RHDO) data; our experience in implementing a non-invasive prenatal diagnosis (NIPD) clinical service.

Beth Young (Birmingham Women's & Children's)

We have developed and implemented an RHDO method for NIPD of multiple single gene disorders (SGD), including spinal muscular atrophy (SMA), Duchenne and Becker muscular dystrophies (DMD/BMD), cystic fibrosis (CF) and congenital adrenal hyperplasia (CAH). Since the launch of the diagnostic service for SMA and DMD/BMD in September 2016 we have received referrals from the UK and further afield for 19 at risk pregnancies. These samples have ranged in gestational age from 8 to 13 weeks, with an average turnaround time of 12 calendar days.We will present results demonstrating a range of factors that have complicated the testing and interpretation of results, including 2+0 SMA carriers, recombination events, consanguinity, low fetal fraction, and dealing with international referrals.We have demonstrated that despite these challenges, NIPD by RHDO is feasible in a clinical setting, increasing accessibility to many more couples with a pregnancy at risk of a SGD.

Spoken: Automation of bioinformatics analysis to improve efficiency of Non-Invasive Prenatal Diagnosis for monogenic disorders in North East Thames Regional Genetics.

Helena Ahlfors (Great Ormond Street)

We offer an ISO 15189:2012 accredited service for non-invasive prenatal diagnosis (NIPD) for monogenic disorders at our Regional Genetics Laboratory. Here we describe automation of our pipeline to increase reliability and scope, decrease costs and improve turnaround times. Excel macros originally used for analysis have been rewritten using bash scripting to not only automate data processing but also include quality control, monitor potential sample contamination and filter out poor quality data. This pipeline has been applied to NIPD for detection of de novo and paternal mutations. Recently the pipeline was expanded to include an R script to perform relative haplotype dosage analysis (RHDO) using pregnancies at risk of cystic fibrosis as an exemplar of testing based on linkage analysis. Analysis time was reduced by automation and incorporation of quality control checks in the pipeline. Validation was completed by direct comparison with our original pipeline to show 100% concordance. The RHDO pipeline was validated in 12 pregnancies with known outcomes and was also 100% concordant. These pipelines have also been applied clinically for families at risk of rare disorders. Automation and robust quality checking processes for our bioinformatic analysis pipeline has allowed expansion of our NIPD service provision, both in referral numbers and test repertoire. In addition to providing resilience for this growing service, efficiencies in analysis time and reduction in risk of operator error have facilitated the delivery of results within a rapid turnaround time.

Spoken: Reproductive options for patients with mitochondrial DNA disease: using mitochondrial donation to prevent disease transmission.

Emma Watson (Newcastle)

Mitochondrial (mt) DNA mutations are responsible for a broad spectrum of chronic, multisystem presentations that can be present at birth or develop later in life. mtDNA disease is progressive, causing debilitating physical, developmental, and cognitive disabilities and without effective curative therapies. Given mtDNA is strictly maternally-inherited, the ability to provide reproductive options following a genetic diagnosis is of considerable importance to patients and their families. Presently within the Newcastle Highly Specialised Mitochondrial Service, we are able to offer prenatal and pre-implantation genetic testing to some patients; however, preventing the transmission of mitochondrial disease by mitochondrial donation offers further reproductive choice for a broader range of mtDNA mutations. Following the introduction of The Human Fertilisation and Embryology (Mitochondrial Donation) Regulations 2015, the first UK treatment licence for mitochondrial donation was granted to the Newcastle team in March 2017. Here I will describe the complexities of mitochondrial disease and genetics, current reproductive options available to women with pathogenic mtDNA variants, specifically detailing the process of mitochondrial donation and how we hope this new IVF treatment will impact families with mtDNA disease in the very near future.

Spoken: Challenges and complexities in the interpretation of mitochondrial DNA (mtDNA) sequence variants.

Steven Hardy (Newcastle)

Mitochondrial disorders are a group of clinically and genetically heterogeneous disorders characterised by biochemical abnormalities of mitochondrial oxidative phosphorylation (OXPHOS). Mitochondria are under bigenomic control, with ~1150 nuclear genes and 37 genes from the maternally-inherited mitochondrial genome (mtDNA) all contributing to normal organelle function. In the era of large-scale next generation sequencing (NGS), the number of rare and novel mtDNA variants continues to increase, representing a significant clinical and diagnostic challenge. Whilst the ACMG guidelines can be robustly applied to the interpretation of variants within nuclear genes, the utility of these guidelines for mtDNA variant interpretation is far more limited and professional guidelines have yet to be produced for this purpose. This is compounded by additional challenges and complexities in mtDNA genetics including: a) heteroplasmy effects due to a multicopy mitochondrial genome within the cell; b) biochemical threshold effects; c) tissue segregation patterns; d) mt-tRNA and mt-rRNA (i.e. non-protein coding) gene variants; and e) variable expressivity and penetrance of mtDNA variants, even within the same family. We present our experience in the interpretation of rare and novel mtDNA variants identified by either NGS complete mitochondrial genome sequencing or data from the GeL 100,000 genomes project.

Spoken: A comprehensive next generation sequencing service for inherited cardiac conditions; a review of the first three years of implementation.

James Eden (Central Manchester University Hospital)

Inherited cardiac conditions result in heart failure, cardiac arrest and sudden death and are estimated to affect 600,000 people in the UK[BHF]. There are high levels of genetic heterogeneity and for many the risk of a familial cardiac condition is highlighted following a sudden and unexplained event in a relative.In collaboration with the Manchester Royal Infirmary Heart Centre, in January 2014 the Manchester Centre for Genomic Medicine (MCGM) introduced a next generation sequencing (NGS) assay to meet the needs of families affected by these conditions.The strategy involves NGS of 71 genes associated with arrhythmic syndromes, cardiomyopathies and aortic dilatation. From May 2017, detection of copy number variation using bioinformatic analysis of sequence coverage has also been implemented.Referrals are made through monthly multidisciplinary team meetings (MDT) attended by Cardiologists, Clinical Geneticists, Genetic Counsellors and Genetic Scientists. The MDT is also used to review all outstanding tests, discuss unusual cases and prioritise cascade testing. In 1400 referrals for NGS we detected an actionable variant in 35-40% of patients. We discuss the challenges of NGS data interpretation and highlight cases of clinical interest.

Spoken: Exome sequencing identifies a new form of autosomal recessive Spondylocostal/spondylothoracic dysostoses.

Melissa Sloman (Royal Devon & Exeter)

Spondylocostal dysostosis (SCDO) is a congenital vertebral malformation syndrome. Autosomal recessive SCDO is caused by mutations in the DLL3, MESP2, LFNG, HES7 and RIPPLY2 genes, and a family with autosomal dominant SCDO has been reported with a stop-loss variant in the TBX6 gene. We undertook exome sequencing in two families with ≥2 affected pregnancies with severe SCDO, employing a couple analysis strategy, testing both parents for heterozygous variants in a shared gene, to preserve the limited material on the affected fetuses. We identified the same two heterozygous missense variants in cis, p.[Gly162Ser;Arg272Gln], and a splicing variant in the TBX6 gene in parents from both families. All affected foetuses were confirmed as compound heterozygotes by Sanger sequencing. We analysed TBX6 in three further probands identifying the p.[Gly162Ser;Arg272Gln] variant in all cases. In one affected fetus this was inherited with a frameshift mutation, and in a male child inherited with a likely pathogenic missense variant. The third proband (male child) had a SNP haplotype in trans that has previously been reported in patients with congenital scoliosis. Our results demonstrate that bi-allelic loss of function variants in TBX6 are a cause of autosomal recessive SCDO and there is a broad phenotypic spectrum.

Spoken: Rapid analysis of whole-genome sequence of children presenting to neonatal and paediatric intensive care.

Alba Sanchis (University of Cambridge)

The Next Generation Children project aims to perform trio analysis of Whole Genome Sequencing (WGS) data of children and parents from the Neonatal Intensive Care Unit (NICU) and Paediatric Intensive Care Unit (PICU) from Cambridge University Hospitals NHS Foundation Trust, in order to investigate the genetic cause of the disease in undiagnosed children, to assess the feasibility of WGS for this purpose and to explore the potential of a rapid turnaround service.Individuals for whom a genetic cause of disease is suspected, including children with neurological problems, dysmorphic features and metabolic disorders, are eligible to be recruited. Children requiring minimal time in NICU, post delivery, and those in PICU who attend due to trauma or the consequence of treatment for malignancies are excluded. The genetic findings from the trio analysis are assessed using a clinical pipeline, where variants are filtered by allele frequency, functional effect and inheritance. Prioritization of genes from a curated disease-associated gene list is used, and potentially pathogenic variants are then evaluated in a Multidisciplinary Team Meeting (MDT) under a clinical view. Only pathogenic and likely pathogenic variants are reported to the families. To date, we have sequenced 37 trios and analysis of these results is in progress.

Spoken: Comprehensive Rare Variant Analysis via Whole-Genome Sequencing to Determine the Molecular Pathology of Inherited Retinal Disease.

Keren Carss (University of Cambridge)

Inherited retinal disease is a common cause of visual impairment and represents a highly heterogeneous group of conditions. Here, we present findings from a cohort of 722 individuals with inherited retinal disease, who have had whole-genome sequencing (n=605), whole-exome sequencing (n=72), or both (n=45) performed, as part of the NIHR-BioResource Rare Diseases research study. We identified pathogenic variants (single-nucleotide variants, indels, or structural variants) for 404/722 (56%) individuals. Whole-genome sequencing gives unprecedented power to detect three categories of pathogenic variants in particular: structural variants, variants in GC-rich regions, which have significantly improved coverage compared to whole-exome sequencing, and variants in non-coding regulatory regions. In addition to previously reported pathogenic regulatory variants, we have identified a previously unreported pathogenic intronic variant in CHM in two males with choroideremia. We have also identified 19 genes not previously known to be associated with inherited retinal disease, which harbor biallelic predicted protein-truncating variants in unsolved cases. Whole-genome sequencing is an increasingly important comprehensive method with which to investigate the genetic causes of inherited retinal disease.

Spoken: Updating penetrance estimates for syndromes with variable phenotypic manifestation.

Adele Corrigan (Viapath)

Following the introduction of array-based testing for copy number variation in the genomes of patients referred for neurodevelopmental disorders, a number of syndromes were discovered that exhibit incomplete penetrance. Examples include 15q11.2 (OMIM 615656) and 16p11.2 (OMIM 611913) deletion syndromes, and collectively, these syndromes are often referred to as susceptibility loci, as the expressivity of associated phenotypic traits is highly variable. These syndromes also make up a large proportion of the findings in these patients, e.g. 16p11.2 represents ~10% of all syndromic findings.Due to the clinical importance of this group of syndromes, attempts have been made to estimate penetrance for each of these susceptibility loci, most notably by Rosenfeld et al (2013; doi:10.1038/gim.2012.164). This systematic attempt was based on a diagnostic cohort of ~30,000 cases compared to ~22,000 controls and had been an incredibly useful resource for the community. However, this study had not been replicated in the literature and therefore it is not possible to assign maximum confidence to the penetrance estimates that it provides.Here we present preliminary data from a study designed to show whether an independent assessment of penetrance produces similar estimates to Rosenfeld et al. Our assessment is based on a diagnostic cohort of ~30,000 patients referred for testing at our genetics centre. Our penetrance estimates are broadly in line with those previously reported. However, some differences were apparent, which may have implications for decisions made using the published data. For example, the incidence of a number of syndromes are far lower in our cohort and therefore penetrance figures presented by Rosenfeld et al may be may be overestimated for our testing population.

Spoken: Lessons from DDD study results: when “de novo, loss of function” doesn’t equal pathogenic.

Sarah Turton (Birmingham Women's & Children's)

As the largest recruiter to the Deciphering Developmental Disorders (DDD) study, the West Midlands Regional Genetics Service has extensive experience in the validation and classification of variants reported back from the study (664 variants from 545 DDD research reports to date). We will summarise the spectrum of candidate variants and present several challenging cases.Protein altering variants are well known to be associated with uncertainty and difficulties in interpretation. However some loss of function (LoF) variants also present challenges in interpretation, and cannot always be classified as pathogenic. The three cases presented here are examples of de novo LoF variants of uncertain significance; and highlight instances where caution must be applied in using ACMG guidelines to classify such variantsWe report:1)A frameshift variant in SRCAP, outside of the 3’ region in which Floating-Harbor syndrome associated truncating, dominant-negative acting variants have been described2)A stop_gained variant in NAA15, a “probable” DDG2P gene associated with congenital heart disease, in a patient with developmental delay3)A stop_gained variant in KDM5B, associated with non-syndromic intellectual disability but with loss of function variants observed relatively frequently in the ExAC database

Spoken: The clinical utility of functional mRNA evaluation of rare genetic variants in diagnostic practice.

Celia Duff-Farrier (North Bristol)

Accumulating evidence implicates non-coding variation and mRNA splicing in human disease. In silico tools predicting intronic mRNA splicing effects have limited sensitivity/specificity, and deep intronic variants remain undetected by most diagnostic assays. The ability to demonstrate mRNA splicing effects in the diagnostic setting is of high clinical utility in genome analysis. We present the development phase of a diagnostic mRNA splicing service at Bristol Genetics Laboratory. 7 cases have been analysed to date with 3 cases ongoing. Specific cases were selected where functional validation would aid variant classification and patient management. Manifesting Barth syndrome female twins, TAZ c.[646+1del];[=], the first report of heterozygous females displaying a Barth syndrome phenotype. Sensory/motor neuropathy KIF1B c.[3121+1G>T];[=], representing the second mutation found in this gene associated with this phenotype to date. Neurodegenerative phenotype WDR45 c.[236-18A>G];[=], the first report of a variant affecting splicing outside of a consensus splice site junction. Hypertrophic cardiomyopathy MYBPC3 c.[1224-52G>A];[=], the first report of an intron 13 variant affecting splicing outside of a consensus splice site junction. Novel insights into the genetic basis for disease were demonstrated in each instance, confirming the clinical utility of mRNA analysis and highlighting the importance of whole genome analysis to ascertain deep intronic variants.

Poster: A mosaic KRAS G12D variant in tissue from a plexiform neurofibroma in a patient with PTPN11 variant positive Noonan syndrome.

Laura Cobbold (St George's)

The Rasopathies are a group of highly variable developmental disorders caused by germline variants in the RAS-MAPK signalling pathway. They are characterised by distinctive dysmorphology, cardiac abnormalities, short stature and developmental delay. Roughly 50% are caused by variants in the PTPN11 gene.A 34 year old male with a clinical diagnosis of Noonan syndrome due to short stature, ptosis, undescended testes, webbed neck and low hair line, presented with a large plexiform neurofibroma causing discomfort.Analysis of blood DNA on a 23 gene Rasopathy panel identified a c.923A>G p.(Asn308Ser) germline pathogenic variant in PTPN11, accounting for the patient's Noonan features but not the presence of the neurofibroma. A mosaic c.35G>A p.(Gly12Asp) KRAS variant was detected at an allele frequency of approximately 7% in tissue from the plexiform neurofibroma.Plexiform neurofibroma are rarely seen in Noonan syndrome. They are common in neurofibromatosis type 1, another Rasopathy. We believe that the mosaic KRAS variant confers a 'second hit' in the formation of the plexiform neurofibroma.

Poster: VASA: ACMG Variant Scoring Assistant: For Sequence Variant Classification and Associated Evidence.

Philip Davidson (St George's)

UK adoption of the ACMG guidelines for genetic variant pathogenicity scoring initiated us to develop a tool to assist clinical scientists during this routine process.VASA is a web-based tool that guides a user through the process of classifying variants against the ACMG categories; recording evidence that supports decisions and calculating the pathogenicity score. The guidelines can be overridden, allowing scientists to exercise judgment where necessary. VASA retains the calculations, variant annotations and associated evidence in a repository of scored variants for reference in future submissions. Different users can score the same variant differently but all scores and associated evidence is available to all users. It is designed to be integrated into existing workflows: variants may be submitted individually or batched by VCF file to create a work list and the tool has an API for interrogating the database. VASA does not store VCF data, so once scoring is complete there is no patient-specific data stored.The tool also has an application in training. Training sets can be uploaded and scored by multiple users, creating a repository of scores that can be used to understand how the guidelines are being applied.

Poster: Development of an NGS Assay for the Detection of Acquired GATA1 Mutations in Neonates and Young Children with Transient Leukaemia or Myeloid Leukaemia in Down Syndrome.

Rachel Dodds (North Bristol)

5-30% of Down syndrome children are born with Transient Leukaemia of Down Syndrome (TL-DS). Although many cases resolve without treatment, TL-DS results in early death in 15-23% cases and 20-23% of survivors will develop Acute Myeloid Leukaemia of Down Syndrome (ML-DS) in the first 4 years of life. TL-DS is driven by pathogenic variants in the haematopoietic transcription factor gene GATA1 and is only seen in conjunction with Trisomy 21. All cases of TL-DS and ML-DS are marked by the presence of an acquired pathogenic N-terminal variant in the key haematopoietic transcription factor gene GATA1, resulting in a truncated GATA1 protein (GATA1s).This STP project developed two different amplicon-based NGS assay methods capable of detecting low-level GATA1 variants on the MiSeq. The first library preparation method used an Illumina Nextera XT kit, and the second a two-stage PCR protocol. Bioinformatic analysis used an in-house somatic pipeline. 17 anonymised research and clinical samples were used for validation. Sequence data confirming different variant types and frequencies will be presented.The first UK routine clinical assay for these variants will be implemented based on this work in summer 2017, with applications for the diagnosis, screening and monitoring of TL-DS and ML-DS patients.

Poster: The SAFE test. A review of NHS NIPT service provision.

Rachel Dunn (St George's)

In June 2015, St George's Fetal medicine unit commenced non-invasive prenatal testing (NIPT) for women booked at St Georges NHS Foundation Trust who receive a high risk result from combined screening and the Quadruple test, as an alternative to invasive testing.In November 2015 St George's opened the SAFE test laboratory, a dedicated NHS NIPT testing laboratory. The laboratory runs the IONA screening test developed by Premaitha Health to determine the likelihood of a fetus being affected by trisomy's 13, 18 and 21. Maternal and fetal derived cell free DNA is amplified and processed using whole genome shotgun sequencing to determine the ratios of chromosomes 13,18 and 21 and therefore the likely risk of the pregnancy being affected. The workflow is monitored and fully traced using the Premaitha Workflow Manager LIMS system and reports are distributed to clinics using an online portal, making the laboratory virtually paperless. Over the past two years the SAFE team have successfully provided further screening to local NHS patients and has branched out to eight hospitals within the region to offer the SAFE test to patients, if the standard NHS scan/blood test is in a high risk category. Since Nov 2015 the SAFE test laboratory at St Georges Hospital has tested over 2700 samples from NHS and Private hospitals/clinics. From these, 3% were reported as high risk, 0.3% Failed and 97% were reported as low risk, with a false negative rate of 0.04% (1 patient was reported as low risk T18, fetus was affected).In the last year (April to April) at St George's hospital the total number of women who have attended the fetal medicine unit for high risks screening results was 184, of those patients 11 (6%) were reported as high risk, meaning a potential 171 (93%) patients avoided an invasive test. Thus reducing costs for hospitals and patients, also reducing anxieties and the risk of miscarriage to couples.

Poster: DMD Deletions and Duplications are Pathogenic..... Aren't They?

Carl Fratter (Oxford)

Whole exon deletions and duplications of the DMD gene are the most frequent causes of Duchenne and Becker muscular dystrophies (D/BMD). When detected in males with muscular dystrophy, such variants are considered to be disease-causing. Does this mean that all DMD whole exon deletions and duplications should be considered pathogenic?In recent years, increased usage of array CGH has led to increased detection of DMD deletions and duplications in probands referred with non-specific clinical phenotypes. These variants appear ostensibly identical to those causative of D/BMD; therefore, interpretation of the clinical significance of these findings can be challenging, particularly if detected prenatally.We describe three recent cases tested at the Oxford Medical Genetics Laboratories, which provide further evidence that not all DMD deletions and duplications are disease-causing. These cases highlight the need for a cautious approach to the pathogenicity classification of any DMD deletion or duplication detected in a proband without muscular dystrophy. Potential mechanisms for reduced pathogenicity, and investigations to aid the classification of these variants, are also discussed.

Poster: The evolution of bowel cancer services at Oxford Medical Genetics Laboratories.

Jessica Gabriel (Oxford)

Introduction of a next generation sequencing (NGS) cancer gene panel has modernised and standardised germline cancer services in Oxford. One of the most significant impacts has been to our bowel cancer services. For germline genetic testing in bowel cancer and polyp patients, the laboratory has moved from sequential interrogation of genes depending on phenotype with a panel as a secondary option, to NGS of a gene panel as the primary approach. Since introduction of the bowel cancer gene panel, we have detected class 5 (pathogenic) variants in 8% of probands. Although most of these are in the genes expected, there have been a few unusual results. The story is similar for class 3 and 4 variants with some representing a challenge in the context of patient phenotype. These results will be discussed in terms of interpretation within the laboratory and impact on patient care. Panel testing in Oxford is improving turnaround times and uniformity of service provision. Routine testing of more genes across a broad range of bowel cancer patients is expanding the known phenotypes associated with variants in certain genes.

Poster: Confirming variants from the DDD study: The Nottingham Experience.

Rebecca Haines (Nottingham University Hospitals)

Variant assessment and interpretation is a major challenge for genetics laboratories as we move towards routine whole exome and whole genome sequencing. We have developed a variant assessment and MDT process to review results from the DDD project and are expanding this procedure to manage 100K genomes results. Since November 2016 we have investigated 127 variants and confirmed 71, leading to a diagnosis in 50% of cases reviewed. For many of the cases where a variant was rejected as unlikely to be pathogenic the original annotation of the variant was on an inappropriate transcript, and these were often found to have an alternate predicted consequence. However, variants often remain as uncertain clinical significance because of the difficulty in gathering evidence to support the interpretation. Many of the reported variants are in genes that have been associated with a non-specific phenotype and in a small number of patients in the literature. This can make interpretation significantly challenging and has required a paradigm shift from our usual practice of interpreting variants in genes associated with well characterised syndromes. We will present our strategy for variant assessment and describe some unusual cases that have challenged our interpretation of best practice guidelines.

Poster: The Affymetrix CytoScan HD Microarray identifies more Genomic Aberrations in Chronic Lymphocytic Leukaemia Patients than Current Testing by FISH and G-Band Analysis in the NUH NHS Trust.

Emily Hodge (Nottingham University Hospitals)

Background: Recurrent genomic aberrations in chronic lymphocytic leukaemia (CLL) can inform prognosis. Current testing at Nottingham University Hospitals (NUH) NHS Trust uses FISH for TP53 and ATM, with supplementary G-banding in TP53-deleted cases. Genome-wide microarray would provide additional information. This study aimed to validate microarray for CLL referrals. Methods: Microarray of peripheral blood DNA from 23 CLL patients used the Affymetrix CytoScan HD platform. Extended and CLL-targeted microarray analyses were compared with previous or concurrent FISH respectively, and previous G-banding.

Results: Extended microarray analysis was concordant with previous FISH and detected additional CLL-implicated aberrations (n=5 patients). Targeted microarray identified recurrent CLL aberrations (n=19/23), including those which would have remained undetected, e.g. genomic complexity, and clinically relevant phenomena that cannot be detected, by FISH or G-banding: copy neutral loss of heterozygosity (CNLOH) and chromothripsis (n=17/23). Targeted microarray was consistent with concurrent FISH (n=17/18). Microarray detected the same and further aberrations seen on previous G-banding (n=1). Conclusion: High resolution genome-wide microarray in CLL provides more information concerning prognostically relevant aberrations, and analysis is less challenging and time-consuming than current testing. These data support the introduction of microarray as a routine CLL service at NUH, to potentially improve current provision and patient care.

Poster: A diagnostic laboratory experience of interpreting variants in exons 2-10 of TP53 detected by next generation sequencing (NGS).

Sophie Laird (Wessex)

Patients with CLL have a variable disease course influenced by different factors. Disruption of TP53 by deletion or mutation is associated with a poor prognosis, a short time to progression, an early need for treatment, and dismal outcome mainly due to refractoriness to standard chemoimmunotherapy treatment. We report on our laboratory experience of implementing TP53 mutation testing by next generation sequencing technologies for patients with chronic lymphocytic leukaemia (CLL) into an accredited diagnostic laboratory, Wessex Regional Genetics Laboratory (WRGL, UK) and discuss our approach to variant interpretation. TP53 mutation testing was implemented in May 2016 and supplements fluorescence in situ hybridisation (FISH) testing which detects deletions of 17p. Sequencing is undertaken on whole blood or bone marrow leucocyte DNA using amplicon-based next generation sequencing (NGS) on the Illumina MiSeqTM platform for exons 2-10 of the TP53 gene. This technique has been validated to reliably detect a mutation above a level of 10% (although it will detect mutations down to 1%) and analysis of the coding sequences includes at least ±6bp into adjacent intronic regions.We have developed a bespoke algorithm for TP53 variant interpretation in order to determine their pathogenicity based upon a number of sources including the European Research Initiative on CLL (ERIC) recommendations and adaptations to published recommendations for somatic and germ-line variant interpretation. Variants are classified according to the following key factors: (1) predicted effect on protein function according to in silico evidence; (2) frequency and classification within locus-specific and population databases; (3) evidence of clinical significance within the published literature. Based on the compiled evidence, variants are assigned into a 5-tier classification system from class 1- benign- to class 5- pathogenic. Due to the reported impact of acquired TP53 mutations on patients' response to standard therapies in CLL, we consider mutations of TP53 that are assigned as likely pathogenic (class 4) or pathogenic (class 5) as "actionable" as their presence directs the treatment of these patients. Variants assigned as class 3 (variants of uncertain significance, US) may indicate the need for close clinical monitoring and are therefore also reported if present at a variant allele frequency greater than 10%; these variants are acted upon at the discretion of the referring clinician. Variants assigned as benign (class 1), such as validated polymorphisms, or likely benign (class 2), such as variants with evidence to indicate neutrality, are not reported. To date, we have identified 21 different variants in 54 patients tested; 15/21 variants were classified as "likely pathogenic" [n=2: p.(Arg181Cys); p.(Ala347Thr)] or "pathogenic" [n=13: p.(Arg248Gln); p.(Tyr234Cys); p.(Cys277Tyr); p.(Lys319*); c.920-2A>G; p.(Arg273Cys); p.(Arg273His); p.(Arg175His); p.(Val218del); p.(Tyr107_Arg110del); p.(Gln317*); p.(Gly245Asp); p.(Arg248Gln)] and so were reported. A major challenge of using NGS for analysis of TP53 in a diagnostic setting has been interpreting variants that are detected at low level. ERIC recommendations state that only mutations present in allelic fraction >10% should be reported for diagnostic purposes until the prognostic and predictive impact of lower level mutations is validated in prospective clinical trials. However, there is strong evidence that TP53 mutations present at sub-clonal levels are associated with the same clinical phenotype and poor survival in CLL and the use of standard therapy would only select the most aggressive clone. Therefore, although the definition of a clinically relevant threshold is still unknown, it is our policy to report variants detected at <10% VAF when they are known pathogenic variants, such as mutations in hot-spot residues, with the recommendation that testing is repeated particularly if the level of lymphocytosis rises. Of note, 13/21 variants identified were present at <10% VAF (range 1~7%); these were classified as "US" (n=6) and therefore not reported or "pathogenic" (n=7) [p.(Arg248Gln) detected at 2%; p.(Tyr234Cys) detected at 3%; p.(Cys277Tyr) detected at 2%; p.(Lys319*) detected at 1%; c.920-2A>G detected at 2%; p.(Arg273Cys) detected at 7%; p.(Arg273His) detected at 3%] and so were reported in line with our reporting policy.

Poster: The Oxford MDM pathway for triage of WES/WGS variants.

Tracy Lester (Oxford)

In May 2017 the Oxford Regional Genetics Service/Genomic Medicine Centre introduced a weekly multi-disciplinary meeting (MDM) for Clinical Scientists and Clinicians to triage variants received from WES and WGS studies such as DDD and the 100K Rare Disease program based on the clinical utility of further confirmation and reporting. The meeting was trialled initially for a period of four weeks using cases referred by local Consultant Clinical Geneticists. Prior to the establishment of the MDM, variants were assessed primarily by clinical scientists with clinical questions being posed on an ad hoc basis by email to the individual referring consultant. Using this pathway, case review was found to be time-consuming, and a large number of variants were confirmed when in fact the clinical phenotype was not in keeping with current knowledge of the gene. We will discuss our experience and the lessons learnt from developing a new MDM pathway, and will present data from pre-meeting and post-meeting variant confirmations to indicate the impact on service activity, classification types of variants reported, and average reporting times.

Poster: Connected Health Cities: The Routine Reporting of Genomic Results Electronically.

Matthew Parker (Sheffield)

As part of the connected health cities (CHC) programme we aim to revolutionise the way inwhich we report genomic findings from all services in Sheffield Diagnostic Genetics Service(SDGS) including but not limited to sanger sequencing screens, cytogenetics, FISH and nextgeneration sequencing. Currently we process over 22,000 reports per year (Figure 1) andthe majority of these reports are currently distributed as paper documents, and significantly,over 4000 of these reports are destined for clinicians in our host hospital - SheffieldChildren's (SCH). The majority of hospital diagnostic departments, such as histopathology,and clinical chemistry currently deliver results electronically. Locally in SCH this is achievedthrough ICE (Integrated Clinical Environment), developed by SunQuest. Through ICE we willbe able to deliver results electronically to 6 hospitals in the South Yorkshire cluster and infuture a further 5 hospitals in Yorkshire are possible. We have now procured a link to ICE toenable us to deliver our results in this way, however, this transition poses significantchallenges. Unlike most hospital departments using ICE we service patients who are notnecessarily patients within our hospital so we have to ensure that we verify these patientsdata against the NHS spine to ensure the fidelity of our results. Initial data suggests 87.5%of patients within our laboratory information management system (LIMS) verify (i.e. matchingNHS number to patient demographics), an application is therefore under development toallow us to handle clashes and merge the results. Patient and clinician engagement is keyand we will be producing surveys to gauge the acceptance of reporting results in thismanner. We have to ensure we meet information governance requirements and internally wehave to change our reporting process, moving away from word documents to plain text HL7based reports. Additionally, within the Yorkshire & Humber genome medicine centre we areexploring this pathway for the whole genome sequencing results received from the 100,000genomes project. Ultimately having genomic test results alongside routine hospital tests willensure that we can decrease turnaround times, make reporting more efficient and furtherpersonalise the treatment of our patients.

Poster: KRAS G12D mosaicism in a patient with linear epidermal naevus and features of Neurofibromatosis type 1.

Vijaya Ramachandran

Mosaic rasopathies are rapidly expanding group of disorders ranging from simple nevoid skin lesions to their systematized forms and multiorgan involvement. We report an 18 year old female patient who presented with bulky raised verruciform lesions extending from the right arm to the hand following a Blaschkoid distribution, extensive café au lait patch on the back, marked scoliosis and small right leg with no movement. Investigations revealed bone lesions, multiple neurofibroma and biopsy confirmed the right sided lesion as epidermal naevus. DNA extracted from skin obtained from affected area was screened for mutations in 23 genes within Ras-MAPK pathway which detected a missense variant c.35G>A p.(Gly12Asp) in KRAS gene but not in DNA from blood. Somatic KRAS mutations frequently occur in lung, colorectal and pancreatic cancer. KRAS G12D has been reported in nevus sebaceus, linear nevus sebaceous syndrome and non-organoid keratinocytic epidermal nevus. This case demonstrates the skin and bone features consistent with neurofibromatosis type 1.