Spoken

Junior ACGS member: -

NIPT as part of the national screening programme in Wales – a 1 year review

Sarah Anderson

Anderson S.E and Morgan S.M

All Wales Genetics Laboratory

As of 30th April 2018, Wales became the first country in the UK to introduce a fully commissioned non-invasive prenatal testing (NIPT) service for the common autosomal trisomies as part of the national screening programme for Down syndrome, Edwards syndrome and Patau syndrome. The introduction of NIPT to the screening programme means that women in Wales, with singleton pregnancies, who have a higher chance screening result for trisomy 13/18 or 21 are now offered NIPT as an alternative to an invasive test as the next stage in the screening pathway. NIPT testing is carried out at the All Wales Genetics Laboratory which utilises Illumina Verifi technology to detect pregnancies at high risk of trisomy 13, 18 or 21. The technology was introduced into the laboratory in 2017 and the NIPT service was ISO15189 accredited the following year. The laboratory has since taken part in EQA pilot NIPT schemes, achieving satisfactory performance to date. We will describe our experience of NIPT test delivery since its introduction as part of the screening programme with audit data for the first full year of service, and in addition we will assess the impact on the uptake of invasive testing in Wales.

Spoken

DECIPHER (https://decipher.sanger.ac.uk) – Enabling the sharing and interpretation of rare disease variants and associated clinical phenotypes

Junior ACGS member: -

Julia Foreman

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DECIPHER is a major global platform for the visualization of genomic and phenotypic relationships and for sharing pseudonymised rare disease patient records. It hosts ~30,000 searchable open-access records containing ~38,000 variants and >90,000 phenotypes. Six consortia facilitate more refined sharing (including the NHS Consortium linking 20 UK Genetics services), sharing >54,000 patient records, including >76,000 variants and their associated >121,000 phenotypes. DECIPHER provides variant interpretation interfaces, including a genome browser, 2D/3D protein browsers and a dynamic patient/variant matching interface, which displays patients with identical/similar variants and their phenotypes. DECIPHER also incorporates a tolerated population variation calculator, which determines whether a variant observed is too common in the reference sample to cause a Mendelian disorder of interest. The pathogenicity interface supports the classification of variants using ACMG criteria and ACGS guidelines. Also included is the ClinVar Bayesian framework for ACMG variant classification, enabling users to see where on the continuum of risk from Benign to Pathogenic a variant lies. DECIPHER currently supports the deposition of CNVs and sequence variants in GRCh37 or GRCh38. During 2019 DECIPHER will become genomic, sharing other types of variation such as aneuploidy, UPD, STRs, inversions and large insertions. DECIPHER shares patient data for discovery and diagnosis. It is a pioneering partner in GA4GH and founder member of the Matchmaker Exchange, which enables the federated discovery of similar entries in connected databases. Since its inception in 2004, DECIPHER has facilitated over 2,000 publications in peer-reviewed scientific literature; a testament to the importance of match-making in rare disease.

Spoken

Junior ACGS member: Yes

Non-invasive diagnosis of retinoblastoma using cell-free DNA from aqueous humour

Amy Gerrish

Amy Gerrish1, Edward Stone1, Samuel Clokie1, John R Ainsworth2, Helen Jenkinson2, Maureen McCalla2, Carol Hitchcott2, Isabel Colmenero3, Stephanie Allen1, Manoj Parulekar2, Trevor Cole1

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Retinoblastoma is the most common eye malignancy in childhood caused by mutations in the RB1 gene. The initial RB1 mutation may be germline or somatic. Distinguishing between these alternative mechanisms is crucial, with wider implications for management of the patient and family members. Modern eye-saving chemotherapy treatment has resulted in fewer enucleations. As a result, tumour tissue required to identify sporadic RB1 mutation(s) is not always available. Intravitreal chemotherapy (IViC) for retinoblastoma involves prior aspiration of aqueous humour (AH), providing a novel sample source for analysis. Using hybridization-based next generation sequencing, we have designed a test capable of detecting somatic mutations of the RB1 gene in cell-free DNA extracted from the AH of retinoblastoma patients. The assay captures sequences across the whole of chromosome 13 with a higher density of probes across 6.5Mb surrounding the RB1 gene. We have developed an in-house bioinformatic pipeline to detect SNVs, INDELs, LOH and CNVs. The results obtained with fluid from enucleated eyes were concordant with tumour tissue in all 10 cases analysed. In addition, AH analysis from 3 patients undergoing IViC successfully identified previously unknown somatic variants including SNV c.751C>T p.Arg251* and two regions of LOH. This proof of principle study indicates that cell-free DNA from the eye fluid of retinoblastoma patients could be used for somatic mutation studies where tumour samples are unavailable. We plan to further develop this assay using our collection of over 40 additional AH samples obtained from a cohort of 11 patients undergoing IViC treatment.

Spoken

Junior ACGS member: -

CNV and SNV analysis of 844 intellectual disability genes in singleton referrals

Hannah Grayton

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Trio analysis of WGS data represents the gold standard genetic test for complex referrals, as all types of genetic variation can be detected and analysed based on inheritance. Our clinical exome pipeline is utilised predominantly for small panel singleton testing, and is not suitable for large panels of genes. This pipeline has now been modified and validated for the detection of SNV, CNV and LOH in a prospective cohort of 150 diagnostic ID referrals, using NxClinical software to interrogate an ID gene panel of 844 genes. The average time for initial assessment of SNVs and whole genome analysis of CNV was 30 minutes per case. This included classification of roughly 50 SNV events per case and whole genome CNV analysis. In half of the cases no variants were identified for further variant analysis. All CNVs previously detected by array, and containing genes sequenced on the clinical exome, were detected using the whole genome CNV analysis approach. Furthermore, pathogenic SNVs and CNVs, which had not been identified from previous more targeted analysis, were detected in 2 and 15 cases, respectively. A single chimeric sample with a 50:50 distribution of the two genotypes was detected and analysed. Our approach clearly demonstrates that even in the instances where trio WGS is not possible, our single assay approach can efficiently detect pathogenic and likely pathogenic variants, and copy number abnormalities involving known OMIM morbid genes, with no significant burden of variants of uncertain significance requiring clinical assessment.

Spoken

WMRGL integrated reporting of targeted NGS and cytogenetic results: review of the first 1,000 samples

Junior ACGS member: -

David Hill

David Hill1, Paula Page1, Nicola Foster1, Anne Cole1, Louise Giles1, Kim Reay1, Christophe Fleury2, Sam Clokie1, Joanne Mason1, Mike Griffiths1, Manoj Raghavan3

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West Midlands Regional Genetics Laboratory collaborated with Illumina to design a hybridisation-based Nextera Rapid Capture Custom Enrichment version of the amplicon-based TruSight Myeloid Sequencing panel. Key additional features included the ability to detect FLT3 internal tandem duplications, KMT2A structural variants and variants in the CG-rich CEBPA gene. The NGS service commenced in October 2017 for routine analysis of AML and high risk MDS, and low risk MDS at clinical request. Since implementation, over 1,000 samples have been reported. We present an audit of the results obtained to date, together with an overview of our integrated molecular and cytogenetic reporting strategy. Overall we have identified a relevant genetic aberration using a combination of cytogenetics and NGS in approximately 95% of AML/?AML patients and approximately 65% of MDS patients. Using NGS alone we have classified approximately 40% of all patients to the adverse genetic risk group according to ELN 2017. As the service has evolved, we have responded to clinical requests to provide a comprehensive array of in silico panels to cover a broader spectrum of myeloid neoplasias including CMML, JMML, low risk MDS and familial MDS/AML. Following 2018 publication of the NHS England National Genomic Test Directory for Cancer, we have re-designed the panel to cover all* mandated genes for the following indications: AML; MDS; MPN; JMML; ALL and familial MDS and AML (with the exception of NF1 in JMML).

Spoken

A pilot study for the application of Next Generation Sequencing in Cystic Fibrosis Newborn Screening

Junior ACGS member: -

Richard Kirk

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Genetic testing forms an integral part of cystic fibrosis newborn screening in the UK. The screening protocol is a delicate compromise between maximising sensitivity for childhood onset disease and minimising the detection of unaffected carriers or the need for a second bloodspot sample. Application of next generation sequencing (NGS), targeting a large panel of CFTR pathogenic variants, may offer a way to minimise these disadvantages. A one-year pilot study was approved and funded by Public Health England to explore this. DNA from bloodspot samples was tested using the Ion AmpliSeq CFTR community panel (ThermoFisher), and Ion GeneStudio S5 Prime (ThermoFisher) platform, for a panel of 332 pathogenic variants, based on the CFTR2 database. A key element of the NGS assay was the development of a bespoke bioinformatics pipeline, incorporating a variant/genotype scoring algorithm in order to reveal only clinically significant genotypes. During the first seven months of study, 152 samples (>90%) were successfully reported within the 3 working day turnaround time, and 5 additional clinical referrals were made on the initial testing as a result of the NGS assay (from a total of 26 ‘non-normal' outcomes). The stringent turnaround times required by the screening protocol present challenges in the event of sample/assay failure. The technical feasibility of this approach is very promising. The study has also provoked discussion regarding the definition of a ‘clinically significant genotype', and the content of the panel. An independent assessment of costs/benefits of this approach will be undertaken upon the conclusion of the pilot study.

Spoken

A process for gaining consensus gene panels in the Genomic Medicine Service using PanelApp

Junior ACGS member: -

Ivone Leong

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PanelApp (https://panelapp.genomicsengland.co.uk) is a publically available knowledge base reviewed by ~200 international experts following gene assessment guidelines to establish consensus diagnostic-grade gene lists spanning ~50% of OMIM phenotypes. It has > 2 million requests and >10,000 unique visitors from around the world per month. Use of virtual gene panels in whole genome or exome sequencing analysis helps prioritise the evaluation of disease-causing variants. Originally developed to support the analysis of the 100,000 Genomes Project, PanelApp is now playing an important role in facilitating the creation of consensus gene panels for the genetic tests offered by the National Health Service England (NHSE) Genomics Medicine Service (GMS). To unify and standardise genetic testing, the Genomic Laboratory Hubs (GLHs) are collaborating to establish high evidence-based consensus panels under the new GMS. PanelApp is used as the platform to help create these consensus panels and is also a repository for collating the evidence, comments and details on panel composition, which further facilitates efficient and transparent discussions between clinical scientists, clinicians and researchers. One major benefit of using PanelApp for this process is that panels in PanelApp are publically available and versioned, allowing for transparency and revisions. To ensure that NHSE GMS panels will remain at the forefront of medical knowledge, additional evidence and reviews from experts worldwide can be collected on panels. Subject to NHSE governance and approval, these may then inform future updates.

Spoken

Exome sequencing of 875 parental/fetal trios with structural abnormalities revealed by ultrasound in the UK Prenatal Assessment of Genomes and Exomes (PAGE) project

Junior ACGS member: -

Dom McMullan

Dom McMullan [1], Jenny Lord [2], Ruth Eberhardt [2], Gabriele Rinck [2], Sue Hamilton [1], Liz Quinlan-Jones [3], Lucy Jenkins [4], Richard Scott [4], Denise Williams [1], Mark Kilby [3], Eamonn Maher [5], Lyn Chitty [6], Matthew Hurles [2]

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PAGE aimed to apply whole exome sequencing (WES) to 1000 trios recruited in the UK-NHS to identify pathogenic variation underlying heterogeneous fetal structural abnormalities detected by ultrasound scan (USS). Whole genome sequencing (WGS) is being carried out on a proportion of cases with complex phenotypes which are negative by WES. Trio WES was conducted after resolution of pregnancy if conventional testing (QF-PCR, chromosomal microarray and/or targeted single/panel gene testing) failed to establish a definitive diagnosis. Variants were triaged via a stringent clinical filtering pipeline established for the UK Deciphering Developmental Disorders (DDD) project using a gene panel adapted iteratively from the DDG2P gene panel throughout the course of the project. Potentially pathogenic variants were assessed and classified by a UK-wide multidisciplinary clinical review panel (CRP), technically validated in NHS accredited labs and reported back to Clinical Genetics units and families where appropriate.
From 875 duos and trios reviewed, diagnostic variants were identified in 97 cases (8·1%). Diagnostic yield varied considerably by phenotypic class, with multisystem phenotypes showing the highest yield (16.4%). The majority of variants are SNVs/indels which would escape targeted detection by conventional testing. Further analysis is predicted to identify new genes and mechanistic associations underlying observed phenotypes as more samples are processed
PAGE aims to catalyse responsible adoption of WES and potentially WGS into routine prenatal clinical diagnostics and lessons learned will be assimilated into the planned introduction of WES for such referrals in the NHS in England in 2019.

Spoken

Pathogenic non-coding variants in inherited retinal disease from the 100,000 genome project

Junior ACGS member: -

Kathryn Oprych

Kathryn Oprych1,2, Emma Clement2, Samantha Malka3, Genomics England Research Consortium, Lyn Chitty2,4,5, Andrew Webster1,3, Gavin Arno1,2,3

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Introduction Inherited retinal diseases (IRDs) are the commonest cause of blindness in working age adults across the UK and Wales. This study aims to identify pathogenic non-coding variants from cases that underwent whole genome sequencing (WGS) in the 100,000 Genomes Project, which contribute towards the missing heritability of IRD. Methods This research was made possible through access to the data and findings generated by the 100,000 Genomes Project. 1276 patients were recruited to the 100,000 Genomes Project from the IRD clinics at Moorfield's Eye Hospital. Following the clinical diagnostic pipeline to identify pathogenic variants in the 313 virtual gene panel for posterior segment abnormalities, we performed non-coding variant analysis to identify candidate pathogenic intronic variants. RT-PCR was used to analyse candidate variants. Results Three patients with an Usher Syndrome phenotype harbouring a heterozygous USH2A coding mutation were identified. Each case had 1-2 candidate intronic variants predicted to cause cryptic splicing. In one patient found to harbour a heterozygous coding mutation (c.1036A>C p.Asn346His), we identified a deep intronic variant (c.4885+375A>G). RNA analysis confirmed the introduction of a 130bp pseudoexon, causing a frameshift and premature termination of the mRNA transcript. This provides a full molecular diagnosis for this patient. Conclusion WGS and detailed analysis of non-coding regions enables the identification of pathogenic non-coding variants that would be missed by focusing on protein-coding regions in isolation. Incorporation of this pipeline into clinical services would improve the diagnostic rates in IRD cases, which is important for guiding appropriate investigations, risk predictions and treatment options.

Spoken

Identifying potential germline variants during somatic testing: a selection of challenging cases from the WMRGL Haemato-Oncology Service

Junior ACGS member: Yes

Kim Reay

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The West Midlands Regional Genetics Laboratory Haemato-Oncology Section offers NGS panels that analyse targeted regions of genes associated with myeloproliferative neoplasia (MPN), acute myeloid leukaemia (AML), myelodysplastic syndrome (MDS) and chronic lymphocytic leukaemia (CLL). Each of these somatic panels contains cancer susceptibility genes that are also implicated in neoplastic disorders. This presentation gives an overview of a selection of cases that have caused us to question when follow-up germline studies should be offered, how these should be performed and how results of these studies should be reported. As the number patients being tested on somatic panels has increased so has the number of potential incidental germline findings, and it has become apparent that there is a need for consensus guidelines for optimum management of potential germline variants; currently being drafted by the ACGS with input from our laboratory and other stakeholders. Our experience has highlighted the challenges of identifying potential germline variants during somatic analysis of haematopoietic and lymphoid tissue disorders and that effective management requires a multidisciplinary approach.

Spoken

Non-Invasive Prenatal Diagnosis for Sickle Cell Disease by Droplet Digital PCR and Next Generation Sequencing

Junior ACGS member: Yes

Joe Shaw

Joe Shaw1, Sophie Sheppard1, Natalie Chandler1, Helena Ahlfors1, Chris Sale2, Michael Parks2, Lee Silcock2, Lyn Chitty1

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Introduction: Sickle cell disease (SCD) is one of the most common single-gene indications for prenatal diagnosis in the United Kingdom. Non-invasive prenatal diagnosis (NIPD) using cell-free-fetal DNA is desired by patient groups, but current NIPD methods for recessive disorders in clinical laboratories require samples from the mother, father and a previously affected child. Droplet digital PCR (ddPCR) and next generation sequencing (NGS) offer the potential to provide NIPD for SCD using only a maternal sample. Methods: NGS and ddPCR assays for NIPD were compared by testing archived maternal plasma samples from pregnancies at risk of SCD. Relative mutation dosage for the HBB c.20A>T pathogenic variant was performed on 18 samples using an NGS workflow from Nonacus Ltd, and on 22 samples using an in–house ddPCR assay. Results: The NGS method generated 10 correct fetal genotype predictions, with 2 incorrect predictions and 6 cases which were inconclusive, whilst the ddPCR assay made 12 correct predictions with 2 incorrect and 8 inconclusive results. Sub-optimal fluorescence amplitude and threshold positioning were identified as causes of incorrect ddPCR results, whilst incorrect NGS results were caused by low read counts following in silico size selection. There was no significant correlation between fetal fractions measured by ddPCR and NGS. Conclusions: ddPCR and NGS offer the potential for NIPD of SCD using only a maternal sample, although incorrect classifications were generated using both methods. Increasing sequencing depth and fluorescence amplitude may improve results for NGS and ddPCR, respectively, and further development is required.

Spoken

100,000 Genomes Project: Validating Whole Genome Sequencing (WGS) for clinical use in Cancer Programme

Junior ACGS member: -

Alona Sosinsky

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The 100,000 Genomes Project aims to improve cancer care for NHS patients in UK through personalised medicine. To date, bioinformatics reports for WGS, with links to potentially relevant therapies and clinical trials, have been produced for thousands of cancer patients and returned to clinicians to help with diagnosis and treatment choices. Currently our bioinformatics analysis of WGS includes somatic small variants, somatic structural and copy number variants (SVs/CNVs), germline pertinent findings, mutational signatures and mutational burden. WGS has the potential to replace multiple standard of care tests as it can detect all types of mutations (SV/CNV/SNV/indels) as well as emerging biomarkers such as mutational signatures and burden in a single test. WGS has been carried out on a research basis in different types of malignancies but if it is to become acceptable as the diagnostic test to inform the clinical management of patients then it must be validated against existing standard of care tests. In this study we use samples submitted as part of the 100,000 Genomes Project to investigate the feasibility of WGS as an alternative to conventional testing. Overall comparison of WGS with the results of NGS panels, cytogenetic FISH tests, Immunohistochemistry tests for Mismatch Repair Deficiency and HER2 status demonstrated Positive Percentage Agreement > 90% and a False Positive Rate < 5%. Further work is required to fully validate all aspects of the WGS analysis pipeline but these results indicate that WGS has the potential to reliably detect clinically relevant biomarkers in the genomes of cancer patients.

Spoken

Junior ACGS member: Yes

Increased diagnostic yield from gene-agnostic trio analysis of 100,000 Genomes Project sequence data

Karen Stals

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The South West NHS Genomic Medicine Centre recruited 1278 index cases with rare disease to the 100,000 Genomes Project. SNV, indel and mitochondrial variants for 811 cases were received from Genomics England via Congenica. Within the assigned gene panels we identified likely pathogenic/pathogenic tier 1/2 variants in 137 cases; a diagnostic yield of 16.9%. A gene-agnostic bioinformatics analysis to filter variants from the 100,000 Genomes Project vcf files according to mode of inheritance and frequency was adapted from our trio exome pipeline. Variant annotation includes synonymous variants predicted to affect splicing and a positive filter for HGMD Pro/ClinVar Pathogenic and Likely Pathogenic variants is applied. This analysis will identify de novo, compound heterozygous, homozygous, X-linked recessive, uniparental isodisomy and mitochondrial variants. Analysis of 106 affected child-unaffected parent trios identified (likely) pathogenic variants in 7 cases (6.6%), increasing overall diagnostic yield to 17.8%. The additional diagnoses included a TBL1XR1 missense variant that was mosaic in the father, ZC4H2 de novo missense variant, a maternally inherited IL1RAPL1 frameshift in an affected sibling, de novo STAG2 frameshift and compound heterozygous frameshift/stop loss PPP1R13L variants. STAG2 was recently published and not included in the Intellectual Disability gene panel. Additional PPP1R13L cases were found through GeneMatcher. HGMD annotation identified a PMM2 founder variant and previously reported synonymous KAT6B variant. Gene agnostic trio analysis combined with enhanced variant annotation increased the diagnostic yield and highlighted additional bioinformatic approaches with potential utility for improving monogenic rare disease diagnosis through the NHS genome sequencing pipeline.

Spoken

Genetic testing and variant classification in Primary Ciliary Dyskinesia reveals considerable genetic heterogeneity despite a high proportion of homozygous cases

Junior ACGS member: -

Simon Thomas

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Primary Ciliary Dyskinesia (PCD) is a clinically heterogeneous condition in which dysfunction of motile cilia causes chronic or recurrent infections of the airways and ears, and organ laterality defects in 50% of patients. PCD has an estimated prevalence of one in 10,000 births, but founder mutations and consanguinity affect the incidence in specific populations. Over 40 genes are associated with PCD and these are predominantly autosomal recessive. Screening of 124 patients using a 29 gene panel identified 38 with a positive molecular diagnosis (31%). Five positive cases have also been returned from the 100KGP. Of the 43 combined positive cases, 23 were homozygotes and 20 compound heterozygotes (three with one SNV and one CNV). A further 13 patients had a single pathogenic or likely pathogenic mutation. A total of 76 variants in 14 genes were classified as pathogenic or likely pathogenic using the ACMG guidelines. 46 variants were identified in only a single family while the remaining 30 had one of 11 recurrent variants. The majority (72%) of variants were both loss of function and rare or absent from gnomAD so both PVS1 and PM2 could be applied. Certain lines of evidence (PM3 and PS4) were also commonly applied while others (PS3, PS2 and PP1) were applied rarely or not at all. The high proportion of homozygotes (in the literature as well as in this study) complicated variant classification. This study demonstrates the genetic heterogeneity associated with PCD and the challenge of applying ACMG guidelines to recessive conditions.

Spoken

Junior ACGS member: -

The analysis of clinically relevant structural variants in 100K cancer genomes

Jamie Trotman

Jamie Trotman, James Drummond, Jonathan Bruty, Laura Freestone, Karola Rehnstrom, Claire Beyeler, Emily Li, Johannes Visser, Mathew Murray, Amos Burke, Liz Hook, James Nicholson, Steve Abbs, James Brenton, Sam Behjati, Lucy Raymond, Patrick Tarpey

Addenbrookes

The introduction of whole genome sequencing (WGS) into routine clinical practice heralds an era of opportunity for the treatment of patients with cancer. This endeavour, builds on the experience of the 100K Genome Project, and the pathways and analysis strategies put in place by local Genome Medical Centres (GMCs). A major and unique advantage of WGS, is the provision of detailed structural variant information, via algorithms reporting discordant read-pairs and copy number events. Structural variants, including activating gene fusions, can present as attractive targets for therapeutic intervention. In the East of England GMC, we have identified known and novel structural variants which have illuminated the histopathology of tumours and influenced the clinical management of patients. In one case, WGS from a paediatric brain tumour identified a novel 40Mb tandem duplication on chromosome 7, apposing exons 1-2 of the ZNF394 gene and exons 10-18 of the BRAF oncogene. The resultant in-frame fusion protein is predicted to be activating, and has presented an opportunity for the administration of a MEK inhibitor as targeted therapy in a young patient. In this presentation we will detail this, and other cases, which highlight the advantage of WGS in harvesting structural variants of valuable clinical utility.

Lightning

Whole genome sequencing in a series of Primary Ciliary Dyskinesia cases identifies molecular diagnoses in DNAH5

Junior ACGS member: Yes

Holly Black

Holly Black1,2, Sophie Marion de Proce1, Alison Meynert3, Mihail Halachev3, Jose L Campos3, Kenneth Macleod4, Don Urquhart4, Stefan Unger4, Tim Aitman1, Pleasantine Mill3

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Primary ciliary dyskinesia (PCD) is a disorder of motile cilia, in which abnormal cilia structure and/or function results in chronic respiratory tract infections, due to impaired mucociliary clearance. In some cases, the phenotype includes male infertility and situs inversus. PCD usually presents as neonatal respiratory distress and, over time, recurrent infections result in bronchiectasis. A diagnosis of PCD is confirmed by assessing ciliary ultrastructure and motility from a nasal brush biopsy. In most cases, PCD is recessively inherited and, to date, around 40 genes have been associated with PCD, accounting for up to 70% of cases. Here, we present the initial results of whole genome sequencing (WGS) of 8 probands with confirmed diagnoses of PCD and parental samples, where available. Initial variant calling was performed for the PCD-associated genes listed on Genomics England PanelApp. This identified three molecular diagnoses of PCD due to biallelic loss-of-function variants in DNAH5, a gene encoding part of the outer dynein arm of the cilia. Loss-of-function of DNAH5 is therefore consistent with the outer dynein arm defect and static cilia phenotypes observed in these cases. Interestingly, in one proband, WGS identified a point mutation on one allele and a 13kb deletion on the second allele, a result that would have been difficult to identify using a targeted amplicon or capture-based sequencing technology.

Lightning

Junior ACGS member: -

Review of FFPE tissue testing in patient with Colorectal Cancer

Naomi Bowers

Naomi Bowers, Helene Schlecht, Andrew Wallace

Manchester University NHS Foundation Trust

Mutation analysis of a patient's pathology sample can be used to distinguish between a diagnosis of hereditary and sporadic colorectal cancer and therefore exclude a diagnosis of Lynch syndrome. However, the majority of archive pathology specimens are formalin-fixed paraffin-embedded (FFPE) tissue, the subsequent analysis of DNA extracted from such FFPE samples is challenging. Unlike the clinically relevant mutation spectrum of genes currently analysed on FFPE tumour DNA, such as KRAS or EGFR, where the distribution and number of mutations is small, thousands of clinically relevant variations in APC, BMPR1A, MSH6, SMAD4, MLH1, MSH2, MUTYH, POLD1, POLE, PTEN and STK11 have been described and these are distributed widely throughout multiple, large coding regions and intron-exon boundaries. Next Generation Sequencing (NGS) methods have the potential to detect variants at low admixture levels due to the clonal nature of the method and also offers a way to reduce the amount of input DNA required. NGS therefore offers a potential solution to this challenging type of analysis. After 2 years of running a diagnostic service for variant screening of FFPE tissue in colorectal cancer patients we present a number of interesting cases where a somatic cause for the colorectal tumour has been shown or where a germline inherited pathogenic variant has been identified.

Lightning

Junior ACGS member: -

Histopathological assessment training for Genomics Clinical scientists

George Burghel

George J Burghel, Michael P Bulman, Jade L Harris, Marta Pereira, Naomi L Bowers, Anne-Marie Quinn, Helene B Schlecht, Andrew J Wallace

Manchester University NHS Foundation Trust

Molecular characterisation of nucleic acids derived from pathology/tumour samples plays an important role in cancer precision medicine. Reliable detection of somatic variants in tumour samples requires careful morphological characterization of the sample in addition to the enrichment of neoplastic cell content (NCC). The level of enrichment required depends on the downstream molecular testing technology, current best practice requires the NCC levels to be at least two times higher than the lower limit of detection of the molecular test in order for a heterozygous non-clonal pathogenic variant to be reliably detected. Therefore, determination of the levels of NCC can harness molecular therapy more reliably and achieve clinically sound interpretation of the testing results, especially negative ones. Pathologists have a unique role in the patient care pathway with an opportunity to integrate data from diverse technologies for patients and clinicians. However, due to increased workload of pathology laboratories and due to rising demand for genomic testing, there is an unmet need to build experience and capacity for NCC estimation in disciplines with no morphological-based expertise. To meet this need, a training programme of pathology assessment for clinical scientists has been established in at the Manchester Genomic Diagnostic laboratory. The programme extends over a period of 4-6 months and is led by a consultant Histopathologist. We present the training experience from Manchester together with some interesting cases to reflect the importance of the histopathological review and the difference that trained clinical scientists could make.

Lightning

Cost effective screening of the PKD1 and PKD2 genes in patients with autosomal dominant polycystic kidney disease (ADPKD)

Junior ACGS member: Yes

Lewis Darnell

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ADPKD genetic testing was rarely performed due to low clinical utility in combination with a relatively expensive test, complicated by the presence of multiple PKD1pseudogenes. Approval of the drug tolvaptan for ADPKD patients with rapid disease progression increased clinical utility and demand for genetic testing as the result aids stratification of patients by their likely speed of disease progression. To reduce the cost of tolvaptan assessment an in-house, cost-effective, and less labour intensive, test was needed. In addition for efficient batching the testing for ADPKD had to be incorporated with other tests into a single workflow. To fulfil these requirements a custom designed next-generation sequencing (NGS) library preparation kit from SOPHiA GENETICS combined PKD1 and PKD2 genes with 36 additional genes which included gene panels for the hereditary cancer NGS testing service and several other genes previously tested by Sanger sequencing. Free access to SOPHiA DDM analysis software dispensed with the need of developing an in-house bioinformatics pipeline. SOPHiA DDM allowed fast, accurate and user friendly analysis of the sequence data including bioinformatic differentiation of PKD1 from its pseudogenes and copy number variant analysis. The efficiencies of the NGS workflow also resulted in a reduction in turnaround times, MLPA use was reduced by over 95% for the genes involved and more even coverage resulted in fewer Sanger repeats compared with previous methods. With the above strategy, over 900 hours of staff time have been saved per year and the cost of ADPKD testing has been significantly reduced.

Lightning

Methylation Sensitive High Resolution Melting (MS-HRM) Assay for the Detection of BRCA1 and BRCA2 Promoter Hypermethylation

Junior ACGS member: -

Gareth Gerrard

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BRCA1 & BRCA2 genes encode key components of the DNA double-strand break repair pathway. Cancers driven by loss of BRCA1/2 are associated with sensitivity to PARP inhibitors (PARPi), such as olaparib, through the synthetic-lethality of concomitantly blockading the single-strand repair pathways mediated by PARP. Monoallelic BRCA1/2 mutations require a 'second-hit' to the unaffected allele, since only tumours with complete abrogation of BRCA1/2 are targetable with PARPi. One recognised second-hit mechanism is gene promoter hypermethylation. We sought to use extant MS-HRM protocols (using kits from MethylDetect ApS, Denmark) to implement a BRCA1/2 promoter hypermethylation assay. 14 triple-negative breast carcinoma (BC) and 13 prostate adenocarcinoma (PA) FFPE samples were sourced from the Imperial College Healthcare Tissue Bank. They were macro-dissected to obtain DNA from paired tumour and normal tissue. 100ng DNA from each was bisulphite-treated in a 20µL reaction and 3µL used in the MS-HRM reaction, along with CpG-flanking primers for either BRCA1 or BRCA2. Kit provided methylation controls were used for IQC. The MS-HRM reactions were run on a Qiagen RotorGeneQ and analysed with the RotorGene v2.3.1.49 software. 7/13 (53.8%) and 0/12 BC samples showed BRCA1 and BRCA2 promoter hypermethylation, respectively; none of the 13 PA samples were hypermethylated for either gene. 1 BRCA1 and 2 BRCA2 samples failed to yield usable results (both BC). The detection of BRCA1 hypermethylation in over half of the BC samples in this limited-scale implementation of a low-cost, rapid and sensitive assay, demonstrates the potential utility of this approach for stratifying patients for PARPi therapy.

Lightning

Targeted microarray design increases detection of clinically-relevant variants across multiple NHS genomics centres

Junior ACGS member: Yes

Jana Jezkova

Jana Jezkova, A Williams, J Heath, D Barrell, S Corrin & S Morgan

All Wales Medical Genomics Service, NHS Wales
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West Midlands Regional Genetics Laboratory - Birmingham Women's NHS Foundation Trust

In recent years, chromosomal microarrays (CMAs) have been widely adopted by clinical diagnostic laboratories for postnatal constitutional genome analysis and have been recommended as the first-line test for patients with intellectual disability, developmental delay, autism and/or congenital abnormalities. Traditionally, array platforms have been designed with probes evenly spaced throughout the genome with increased probe density in regions associated with specific disorders and resolution at the level of whole genes or multiple exons. However, this level of resolution often cannot detect pathogenic intragenic deletions or duplications, which represent a significant disease causing mechanism. Therefore, new high-resolution array CGH have been developed with probes targeting single exons of disease relevant genes. Here we present a study on 31,314 patient samples from a consortium of diagnostic genomic centres in the UK assayed by either array CGH of a traditional design (Cutosure v2) or by an array CGH with enhanced exon-level coverage of genes important for developmental disorder research (Cytosure v3). The new targeted design used in Cytosure v3 array is able to call small likely pathogenic and pathogenic intragenic aberrations that would have been missed on the CytoSure v2 array. Our results demonstrate that high-density targeted array CGH is a powerful tool for detection of intragenic deletions that leads to a significant improvement in diagnostic yield.

Lightning

Junior ACGS member: -

Detection of Microdeletions by NIPT

Kathryn Jones

Kathryn Jones

Cheshire and Merseyside Regional Genetics Laboratory

NIPT technologies have allowed the detection of fetal chromosome aneuploidies from a maternal blood sample, reducing the need for invasive testing. Sensitivities have improved to the degree that smaller, sub-chromosomal fetal copy number changes may now be detected. This proof of principal study uses artificial NIPT-like samples creating using cell lines containing microdeletions spiked into a normal female background at known percentages. The samples were then sequenced using the Ion Torrent Platform and then analysed using different bioinformatics pipelines. Results have shown that larger deletions are detectable at low fetal fractions, but smaller deletions are only visible at higher fetal fractions. Although these services are available in a private setting from some companies, caution should be used when considering the use of this in a Clinical setting due to the rare nature of the microdeletions and the risk of invasive testing for women with false positives.

Lightning

Junior ACGS member: -

Somatic Variant Interpretation

Siân Lewis

Siân Lewis

All Wales Medical Genetic Laboratory

The demand for next generation sequencing (NGS) in somatic testing is increasing. Large gene panels are able to offer increasing information on patients' diagnosis, prognosis and best treatment options across more cancer types than ever before. In 2018 UK specific variant interpretation guidelines were released by the ACGS. The introduction of these guidelines for germline disorders has highlighted the needed to adopt guidance to standardise the interpretation of somatic sequencing variants. However, this guidance is limited to germline testing and is not suitable for the interpretation of somatic sequencing variants. In 2015 the American Association of Molecular Pathologists (AMP) released guidelines for the interpretation and reporting of sequence variants in cancer. These guidelines use a four tier system to determine the actionality of somatic sequencing variants. A variant is considered actionable if actionable if it predicts treatment response to a specific therapy, affects the patient's prognosis or diagnosis or indicates that the patient requires surveillance for the early detection of cancer. Over the last 6 months the All Wales Medical Genetics Laboratory has worked at adapt the AMP guidelines into routine service in both the solid tumour section and the haematological malignancies section. The adoption of these guidelines has helped to standardise and streamline somatic variant interpretation across the somatic sections of the laboratory.

Lightning

Junior ACGS member: -

Preparing Solid Tumour Reports for the Genomic Era: A Service Development

Siân Lewis

Siân Lewis

All Wales Medical Genetic Laboratory

The demand for more genomic information in cancer is rising with large gene panels able to offer increasing information on patients' diagnosis, prognosis and best treatment options across more cancer types than ever before. Genomics can now offer exciting opportunities for the management of cancer. To ensure that testing is meeting the needs of the MDTs it is important that the information within in the genomic report needs to be carefully considered. Reporting of genetic results has been a neglected topic as historically if the testing was requested by a different specialism such as oncology it was a simple single gene testing that did not require complex interpretation. The genomic era will mean more extensive complex genetic results for oncologist providing more than just information of one treatment option. This service development uses a Plan Do Study Act (PDSA) cycle to develop new reports for an expanded solid tumour panel and set up a process of continuous development. These reports aim to meet the needs of oncology service users while being streamlined and futureproofed. The new reports were developed using surveys circulated to the 67 oncology MDTs across Wales, to staff within the laboratory and 2 workshops. The new reports will be implemented with the launch of the new panel later this year. This PDSA cycle will form the basis for a yearly review of solid tumour reporting to ensure that the reports continues to meet the needs of MDTs.

Lightning

Junior ACGS member: -

Assessing whole genome sequencing as a diagnostic test for mitochondrial disease

Frankie Macrae

Frankie Macrae, Wei Wei, Stephen Burr, Zoe Golder, Howard Martin and Patrick Chinnery.

Addenbookes

Background: Mitochondrial diseases are a heterogeneous group of disorders that can be caused by pathogenic variants in the mitochondrial (mtDNA) or nuclear DNA. Current approaches for diagnosing mitochondrial disease involve different complementary methods including biochemical, histochemical and molecular genetic testing. Recently, it has been shown that whole genome sequencing (WGS) is able to detect mtDNA variants, with ~1200x coverage across the entire mitochondrial genome, making it an attractive potential first-line test in this group of patients. However, this has not been validated in a diagnostic setting. Aims: To assess the performance of WGS compared to current molecular genetic testing methods with respect to single nucleotide variants (SNVs) and mtDNA copy number. Methods: WGS sequencing data and mtDNA copy number estimations were compared to results generated from deep mtDNA sequencing and droplet-digital PCR in 23 patient samples. Results: We show that WGS produces results which are consistent with current testing methods, with respect to high-frequency SNVs (100% experimental sensitivity) and mtDNA copy number (R2=0.9693, p=1.31×10-16). Conclusions: This data provides some evidence that can be put towards the implementation of WGS as a diagnostic test for mitochondrial disease. Further work is required to assess the performance of WGS with respect to other types of mtDNA pathogenic variants, including small indels and mtDNA rearrangements (deletions, duplications and inversions) as well as its quantification of heteroplasmy levels.

Lightning

Junior ACGS member: -

Focused Exome Pool-Seq of parent offspring Trios in previously negative cases

Kathleen Murphy

Manchester Centre for Genomic Medicine

Background This study investigates an adapted trio focussed exome sequencing (FES) approach in rare paediatric disease of suspected de novo origin, including developmental delay, congenital and dysmorphic abnormalities. This cohort has undergone numerous sequential genetic investigations in the search for a molecular diagnosis. As next generation sequencing (NGS) of trio exomes comes at a significant cost versus singleton analysis, Pool-Seq (sequencing multiple parents in an equimolar pool) may offer a cost-effective means of providing inheritance data fit for the diagnostic setting. Pooling however, presents challenges such as estimation of allele frequencies, calling variants, and differentiating them from technical artefacts. Methods FES of 10 proband and sex-matched Pool-Seq of parental samples was performed. Firstly, allele frequencies were assessed within the parental pools by quantifying levels of rare Single Nucleotide Polymorphisms (SNPs) unique to each proband and thus expected to be present in a single parent within the pool. Variant prioritisation and de novo analysis were subsequently performed using Sapientia (Congenica) to establish the clinical utility of Pool-Seq trio analysis. Findings and conclusions We successfully detected rare SNPs with a mean frequency of 8.64-10.65% in parental pools. Trio filtering and phenotypic information allowed prioritisation of variants and assessment of the inheritance of candidate variants, including detection of 2 de novo diagnoses (confirmed by Sanger sequencing), 2 additional candidate variants (X-linked and recessive inheritance), as well as reclassification of a variant previously detected by panel analysis, from variant of unknown significance to benign.

Lightning

Junior ACGS member: Yes

The detection of novel Alu insertions in cancer susceptibility genes

Evgenia Petrides

Evgenia Petrides, Jenna Ridley, Michael Bowman, Tina Bedenham, Treena Cranston

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In the Oxford Genetics Laboratories we have recently validated the SOPHiA Genetics Hereditary Cancer Solutions (HCS) kit along with bioinformatic analysis using their Data Driven Medicine (DDM) platform. This allows molecular analysis of 38 cancer susceptibility genes. The capture-based Next Generation Sequencing (NGS) methodology provides 100% coverage within all the regions of interest as well as reliable dosage analysis. We have introduced the HCS into our constitutional cancer service and it has proved to be more sensitive than our previous methodologies. Here we present interesting cases where this methodology, along with further investigations, allowed the detection of large genomic rearrangements. Two novel Alu element insertions of approximately 350 bp have been characterised within the coding regions of exon 16 in BRCA2 and exon 3 in SDHB. Both Alu insertions were not detectable using our previous NGS technologies. Pathogenic Alu element insertions are rarely reported. However, their occurrence is expected to be significantly underestimated as they are undetectable with most conventional screening methods.

Lightning

Junior ACGS member: -

Longitudinal changes in mutational burden associated with disease progression in myeloid malignancies

Jan Taylor

Jan Taylor, Paul Glover, Suzan Van Hoppe, Paul Evans, Catherine Cargo

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The Haematological Malignancy Diagnosis service has been routinely sequencing patients presenting with a confirmed, new diagnosis of either MDS, MDS/MPN overlap, CMML or AML since the autumn of 2015. The HTS panel is targeted to frequently mutated regions of genes that are commonly implicated in the literature for these myeloid disorders. Acceptance of NGS testing as part of the available diagnostic test repertoire has seen a widening request for the test to include pre-diagnostic samples, differential diagnostics, refractory or relapse disease, post-treatment and transformed disease. We have collected a cohort of over one thousand patients for whom samples have been sent for sequencing on more than one occasion. Here we present a summary of some of the trends seen in the mutational profiles of patients with longitudinal samples in these different scenarios, highlighting some interesting cases with complex mutational profiles. This study shows that targeted panel sequencing provides additional important molecular evidence for disease monitoring in addition to standard testing pathways, and can assist the understanding of clonal disease evolution in addition to initial diagnosis and prognostication.

Lightning

Assimilation of high-resolution HLA alleles from low-resolution serological typing; a computational approach

Junior ACGS member: Yes

Adriana Toutoudaki

Toutoudaki A, Turnbull H, Garner M, Peacock S, Kosmoliaptsis V, Abbs S.

Addenbrookes

HLA typing is an essential procedure for solid organ transplant allocation to achieve optimal patient outcomes. Time constraints limit current practice to low-resolution HLA typing which may result in sub-optimal tissue matching. Algorithms currently in development have the potential to improve matching by determining donor-recipient immunogenicity scores; however this requires high-resolution HLA typing information. Therefore we have developed an algorithm to convert existing low/intermediate-resolution HLA typing information into high-resolution, to be subsequently used to generate immunogenicity scores. Common HLA haplotypes, associations and allele frequencies within the Caucasian population were assessed through web databases and used to create a set of rules (n=115) which were incorporated into an assimilation table. A computational algorithm has been developed which manipulates multiple HLA types simultaneously and transforms low-resolution HLA typing into high-resolution using the assimilation table as reference. This method was tested on an existing dataset genotyped by NGS (n=104) to evaluate rule validity. The success rate by locus was: HLA-A (95%); HLA-B (86%); HLA-C (94%); HLA-DRB1 (78%) and HLA-DQB1 (73%). Overall the algorithm performed well, particularly for Class I loci, with some common errors identified in specific loci. Considering the complexity of the HLA system and ethnic variation, correct assimilation of high resolution HLA alleles from low resolution data is challenging. This initial proof of concept indicates it is possible and further development could lead to a useful tool for research.This algorithm will be applied retrospectively to a cohort of transplant patients in order to evaluate its potential for clinical use.

Lightning

Junior ACGS member: -

The 100,000 Genomes Project; successful cases and cautionary tales – the Wessex experience

Amy Webb

Amy Webb, Simon Thomas (Wessex Regional Genetics Laboratory), David Hunt (Wessex Clinical Genetics Service), Andrew Douglas (Wessex Clinical Genetics Service).

Whole Genome Sequencing (WGS) is heralded as a revolutionary advance in healthcare but how beneficial is this approach? Our Genomic Medicine Centre (GMC) has reported many causal variants found by the 100,000 Genomes Project (100KGP) where previous testing was unsuccessful. However, cases where the 100KGP failed to identify a known causal variant (or unwittingly filtering it out) have also been seen. This poster highlights several variants identified by the 100KGP but missed by other means: including a heterozygous missense DSP variant (not identified by single gene testing or the DDD project); the first causal 100KGP CNV confirmed in our GMC, a homozygous deletion involving SLC19A3 (previously not identified by extensive genetic testing); and an ALOX12 variant missed by whole exome sequencing. In contrast this poster also details several cases identifiable in the raw data but "missed" by the 100KGP. Many of these result from the 100KGP variant tiering and filtering strategy, including an NSD1 splicing mutation at position -14, a family where affected individuals on both sides of the family caused a dominant COL4A5 variant to be filtered out and a case where compound heterozygous DNAAF4 variants (one CNV and one SNV) were both missed. In summary, whilst many patients have undoubtedly benefitted from the 100KGP, possible diagnoses could be missed if WGS is considered a one-test-fits-all solution. WGS is a powerful tool when previous conventional testing has proved unsuccessful but a negative WGS result should not denote the end of genetic investigation when a genetic condition is clearly indicated.

Lightning

Junior ACGS member: Yes

A new diagnostic approach to calling CNVs from low read-depth genome sequencing data

Bethany Wild

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Low read-depth genome sequencing (GS) is frequently demonstrated to be a cost-effective, resilient alternative to cytogenomic microarrays (CMA) for CNV detection. We aimed to assess this technology for poor-quality product of conception (POC) samples, in comparison to the current standard of care. GS data was downsampled to a series of read depths and processed with several CNV callers to evaluate performance. These included: WisecondorX, Canvas and a caller developed by the Leeds MRC Medical Bioinformatics Centre. After optimising the algorithms by modifying the seed length or window size, we demonstrated detection of CNVs down to 0.3Mb at a read depth of 0.05X. Samples with known CNVs were prepared using the NEXTFELX Rapid DNA-Seq kit, sequenced on the Illumina NextSeq platform and analysed using the refined pipeline. Samples can be sequenced to roughly 0.225X to match the current cost of CMA analysis, which has previously been indicated to be comparable to CMA resolution. The algorithms performed equally well for POC samples detecting CNVs >0.3Mb. WisecondorX was demonstrated to deliver a low false positive rate and demonstrates sex-awareness for CNVs on the X chromosome. In conclusion, low read-depth GS can detect CNVs down to 0.3Mb at a read depth of just 0.05X. This allows many samples to be multiplexed on one NGS run, making GS a cost-effective option for diagnostic laboratories. Although issues surrounding mapping repetitive regions and breakpoint accuracy need to be resolved, low read-depth GS has the potential to deliver an improved service for poor-quality samples.

Poster

Junior ACGS member: -

Rapid mitochondrial genome (mtDNA) sequencing: facilitating rapid diagnosis of mitochondrial diseases in paediatric acute care

Lauren Akesson

*Lauren S. Akesson1,2,3, *Stefanie Eggers1, Clare J. Love1, Belinda Chong1, Matthew F. Hunter3,4, Emma I. Krzesinski3,4, Natasha J. Brown1,2, Tiong Y. Tan1,2, Christopher M. Richmond1, David R. Thorburn1,2,5, John Christodoulou1,2,5, **Zornitza Stark1,2,5, **Sebastian Lunke1,2,5

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Introduction: Standard rapid genomic testing techniques analyse nuclear DNA variants using exome and/or genome sequencing (ES/GS). Rapid mtDNA analysis is not routinely available, particularly in centres performing ES, which does not deliver clinical-grade mtDNA sequencing. We describe our experience using rapid mtDNA sequencing in tandem with an ES-based rapid genomic diagnosis program as part of the Australian Genomics Acute Care flagship. Methods: Two infants presenting with persistent lactic acidosis and bone marrow failure were recruited for rapid genomic testing. With clinical suspicion of mitochondrial disease, both infants underwent rapid ES and mtDNA sequencing in tandem, the latter using Nextera libraries from a full length mtDNA amplicon. Results: ES was non-diagnostic in both infants. mtDNA sequencing identified a single large mtDNA deletion in both infants, diagnostic of Pearson syndrome (MIM 557000). Diagnostic reports were issued within 73 hours 55 minutes and 54 hours 25 minutes, respectively. Both infants avoided invasive bone marrow biopsies and a range of other investigations. Conclusions: Rapid mtDNA sequencing in tandem with ES results in additional diagnoses in seriously ill children with suspected mitochondrial pathology, suggesting that ES alone may be insufficient in this setting. When designing rapid genomic diagnosis programs, centres should consider incorporating mtDNA amplification and analysis in individuals with suspected mitochondrial pathology, by combining ES and mtDNA sequencing in tandem, or analysing mtDNA data from GS, which captures the mitochondrial genome.

Poster

Junior ACGS member: Yes

Automated reporting of negative results for the 100,000 Genomes Project

Andrew Bond

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3 - Leeds Genetics Laboratory, St James's University Hospital, Leeds, UK

The South London GMC has recruited over 8,000 rare disease samples for the 100,000 Genomes Project (100KGP). A significant proportion of the probands (~40%) will not carry any tier 1 or 2 variants and will therefore be issued a negative result. Genomics England have developed and made available to GMCs, the CIPAPI, which holds patient and variant information for 100KGP in a format that can be queried programmatically. The South London GMC hub lab at Guy's Hospital are developing a solution for these negative cases using a fully automated system that will issue these results as soon as the patients come off the Genomics England pipeline, with no manual intervention required. Where possible, the solution has taken advantage of the JellyPy and GEL2MDT code repositories, which are collaborative efforts between NHS bioinformaticians to share methods and code to interact with Genomics England infrastructure. The solution identifies any negative cases and pulls together the patient's phenotype and testing details from CIPAPI with patient identifiers and demographics from the lab LIMS, to create a negative result letter according to published templates, and then emails out the letter as well as recording this activity in the lab LIMS. This solution has so far been implemented for Guy's and St Thomas' and effectively reduced the workload associated with these cases to zero. As it has been built using JellyPy, it will be offered to other GMC laboratories for local implementation.

Poster

NGS panel testing for albinism and nystagmus: missing heritability, modifiers and potential polygenic effects

Junior ACGS member: -

Jonathan Callaway

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Albinism encompasses a genetically heterogeneous group of congenital conditions which are characterised by hypopigmentation of the hair, skin or eyes. Pigment is believed to be essential for the normal development of the eye and without it albinism patients can suffer from a range of ocular defects, including nystagmus. At the Wessex Regional Genetics Laboratory in April 2018 we implemented a 31-gene NGS panel for congenital albinism and nystagmus and here we present our findings from the first 100 patient screens. We have confirmed a molecular diagnosis in 10 cases and have noted three additional findings of interest: 1. Most of the genes on our panel exhibit an autosomal recessive pattern of inheritance; we have identified 23 cases whereby only one pathogenic or likely pathogenic variant has been detected, which is suggestive of missing heritability. 2. In 13 of these 23 cases, the patients have one pathogenic TYR variant but are also heterozygous for two previously-reported potential modifier variants: TYR c.575C>A p.(Ser192Tyr) and TYR c.1205G>A p.(Arg402Gln). 3. We have three cases where two heterozygous pathogenic or likely pathogenic variants have been detected in different genes, suggestive of a polygenic effect. In summary, our data highlight the complexity associated with interpreting genetic test results for albinism owing to missing heritability and the involvement of potential modifier variants as well as possible polygenic effects. Additionally, classifying variants in autosomal recessive disorders according to the ACMG guidelines can be challenging and this will be discussed.

Poster

A targeted Next Generation Sequencing approach to detect activating and resistance mutations in the circulating tumour DNA of cancer patients

Junior ACGS member: Yes

Julian Cano-Flanagan

Julian Cano-Flanagan1, Claire Faulkner1, Kirsty Russell1, Paula Waits1, Christopher Wragg1

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Circulating tumour DNA (ctDNA) testing of patients with cancer has a wide range of clinical applications and is becoming complementary to genetic testing of tumour tissue. Patients who are unable to have a tissue biopsy, for testing to determine eligibility for targeted treatment have the opportunity to be tested via a less invasive "liquid biopsy". Those on targeted treatment may benefit from testing for a resistance mutation a tumour may have acquired against the therapy. The level of ctDNA in plasma can also be used to monitor a patient and provide prognostic information. Laboratories currently offer testing using sensitive PCR techniques to target specific actionable mutations. Next generation sequencing (NGS) has the advantage of screening many genes simultaneously and could therefore pick up a wider repertoire of actionable lesions. The aim of this project was to recruit patients with NSCLC and melanoma, evaluate a method of extracting cell free DNA in the laboratory, evaluate a Next Generation Sequencing (NGS) panel's ability to detect a broader range of clinically actionable mutations and determine its level of sensitivity. This study has shown the potential for using a targeted NGS assay for detecting clinically actionable mutations in the ctDNA of cancer patients. In particular the utility of detecting an activating mutation in the ctDNA of a NSCLC was demonstrated. The assay was able to detect variants down to an allele frequency of 1%. A further NGS run will evaluate the suitability of the assay as a monitoring tool and to detect resistance mutations.

Poster

Clinical exome sequencing in patients with rare genetic disease found to have loss of heterozygosity by microarray

Junior ACGS member: Yes

Lewis Darnell

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SNP microarray results can detect regions of the genome exhibiting loss of heterozygosity (LOH), which may suggest consanguinity in the parental relationship. In patients with a suspected rare genetic disorder and consanguineous parents there is an increased risk of the disorder being caused by a rare, homozygous recessive variant. The associated disorders are often very rare and hard to identify by the phenotype alone. We investigated the utility of following up a LOH microarray result with clinical exome sequencing using the SOPHiA GENETICS Clinical Exome Solution in 11 families. The proband and any affected siblings were sequenced with the data analysis being performed using SOPHiA DDM software and focussing on rare, homozygous variants. A diagnosis was made in four families and in another family the genetic result explained the biochemical results of the proband, but not the full familial condition. Using the presence of LOH as an indication to activate proband only clinical exome sequencing and specific analysis for rare homozygous variants is a time and cost-effective diagnostic testing method with a good diagnostic yield. The use of the SOPHiA GENETICS Clinical Exome Solution and SOPHiA DDM enables fast, high quality library preparation with simple, user friendly data analysis without the need for a specialist bioinformatician. An early diagnosis prevents a long diagnostic odyssey for patients and their families, potentially allows for targeted treatment or monitoring for the patient while providing the family with timely reproductive testing options.

Poster

Streamlining the process of Sanger sequencing in a diagnostic laboratory by HotStart TouchDown PCR

Junior ACGS member: -

Juan Del Rey Jimenez

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1- SW Thames Regional Genetics Laboratory at St George's Hospital, London

DNA amplification via polymerase chain reaction (PCR) is a critical step for Sanger based DNA sequencing. GC rich sequences (>60% GC content) are problematic to amplify due to extra hydrogen bonding and propensity to form secondary structures resulting in resistance to efficient denaturation and annealing. In our laboratory, primers for GC-rich and non GC-rich amplicons were processed separately as GC-rich amplicons were optimised to work at different PCR cycling temperatures and required additives that enable efficient denaturation. Under the scope of a diagnostic laboratory, having two different processing protocols added delay to the result and created a bottle neck in diagnosis. Combining HotStart polymerase and TouchDown PCR (TD-PCR) we have developed a process for specific PCR amplification of any amplicon independent of GC content. We have validated the combined HotStart polymerase and TD-PCR protocol for batch processing of a variety of amplicons that differ in GC content in order to streamline the sequencing workflow. We tested 633 normal controls and 125 different positive controls for a broad range of genes and exons. Missense, nonsense and synonymous variants as well as deletion and duplications were tested in either exonic or intronic regions. Sensitivity and specificity values were obtained showing that HotStart TD-PCR is a reliable method in diagnosis.

Poster

Validation of whole genome findings using qPCR – a service within the All Wales Medical Genomics Service (AWMGS)

Junior ACGS member: -

Jade Heath

J Heath, S Corrin, A Williams, J Jezkova, D Barrell, and S Morgan

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Genomics techniques such as exome sequencing, whole genome sequencing (WGS) and array comparative genomic hybridisation (aCGH) have become the frontline tests for a vast range of referrals within genetics laboratories. A significant challenge of these techniques is the ability to confidently identify small scale copy number changes. This can result in the need for further testing to fully interpret or validate certain complex findings. We have developed an in-house real-time qPCR assay as a method for validating imbalances, including single exon imbalances, detected by whole genome methods such as whole exome sequencing. Here we present several cases where this service enabled the confirmation of an imbalance, as well as targeted testing in the parents. Within the laboratory this service has proven to be efficient and cost effective.

Poster

Junior ACGS member: -

Improving array performance in GC rich regions

Jade Heath

J Heath, S Corrin, A Williams, J Jezkova, D Barrell, and S Morgan.

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Array comparative genomic hybridisation (aCGH) is the gold standard test for genome-wide CNV detection. However, achieving consistent coverage right across the genome can be a challenge. Probes designed to targeted regions of the genome with high a GC content can be prone to non-specific binding and producing non-informative signals. In our past experience, this has led to inaccurate calling and splitting regions, complicating interpretation and reporting. Our laboratory currently uses the OGT CytoSure Constitutional v3 platform. This array includes probes for exon level coverage of up to 502 developmental delay genes identified by both the DDD project and ClinGen. In order to overcome the issues associated with GC rich probes, OGT is investigating an updated microarray which includes shorter probes and updated gene content. They are also investigating the utility of adding a GC modifier reagent. Here we present the results of a trial to determine if the performance of the new OGT array is improved in GC rich regions. This includes both the addition of a GC modifier and use of the new OGT array platform.

Poster

Junior ACGS member: Yes

Complex variant interpretation for an emerging PPM1D-related syndrome

Jana Jezkova

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PPM1D encodes a protein phosphatase that regulates DNA damage response and controls global heterochromatin silencing. To date, pathogenic truncating variants in the last and penultimate exons (5 and 6) of the PPM1D gene have been reported in 17 patients with mild-to-severe intellectual disability (ID) and/or developmental delay. However, loss-of-function variants in exons 5 and 6 are also reported in gnomAD and the role of the region in protein function is not well characterised. Truncating PPM1D somatic variants have also been reported in breast and ovarian cancer patients. For these reasons, the use of the loss-of-function PVS1 criterion becomes problematic when interpreting PPM1D truncating variants using the ACGS Best Practice Guidelines for Variant Classification. Here we report an additional patient with a novel truncating nonsense NM_003620.3:c.1451T>G p.(Leu484X) variant in exon 6 of the PPM1D gene. Comparison with previously described cases revealed common phenotypic features including learning difficulties, anxiety, broad mouth, low set ears and small hands and feet. However, currently this variant remains classified as a variant of uncertain significance. This report adds to the growing evidence available for the PPM1D-related ID syndrome and further supports the notion that patients with pathogenic PPM1D variants can present with variable clinical features.

Poster

Junior ACGS member: Yes

Approaches for trimming primer sequences from amplicon NGS reads

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Amplicon based NGS assays produce raw data where the region of interest is flanked by the primer sequences. To ensure the primers do not interfere with variant detection additional bioinformatics steps are required. Bioinformatics tools can be applied to trim or mask primer sequences at various stages of the bioinformatics pipeline, for example, BAMClipper removes primer sequences post alignment, whereas Trimmomatic works on unaligned fastq files. Pre-alignment primer trimming approaches includes removal of a set number of bases from the start or end of reads or look for given primer sequences but this approach is crude and as the length of primers can differ may result in incomplete removal of primers or removal of data from the region of interest. Post-alignment trimming allows the primer sequences to be utilised during alignment, improving variant detection at the primer-amplicon boundary and enables more sophisticated approaches to primer trimming utilising genomic coordinates. Variant calling should not be performed on primer sequences so primer trimming is particularly important where amplicons are tiled as variants may be covered by the primers of neighbouring amplicons. Variants may affect the amplification of alternate alleles and if primers are not excluded from variant calling the variant allele frequency will be underestimated, enriched for the wild type allele and the read depth inflated with uninformative reads. We describe a number of approaches for primer trimming and the implications of these for clinical NGS pipelines.

Poster

Junior ACGS member: Yes

Development of a targeted NGS assay for myeloproliferative neoplasms

Clare Maurer

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Addenbrookes

Myeloproliferative neoplasms (MPN) are a group of rare heterogeneous bone marrow disorders characterised by the uncontrollable production of one or more myeloid cell lineages. Polycythemia vera, primary myelofibrosis and essential thrombocythemia are subtypes of MPN associated with acquired mutations in JAK2, CALR and MPL. Current testing for MPN at the Cambridge University NHS Hospitals Haemato-pathology and Oncology Diagnostic service involves sequential mutation testing using multiple laboratory techniques. The aim of this project was to design a targeted next-generation sequencing (NGS) assay to sequence the affected regions in order to improve efficiency and cost plus manage increasing demand. The method involved designing PCR primers to capture the following regions; JAK2 exon 12, JAK2 exon 14, CALR exon 9 and MPL exon 10, followed by NGS. The assay has been optimised with a multiplex approach in a single PCR reaction to obtain even and high depth of coverage to call somatic variants. DNA samples from 12 anonymised MPN patients with genetic variants in one of the four regions were tested and results demonstrated detection of the known genetic variants with 100% concordance. Further development will focus on screening a breadth of mutation genotypes and sensitivities to determine the limit of detection, as clinical guidelines state the assay detect a depth of ≥1% mutation burden. On-going development of a bioinformatics pipeline is required to call larger insertions and deletions. Implementation of this targeted NGS assay into routine diagnostic use will aid in the accurate diagnosis of MPN with reduced turn-around times.

Poster

ACGS audit of invasive prenatal samples received by NHS Regional Genetics Laboratories for confirmation of NIPT findings over a 5 year period

Junior ACGS member: No

Sian Morgan

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UK NHS Regional Genetics Laboratories were invited to share data on invasive prenatal samples sent for confirmation of non-invasive prenatal testing (NIPT) findings. Seventeen Laboratories submitted data representing 1272 NIPT results over a 5 year period with sample numbers increasing steadily across that time. The majority of samples received (93%) were for confirmation of high chance trisomy 13, 18 or 21. 65% of invasive samples received were amniotic fluid samples with the remaining 35% CVS.
The results of invasive testing did not confirm the NIPT finding in just 9% of cases; 2.4% for trisomy 21(23/949), 11% for trisomy 18(18/160) and 41% for trisomy 13(30/73). Only 52%(32/67) of invasive samples received due to an increased chance of sex chromosome aneuploidy confirmed the result, the remainder being false positives. 80%(8/10) of samples referred following NIPT results indicating less common genomic imbalances such as microdeletion syndromes, rare autosomal aneuploidies or triploidy went on to have normal invasive sampling results.
Data shows that, on the whole, invasive sampling is being performed appropriately; however the decision on whether to take an amniocentesis or a CVS appears to mainly be taken without consideration of the genomic nature of the NIPT finding. NIPT has a high positive predictive value (PVV) for trisomy 21 and trisomy 18, but a lower PPV for trisomy 13 in the combined population. The PPV for trisomy 13 and monosomy X increases following abnormal scan findings. The distribution of false positive results was random with respect to maternal age.

Poster

Junior ACGS member: Yes

Molecular testing of CADASIL in SWTRGL

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an adult-onset disorder characterised by migraine, recurrent subcortical strokes, cognitive impairment, psychiatric disturbances and dementia. The average age of onset is 45 and fully penetrant by age 60. The disease is caused by pathogenic variants in the NOTCH3 gene on chromosome 19 that has 33 exons and contains 34 EGF-like repeats of 40-50 amino acids present in the extracellular domain. Each repeat has six conserved cysteine residues and the vast majority of causative mutations found in CADASIL patients result in the creation or loss of a cysteine (Cys) residue in this gene. Here we present how the molecular testing of CADASIL is conducted in SWTRGL and the rate of accurate diagnosis achieved during the history of this disease service. The clinical manifestations of patients referred to this laboratory are described, including an audit of type of cases received: diagnostic, confirmation, predictive and prenatal. Data about mutation clustering and most common Cys changes found in SWTRGL will also be presented including variants of uncertain significance (VUS) that have been reported in house. Interesting cases will be discussed and compared to relevant existing literature.

Poster

Value of UroVysion FISH in non-muscle invasive bladder cancer surveillance: first UK experience

Junior ACGS member: -

Iryna Temple

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Between 60 to 70% of patients with intermediate/high-grade non-muscle invasive bladder cancer treated with transurethral resection and intravesical Bacillus Calmette-Guérin immunotherapy in combination with electromotive Mitomycin C (Di-Stasi regimen) will recur and, at present, treatment response cannot be predicted. Current follow-up involves three-monthly flexible cystoscopies (FC) under local anaesthetic and equivocal results require further white and blue light cystoscopy (LC) under general anaesthetic. However, LC interpretation can be compromised by the level of inflammation resulting in unnecessary biopsies of inflamed but non-malignant tissue. Abbott UroVysion is a fluorescence in situ hybridisation (FISH) probe-combination developed to identify bladder cancer cells in the urine, through the detection of cells carrying polysomies of chromosomes 3, 7 and 17 and/or loss of 9p21. We report the validation, implementation, challenges and preliminary findings of a single-centre prospective study aimed to assess the value of Urovysion-FISH performed in combination with FC at different time-points during the follow-up of patients undergoing Di-Stasi. Out of the 25 patients tested by FISH, 10 completed FC. FISH showed no evidence of residual tumour cells in any of these 10 samples and FC showed no recurrence in 7 out of 10. Three patients had a suspicious patch on FC; however, biopsy examination from this patch showed non-malignant bladder tissue. All ten patients remained recurrence-free to date (range 1-28 months, mean 8 months). These preliminary results suggest that UroVysion-FISH has the potential to reliably assess absence of disease post Di-Stasi and likely reduce the number of superfluous biopsies of inflamed bladder tissue.

Poster

Identifying genetic variants that underlie hereditary Hearing Loss within the 100,000 Genomes Project

Junior ACGS member: -

Letizia Vestito

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Although mutations in over a hundred genes can cause hereditary hearing loss (HHL) in humans, less than 50% of HHL is explained by mutations in known genes. Moreover, their respective contributions to the frequency and type of inherited deafness are largely unknown. The opportunity to identify novel hearing genes by whole genome sequencing through the 100,000 Genomes Project has the potential to bridge this gap in our current knowledge. We have developed an analysis pipeline with the aim of identifying causal variants in probands with HHL utilising Exomiser and Genomiser to re-analyse sequences from individuals with no primary finding (NPF). A number of probands were recruited to the study with hearing or ear abnormality as a 'primary phenotype' and many more individuals had hearing or ear abnormality as a 'secondary phenotype'. We report the case of two sisters with hearing loss, microcephaly, intellectual disability, global developmental delay, delayed motor development and various other dysmorphic features. The two sisters were identified to have a likely pathogenic intronic mutation in TAF6 gene, a rare cause of Alazami-Yuan Syndrome.

Poster

Hiding in plain sight: Inherited pathogenic variants that are expanding clinical phenotypes in autosomal dominant syndromes

Junior ACGS member: Yes

Angharad Williams

Angharad Williams, A. Kamath, A. E. Fry, J. Jezkova, J. Heath, D. Barrell, S. Corrin and S. Morgan

All Wales Medical Genomics Service, NHS Wales

At the All Wales Medical Genomics Laboratory, we provide an Intellectual Disability service including singleton clinical exome sequencing to further investigate patients referred from Clinical Genetics. Pathogenic variants associated with three autosomal dominant syndromes (Cornelia de Lange syndrome, KBG syndrome and Neurofibromatosis Type 1) were detected and were subsequently determined to have been inherited. These parents had not previously presented for genetic testing, although some mild phenotypic features were noted following further clinical evaluation. Here we present two families, including one proband with a pathogenic variant inherited from either parent. These cases demonstrate the importance of carefully considering genomic testing and variant filtering strategies. Strategies that filter out inherited variants may miss inherited, milder disease alleles. Therefore a list of known likely pathogenic and pathogenic variants to be always included in analysis could prove extremely useful. Alternatively, genomic testing pathways could incorporate inherited autosomal dominant variants for reiterative analysis where a diagnosis has not been found using the de novo route. Cases such as these are leading to the expansion of clinical phenotypic description in many previously well characterised disorders. Further work could include investigating possible high level mosaic parents with sensitive molecular techniques.

Poster

Junior ACGS member: Yes

Accurate Transmission Risk Assessment in Mosaic Neurofibromatosis Type 2 (NF2)

Jonathan Williams

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Pathogenic NF2 variants detected in sporadic vestibular schwannomas (VS) are frequently confined to the tumour. Such findings represent a challenge for genetic counselling in young adults, as rarely the isolated VS is the first manifestation of mosaic NF2, even though the pathogenic variant is undetectable in blood. An NF2 patient presenting age 20-29 with a unilateral VS, but no NF2 mutation in blood, has a 7% empiric offspring risk of a child with constitutional NF2 [1,2]. As the isolated VS may be the first feature of mosaic NF2, there is potentially a small offspring risk for these patients. Here, we present the case of a 29 year old male presenting with likely multi-focal, unilateral VS. Initial analysis of heterogeneous tumour DNA using targeted NGS and MLPA (performed at the Manchester Centre for Genomic Medicine) detected two pathogenic single nucleotide variants in NF2 in conjunction with a large deletion incorporating the entire gene. Using a combination of ultra-deep NGS and array-CGH conducted on DNA extracted from semen we were able to reduce this individual's transmission risk to <0.5%. This estimate has been further refined using a haplotype phasing technique to determine the natural history of tumour development with respect to the NF2 variants detected. This case highlights both the feasibility and clinical utility of germline testing in an NHS laboratory. The techniques utilised are now being further developed to validate findings from the PREGCARE study being led by Professors Anne Goriely and Andrew Wilkie of the Weatherall Institute of Molecular Medicine. Reference [1] Evans et al., 2007. Journal of Medical Genetics, 44, pp.424-428 [2] Evans DG, Raymond FL, Barwell JG, Halliday D, 2012. Clinical Genetics, 82, pp.416–424.