NHS

Birmingham Women's and Children's

NHS Foundation Trust

WMRGL STP CONFERENCE 2023

Thursday, 31st August 2023

Edgbaston Park Hotel - 53 Edgbaston Park Rd, Birmingham B15 2RS)

WMRGL STP Conference

09:30 to 17:00 on 23/08/2022 at Edgbaston Park Hotel (53 Edgbaston Park Rd, Birmingham B15 2RS).

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Programme:

WMRGL STP Conference

09:30 to 17:00 on 31/08/2023 at Edgbaston Park Hotel (53 Edgbaston Park Rd, Birmingham B15 2RS).

Welcome and sign in Complementary refreshments will be served to all guests. This will include Tea, Coffee and pastries. During this time, the guests will be allowed to mix and network	09:30	10:00
Careers Panel A panel formed of some of our accomplished Clinical Scientists to lead a conversation about careers after the STP programme.	10:00	10:30
Quality Session - Risk, detecting and reporting problems Speaker: Carly Mogg	10:30	11:15
Mid-Morning break (Complementary tea, coffee, cereal bar and fruits served)	11:15	11:30
Whole Genome Sequencing in Rare Disease Speaker: Gavin Ryan	11:30	12:15
Lunch Break Hot and cold buffet served at the hotel restaurant	12:15	13:00
Solid Cancer WGS - Referral pathway, analysis and interpretation Natasha Vafadar	13:00	13:45
Whole Genome Sequencing in Clinical Practice - Meeting the Challenge Peter Marks	13:45	14:30
Selected oral presentations from abstracts		
Implementation of a methylation array service for glioma samples. Speaker: Amy Beastall	14:30	15:00
Microarray audit to determine the range and frequency of copy number variants detected across different neonatal clinical cohorts. Speaker: Christina B. Joseph	15:00	15:30
Poster sessions 12 posters will be selected from the abstracts submitted. The guests will be encouraged to network. Complementary tea, coffee and a sweet treat served	15:30	16:30

Careers Panel.

Moderator: Lorraine Hartles-Spencer

Speaker: Jennie Bell, Gavin Ryan, Natasha Vafadar and Lorraine Hartles-Spencer.

Notes:

Quality Session - Risk, detecting and reporting problems.

Speaker: Carly Mogg

Notes:

Whole Genome Sequencing in Rare Disease.

Gavin Ryan

Notes:

Solid Cancer WGS - Referral pathway, analysis and interpretation.

Natasha Vafadar

Notes:

Whole Genome Sequencing in Clinical Practice - Meeting the Challenge.

Peter Marks

Notes:

List of abstracts

Implementation of a methylation array service for glioma samples. (Selected for Oral Presentation)

Amy Beastall.

Gliomas are a diverse group of tumours originating from glial or supporting cells of the central nervous system and they can be broadly categorised as diffuse and non-diffuse gliomas (1). Previously, gliomas have been diagnosed based on histology alone according to the criteria in 2007 WHO and this served as the gold standard for glioma diagnostics for decades (2). However, significant interobserver variation emerged upon grading glial tumours and this has the potential to detrimentally impact patient care since treatment decisions are based on histological diagnosis. In the last decade the knowledge of molecular subtypes of gliomas has expanded and led to the 2016 WHO revision which integrates histopathologic and molecular features into a combined diagnosis (3). The WHO 2021 revision further advances the role of molecular diagnostics in CNS classification. For example, IDH genotype is now critical for the diagnosis of specific entities of adult diffuse glioma: Astrocytoma, IDH mutant; Oligodendroglioma, IDH mutant and 1p/19q codeleted; and glioblastoma IDH wildtype (4).

The combination of histological assessment with conventional molecular testing is the first line diagnostic approach for the majority of intrinsic tumours. However, for tumours with unusual, non-specific or non-representative histology and where molecular testing is not diagnostically informative, a new method of methylation-based classification has shown to produce a more accurate and clinically relevant diagnosis (5).

Aberrant DNA methylation is a hallmark of tumour initiation and progression with methylation patterns commonly being used as markers for cancer detection, prognosis and prediction of treatment response. Methylation profiles of tumours are highly robust and reproducible in a clinical setting and have been used to subclassify CNS tumours, for example, ependymoma's, meningioma's and medulloblastomas (5). The NHS Wales AWMGS has provided molecular profiling for Gliomas since 2017. In addition to genotyping using NGS, the laboratory currently uses bisulphite conversion and methylation-specific pyrosequencing to determine methylation levels of the promoter region of MGMT, which is a predictive marker for response to alkylating chemotherapy.

Recently, a new approach for tumour diagnostics has been developed and is based on genome wide DNA methylation profile analysis using the Illumina EPIC BeadChip methylation array. A brain tumour methylation classifier has been developed by a group in Germany which identifies distinct DNA methylation classes and subclasses of CNS tumours (6). Results from the EPIC BeadChip arrays are uploaded and compared to the methylation data of a reference cohort of 2800 neuropathological tumours. This approach has been used diagnostically on more than 500 tumour samples and has led to the confirmation, refinement or establishment of new diagnoses leading to an impact on treatment and clinical management. The National Genomic Test Directory states that all neurological tumours are eligible for methylation analysis provided that the neuropathologist review or specialist paediatric pathology review indicates that molecular assessment will aid diagnosis or management (7).

1. Perry A, Wesseling P. Chapter 5 - Histologic classification of gliomas. In: Berger MS, Weller M, editors. Handbook of Clinical Neurology. 134: Elsevier; 2016. p. 71-95.
2. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, et al. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol. 2007;114(2):97-109.
3. Wesseling P, Capper D. WHO 2016 Classification of gliomas. Neuropathology and Applied Neurobiology. 2018;44(2):139-50.
4. Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D, et al. The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. Neuro Oncol. 2021;23(8):1231-51.
5. Jaunmuktane Z, Capper D, Jones DTW, Schrimpf D, Sill M, Dutt M, et al. Methylation array profiling of adult brain tumours: diagnostic outcomes in a large, single centre. Acta Neuropathol Commun. 2019;7(1):24.
6. Capper D, Jones DTW, Sill M, Hovestadt V, Schrimpf D, Sturm D, et al. DNA methylation-based classification of central nervous system tumours. Nature. 2018;555(7697):469-74.
7. NHS England. National Genomic Test Directory for Cancer 2022 [Available from: https://www.england.nhs.uk/publication/national-genomic-test-directories/.

Microarray audit to determine the range and frequency of copy number variants detected across different neonatal clinical cohorts. (Selected for Oral Presentation)

Christina B. Joseph[1] and Sally Jeffries[1].

[1]West Midlands Regional Genetics Laboratory, Birmingham Women's and Children's NHS Foundation Trust

Chromosomal microarray (CMA) has been the gold standard technique in the UK since 2010 to detect genomic imbalances for a wide range of referrals including neonates with congenital anomalies. There are currently no large, published datasets of copy number variants (CNVs) observed across all neonatal clinical cohorts using CMA. The West Midlands Regional Genetics Laboratory (WMRGL) has completed >1000 neonatal microarray analyses using the Illumina Global Screening Array (GSA) platform.

The overarching aim of the project was to carry out variant review using the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen) technical standards for CNV interpretation, followed by retrospective audit to determine the range and frequency of pathogenic/likely pathogenic copy number variants detected in neonatal patients who had SNP microarray testing.

Variant review in 998 patients led to reclassification of two likely pathogenic variants to pathogenic. Classification did not change for any reported variants of uncertain significance. An average diagnostic yield of 10% was established by retrospective analysis with higher rates seen in patients with immune system abnormality (30%) and neurodevelopmental abnormality (17%). 'Abnormality of the face' and 'all growth abnormality' were two of the most common phenotype categories received for referral. The most frequent type of pathogenic/likely pathogenic CNV identified belonged to recurrent microdeletion/duplication syndrome regions. Mosaic variants were reported in 0.1% (9/998) of patients.

With the move towards whole genome sequencing (WGS), information from this project is critical to inform the requirements of WGS with respect to CNV detection and sensitivity requirements for detection of mosaicism. This would ensure appropriate testing pathways are put in place during the transition of moving away from array technology to WGS only. Future analysis using CMA data from previous platforms will help to provide more accurate results.

Implementing mosaicism testing to stratify recurrence risk in families with a de novo rare disease diagnosis.

Connor Davieson.

So-called ‘rare diseases’ collectively affect 1 in 17 people in the UK, posing a significant healthcare burden. Many of these diagnoses are attributed to ‘de novo’ genetic variants (seen in patients but not their parents), which affect up to 1/213 live births (The Deciphering Developmental Disorders Study, 2017). These families are counselled around having a 1-2% recurrence risk for future pregnancies, but in reality, risks range from negligible in >90% of families, to up to 50% in families with parental germline mosaicism. This could significantly impact care; high recurrence risk families should be prioritised for genetic counselling and prenatal testing in subsequent pregnancies, whereas low risk families could avoid unnecessary invasive prenatal tests. However, currently parental germline mosaicism cannot be investigated, so personalised recurrence risks are not available. The recently-published PREGCARE study demonstrates a testing strategy to identify high-recurrence risk families attributed to parental mosaicism (Bernkopf et al, 2023). This includes testing of sperm to calculate bespoke recurrence risks in paternally derived cases. In collaboration with the PREGCARE group, this project aims to prepare for the implementation of parental mosaicism testing in the NHS. A new protocol will be assessed for the extraction of DNA from semen samples. Ultra-deep sequencing of DNA from diverse participant tissues (including paternal semen) will be used to clinically verify mosaicism reported by the PREGCARE study. Alongside this, existing NHS services and published literature will be used to design eligibility criteria, assess costs of testing and estimate future workloads. To estimate future service workloads, West Midlands Regional Genomics Laboratory (WMRGL) services were audited for historic case detection rates. Relevant families were frequently identified: data from the R21 fetal exome sequencing service identified an average of 1.63 eligible cases per month, whereas data from a review of the NHS Whole genome sequencing service identified an average of 1.45 cases monthly. Extrapolation to reflect national contributions yields a monthly case detection rate of ~19 in England, although true future workloads will depend heavily on uptake, which is difficult to predict. From the literature, three clinical factors were identified that increase prior risks of parental mosaicism, including having had multiple affected children. Additional genetic factors were also identified, for instance a group of variants in which the likelihood of parental mosaicism is lower. These risk factors were applied to the eligible families outlined previously; 3 of 39 identified R21 families (7.8%) and 2 of 42 identified WGS families (4.8%) had additional features suggestive of parental mosaicism. This is consistent with the proportion of cases attributed to parental mosaicism in the literature, suggesting that this is an appropriate strategy for prioritising families to test in a future service. Although technical outcomes are pending, this work so far highlights the scale of this issue, and lays important groundwork for future service design. The families identified in the WMRGL would make an ideal future pilot population, marking significant progress towards a first-of-its-kind clinical service, and contributing to the resolution of a major unmet clinical need.

Bernkopf, M., et al. (2023) 'Personalized recurrence risk assessment following the birth of a child with a pathogenic de novo mutation', Nature Communications, 14(1), pp. 853.
The Deciphering Developmental Disorders Study. (2017) 'Prevalence and architecture of de novo mutations in developmental disorders', Nature, 542(7642), pp. 433-438.

Method verification of alpha-1-antitrypsin assay for the differential diagnosis of patients with neonatal jaundice and a retrospective clinical audit on the relationship between alpha-1-antitrypsin levels, phenotype/genotype, and clinical outcome in patients with alpha-1-antitrypsin deficiency.

Summer Louise Ajayi.

Biliary Atresia is a rare disease of infancy and is one of many causes of neonatal jaundice. It is a destructive inflammatory obliterative cholangiopathy which affects varying lengths of both the intrahepatic and extrahepatic bile ducts. If left untreated, it can progress onto cirrhosis of the liver and eventually death, by the time the child is 2 years of age. Biliary atresia can be classified according to the highest level of proximal biliary obstruction - Type 1 has luminal patency until the region of the common bile duct and proximal cystic biliary ducts, type 2 has patency until the region of the common hepatic duct and type 3 presents with the most proximal section of the extrahepatic biliary tract within the porta hepatis being completely blocked. The cause of biliary atresia is unknown; however, it is considered to be multifactorial in nature with genetic, inflammatory, infective, and toxicological factors playing a role in the obliterative cholangiopathy. Surgery is the most effective form of management of biliary atresia, of which a Kasai portoenterostomy technique is performed that achieves clearance of jaundice, restoration of excretory and synthetic liver function and the healthy growth and development of the infant. The Kasai portoenterostomy involves the complete excision of the extrahepatic biliary tree, exposing the microscopic ductules that remain after the porta hepatis is transected. There is a clinical triad of features of biliary atresia that present after birth; jaundice, acholic stools/dark urine and hepatomegaly. The clinical features presented are non-specific and so a differential diagnosis is required, where other medical causes of cholestasis are excluded to prevent infants from undergoing an unnecessary surgical procedure. The main differential diagnosis is Alagille syndrome, sclerosing cholangitis with neonatal onset, cystic fibrosis, progressive familial intrahepatic cholestasis and alpha-1-antitrypsin (A1AT) deficiency. A1AT deficiency is an autosomal co-dominant genetic disease that affects the A1AT gene which is responsible for the production of the protein A1AT that is highly expressed by hepatocytes. The protein is secreted into blood and the enzyme is involved in the inhibition of neutrophil proteases in a bid to protect the host tissues from non-specific injury after inflammation. A deficiency in the production of this protein can cause, liver disease, hepatocellular carcinoma, and cirrhosis.

Birmingham Children's Hospital (BCH) is the UK's premier paediatric liver unit and is a specialist referral site for liver disease. Currently, A1AT levels and A1AT antitrypsin phenotype requests received are sent away to an external laboratory that process the tests twice a week. Urgent requests for alpha-1-antitrypsin levels are sent to another external laboratory via taxi for neonates/very young infants where a possible surgical investigation is required urgently. A method verification of the incorporation of an inhouse A1AT assay will be performed, to improve the efficiency of the availability of the test result and consequently improve the outcome of patients, with a potential reduction in send-away and transportation costs.

A clinical audit on the clinical outcome of patients who were previously treated for A1AT deficiency at BCH and have transitioned onto adult care to observe patterns in A1AT levels and phenotypes/genotypes to provide prognostic data to patients and families of children who are being treated for A1AT deficiency.

Analysis of tools used for the annotation and filtering of non-coding variants, and their optimisation for use in a clinical diagnostic service.

Aishah K. Westwood.

Whole genome sequencing (WGS) in diagnostic laboratories predominantly focuses on protein-coding regions, while non-coding regions of the genome are underexplored, despite their roles in complex diseases. In addition, popular computational tools offer limited annotations for non-coding variants and guidelines for the classification of variants did not exist for variants in non-coding regions, like they do for those in protein-coding regions, until recently. This study investigates two specialised tools, UTRannotator and SpliceAI, for their ability to annotate and distinguish between benign and pathogenic non-coding variants in diagnostic WGS runs. Intronic and 5'UTR variants were selected at random from the ncVarDB, and added to typical WGS runs for annotation through post processing. When considered alongside annotations provided by VEP and Slivar, SpliceAI performs with a 98.3% sensitivity and 100% specificity. UTRannotator exhibits a sensitivity of 92%, and a specificity of 96%, when annotations provided by VEP and Slivar are also considered. These tools will be incorporated into the All Wales Medical Genomics Service (AWMGS) pipeline to introduce non-coding variant annotation and interpretation, and guidelines for the classification of these variants will be adopted, with changes where necessary, to support and improved diagnostic service.

Detection of gene fusions in ctDNA in lung cancer.

Hamida Mohammed[1] and James Beasley[1].

[1]Birmingham Women's and Children's NHS Foundation Trust

ctDNA extracted from the plasma of cancer patients has many benefits when compared to standard diagnostic procedures such as tissue biopsy. Liquid biopsy based on ctDNA analysis has a rapid turnaround time, samples can be easily obtained, it is non-invasive and has a high concordance with tissue biopsy (Tivey et al., 2022). Results support diagnosis, prognosis and treatment monitoring for patients.

In the last few years there has been a push to deliver large NGS panels using ctDNA. The majority of ctDNA assays are designed to detect SNV only and not gene fusions which is why currently in the NHS ctDNA testing is done in very targeted and specialised cases.

The NHSE Cancer Genomics Test Directory for Non-small Cancer (NSCLC) soon requires genetic testing for driver mutations using ctDNA assays that are able to detect fusions (Blackburn et al., 2017). Currently detecting fusions in ctDNA is challenging and data shows that only 50% of fusions detected by ctDNA show a concordance with tissue testing (Mondaca et al., 2021).

The aim of this project is to assess the capability of two different assays in their ability to detect gene fusions in ctDNA. PGDx elio plasma resolve and Agilent resolution ctDNA are comprehensive, sensitive NGS assays that identify key genomic alterations in a range of cancer genes including SNVs and gene fusions. The project will include plasma separation from blood and extraction of ctDNA from plasma samples using the Genexus Purification System and the Qiasymphony DSP Virus/Pathogen Kit. The project will also include obtaining commercial control samples for testing with the intention of using patient samples at a later date. Our finding will support in ensuring compliance with future test directory requirements and improve care for cancer patients by increasing access to genomics testing throughout their clinical pathways.

Tivey, A. et al. (2022) “Circulating tumour DNA — looking beyond the blood,” Nature Reviews Clinical Oncology, 19(9), pp. 600–612. Available at: https://doi.org/10.1038/s41571-022-00660-y.

Mondaca, S. et al. (2021) “Clinical utility of next-generation sequencing-based ctdna testing for common and novel Alk Fusions,” Lung Cancer, 159, pp. 66–73. Available at: https://doi.org/10.1016/j.lungcan.2021.06.018.

CNV reanalysis of exome data: Expanding the diagnostic yield for rare diseases.

Isabel Reid[1] and Louise McClelland[1].

[1]West Midlands Regional Genetics Laboratory, Birmingham Women's and Children's NHS Foundation Trust

Introduction: The segregation of copy number variant (CNV) analysis and submicroscopic analysis, for single nucleotide variants (SNVs) and small insertions/deletion (indels), has been demonstrated to have a significant impact on diagnostic yield and overall time to diagnosis (1). Due to current testing strategies, a large subset of patients are offered frontline SNV testing and would only receive CNV specific testing if results are: indicative of larger changes, inconclusive or require additional confirmation - thereby increasing the chance of a missed diagnosis, due to an undetected CNV.

Routine clinical implementation of whole exome sequencing (WES) has proven highly beneficial in expanding diagnostic yield in a wide cohort of patients. However, CNV analysis using WES is yet to be routine, despite advancements offering increased read lengths and sequencing depths to enable efficient detection of both variant types. The West Midlands Regional Genetics Laboratory (WMRGL) has recently developed and implemented a bioinformatics pipeline to facilitate CNV calling of exome data, using a read depth approach (2).This simultaneous detection of CNVs and SNVs, offers elimination of the need to perform multiple assays on one patient and a means of speeding up the diagnostic process for many (3). The main aim of this project is to evaluate the diagnostic yield of WES-CNV calling through determining the range and frequency of pathogenic/likely pathogenic CNVs observed across different clinical indications, using exome data that has been previously run on the Novaseq for SNV analysis.

Methods: Novaseq exome data will be audited to review clinical indications and previously assigned gene panels. Data will be further reviewed to refine the patient cohort to a specific selection criteria: a previously negative result (with/without previous microarray testing) and/or a single variant in an autosomal recessive gene, and human phenotype ontology (HPO) terms will be assigned according to referral information. A minimum of 100 patients' FASTQ files will be manually reuploaded to the Congenica platform and CNVs called using the embedded ExomeDepth software. CNVs detected will be interpreted using the ACMG-Clingen CNV guidelines. Pathogenic/likely pathogenic variants will be confirmed by microarray, reported and used to determine diagnostic yield within the cohort.

Results: The project is ongoing, as of July 2023, exome data from 100 patients has been reuploaded to Congenica (patient cohort size is anticipated to increase). CNV interpretation is currently ongoing and results will be available by the end of 2023.

Conclusion: Data obtained from this project will supplement previous findings on the diagnostic yield of exome-based CNV detection (4), identifying clinical indications that may benefit most from the simultaneous detection of CNVs and SNVs. Given the recent implementation of the WES-CNV calling pipeline at WMRGL, this data may assist in identifying technical limitations and means of refining testing strategies in specific subsets of patients, with the overall aim of reducing the 'diagnostic odyssey', maximising quality of care and improving patient outcomes.

Mu, W. et al. (2019) "Detection of structural variation using target captured next-generation sequencing data for genetic diagnostic testing," Genetics in Medicine, 21(7), pp. 1603–1610. Available at: https://doi.org/10.1038/s41436-018-0397-6.

Nonacus (2021) Exome CG - Whitepaper [Preprint]. Available at: https://nonacus.com/exome-cg/ (Accessed: July 28, 2023).

Royer-Bertrand, B. et al. (2021) "CNV detection from exome sequencing data in routine diagnostics of rare genetic disorders: Opportunities and limitations," Genes, 12(9), p. 1427. Available at: https://doi.org/10.3390/genes12091427

Pfundt, R. et al. (2017) "Detection of clinically relevant copy-number variants by exome sequencing in a large cohort of genetic disorders," Genetics in Medicine, 19(6), pp. 667–675. Available at: https://doi.org/10.1038/gim.2016.163.

Design and implementation of a novel custom Genexus panel to meet the demands of high volume, fast turnaround somatic solid cancer services.

Lorenzo Magini[1,2], James Beasley[2] and Samantha Butler[2].

[1]University of Manchester; [2]Core Solid Cancer, Birmingham Women's Hospital

Over the past year, the BWH Solid Cancer team has recognised the need to enhance its service due to an increase in the volume of samples and the short turnaround times required for the adult National Genomic Test Directory. The rise in sample numbers can be attributed to advancements in treatments and actionable gene variants. One such treatment is the recently approved PARP inhibitor. As a result, the demand has exceeded the laboratory's current technologies, particularly for BRCA and POLE testing that are conducted using TSO500 and Truesight170. Although the Oncomine Precision Assay has proven useful, it does not include coverage for BRCA1, BRCA2 and POLE, and these can only be delivered using very large NGS panels. Consequently, the failure rate is higher, and greater read depths cannot be achieved. To address this issue, the team requires a custom panel that can discover clinically targetable variants in a smaller number of genes found in solid cancers such as breast (PIK3CA, BRCA1, BRCA2), ovarian (BRCA1, BRCA2), pancreatic (BRCA1, BRCA2), prostate (BRCA1, BRCA2, ATM, CDK12), and endometrial (POLE) cancer. The immediate goal of this project is to create a gene panel that can target clinically actionable variants in a smaller number of genes present in these cancers. Once developed, the panel will need to be validated and introduced into the service. This goal will be achieved by designing an Oncomine Tumor Specific Panel (OTSP) on the Ion AmpliSeq Designer Thermofisher platform. This panel will include the most important genes related to the tumour. To ensure its accuracy, the panel will be tested on control and pre-characterised samples. The results will be compared with previous reports to validate the panel. Finally, the accuracy of the panel will be assessed by calculating sensitivity, specificity, positive predictive value, and negative predictive value. At present, preliminary data are not available, but the team expects to achieve an x500 coverage on the majority of genes (particularly BRCA), and improve turnaround times significantly.

An Audit and Service Evaluation of the R21 Prenatal Exome Sequencing Service.

Megan Horton-Bell and Steph Allen.

Whole exome sequencing is becoming increasingly integrated within prenatal diagnostic services and is an important tool in identifying genetic aetiologies of foetal structural abnormalities. The West Midlands Regional Genetics Laboratory has been providing the R21 prenatal exome sequencing service to patients within the Central and South, North West and Yorkshire and North East regions of England since 01/10/2020. This audit aimed to review the service provision over the first two years (01/10/2020 to 30/09/2022). In total, 555 referrals were received and 357 proceeded to testing. 105 diagnoses were reported, representing a diagnostic yield of 29.4%. Pathogenic variants were identified in 71 genes, 16 of which were recurrent. 6/15 reported variants of uncertain significance were later reclassified to likely pathogenic, raising the diagnostic rate to 31.1%. Abnormalities of prenatal development and the central nervous and musculoskeletal systems were the most frequently reported phenotypes, and skeletal dysplasias were the most common disease category. A review of nuclear encoded mitochondrial genes identified 51 that were suitable for addition to the R21 Fetal anomaly panel. The service provides clear clinical benefit to parents and their healthcare team and allows for informed decision making regarding their pregnancy. However, continued development of the service is required, to ensure equity of access and to stay abreast of new associations between genes and prenatal phenotypes.

Investigating the use of the Mastermind genomic search engine in existing variant interpretation pathways.

Pavandeep K. Sidhu and Jessica Woodley.

Variant interpretation according to guidelines such as those set by the ACMG (Richards S, et al., 2015) is an important part of the work of NHS genomics laboratories throughout the UK. Locating literature to aid in this is a difficult and time-consumiing part of this process, with scientists making use of platforms such as Google Scholar, PubMed, or services like the Human Gene Mutation Database (HGMD). Launched in 2019 by Genomenon, Mastermind is a platform that indexes literature relating to Mendelian disease to allow for more sensitive, accurate and time-efficient literature searching for variant classification (Chunn LM, et al., 2020). Increased sensitivity of variant classification and more time-efficient literature searching would help NHS laboratories increase diagnostic yield on patient samples and reduce patients' diagnostic odyssey. However, these claims are as yet untested in a clinical NHS setting and the use of the platform comes with a cost implication that requires justification. This research aims to validate the claims of the Mastermind platform by undertaking variant interpretation with and without the platform to provide a direct comparison of its benefits over other literature-searching methods. The data gathered will then be used to perform a cost-benefit analysis for the use of the Mastermind platform to determine if it should be adopted into existing variant interpretation protocols. As the project is currently underway, there are no findings to discuss as of yet. Ultimately, if the claims made by the makers of the platform prove to be true, there would be a significant impact on NHS genomic scientists in helping them to meet turnaround times and reduce extensive case backlogs across the UK.

Chunn LM, Nefcy DC, Scouten RW, Tarpey RP, Chauhan G et al. Front. Genet. 2020;11:577152.

Richards S, Aziz N, Bale S, Bick D, Das S et al. ACMG Standards and Guidelines 2015;17(5):405-424.

Pipeline rewrite and cloud deployment of the existing Universal MiSeq Pipeline using Nextflow.

Rachael Ayegba[1].

[1]Trainee Clinical Scientist (Bioinformatics – Genomics), University of Manchester, email: rachael.ayegba@nhs.net

PREMIS OF THE STUDY: Genomic analysers generate large amounts of data. This data amounts to approximately 1TB/week (predicted for 2021) and increasing on a yearly basis by an unknown amount. A lack of storage space negatively affects the performance of genomic laboratories and can result in the disruption of the Bioinformatics Service as well as other services within the laboratory. Cloud platforms offer a solution to this problem, and other benefits, including reproducibility of pipeline analysis and the provision of global access to said pipelines and the data they generate. As a result, cloud platforms are beneficial for large genomics collaborations, and prove to be a valuable tool in aiding the breaking down of data silos and the promotion sharing of genomics data. The cloud is also the substrate for the National Institute of Health (NIH) Data Commons Pilot; an effort to increase availability and utility of data and software from NIH-funded efforts. The move to cloud platforms will aide in streamlining the data processing and storage at the West Midlands Regional Genomica Laboratory (WMRGL), thereby ensuring the long-term safe storage of our NGS data. Additional benefits of this change include the reduction of long-term storage cost, especially with the ever-increasing NGS data generated, and the ability to access to pipelines used by other laboratories, the ability to work on pipelines that have been created collaboratively, as well as the sharing of data within the Genomic Laboratory Hubs (GLH) and other healthcare organisations where appropriate.

MEHTODS: The main aims of the project are to: (1) re-write the pipeline in nextflow and (2) deploy the pipeline on a cloud platform. As such the project can be split into 2 sections, respectively. Once the pipeline re-write has been completed, in-house validation data set, containing clinical samples, will be used to test the pipeline. This data set is obtained from runs previously processed by other versions of the pipeline and the results are already known. As the development process progresses iterative testing will be undergone on each tool or technique added, comparing results or outputs with those expected. Genome in a bottle data will also be used for validation. The Genome in a Bottle (GIAB) Consortium is hosted by the National Institute of Sceine and Technology (NIST) and provides reference material which can be used to adapt next-generation sequencing tests into clinical practice. These reference materials are based on NA12878 DNA from Coriell and are useful for validating NGS pipelines as they allow for comparison between the pipeline findings and the highly confident small variant (SNP and Indel) calls from GIAB.

TP53 disruptions detected in West Midlands CLL Patients from 2019 to 2022, real world data including SNV and CNV data by NGS.

Rofida Al-Thubhani.

The presence of TP53 aberrations due to deletion of 17p and/or TP53 gene variants is an important risk factor for CLL patients. According to the international workshop on Chronic Lymphocytic Leukemia iwCLL, patients with cells that carry del(17p) and /or TP53 mutations have inferior prognosis and appear relatively resistant to standard chemo-immunotherapy agents and have a very poor outcome. In the era of new therapies including BTK inhibitors, there have been improvements in progression-free survival, but long-term survival outcomes remain poorer for patients with TP53 aberrations. TP53 deletion/variant mutation is a core test on the NHSE National Genomics Test Directory for CLL patients who are about to undergo treatment. It can be difficult to determine at the point of referral if the sample/ time point is suitable for testing.

In the WMGLH, only confirmed CLL referrals with indications for treatment or signs of disease progression are tested. It is not appropriate to test patients at initial presentation or where diagnosis is not confirmed. Currently, TP53 deletions are detected by FISH and TP53 variants using a targeted CLL NGS panel, requiring cell culture and DNA extraction from every sample. The NGS panel (OGT SureSeq CLL + CNV) also has the capacity to detect copy number aberrations in 17p, 11q 13q and chromosome 12.

Validation work has shown that NGS can detect most deletions detected by FISH, along with further small deletions beyond the sensitivity of interphase FISH. However, as these two techniques detect CNV in very different ways, it is difficult to do an exact like for like comparison between the results of the two techniques.

To support our understanding of the WMGLH CLL patient cohort, a retrospective audit of all patients received for TP53 aberration testing at WMRGL will be performed. This audit will examine how standard care is delivered for CLL patients at the testing time point and how this can be improved. There is a proposed plan to change the method of detecting 17p CNV from FISH to NGS and the outcomes from this audit will support this.

Abstract for STP Conference 2023: Audit of the first 18 months of the Somatic Solid Cancer NGS Panel Testing Service.

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At West Midlands Regional Genetics Laboratory (WMRGL), the expansion of medium and large NGS panels in acquired solid cancer has been a focus of the team since April 2021. NGS testing for the Central and South genomic laboratory hub (GLH) has been largely centralised to WMRGL. Since the implementation of larger cancer panels, it has been found that driver mutations may not be mutually exclusive. Until recently, genetic testing in cancer was dedicated to the detection of individual driver genes that were identified by recurrent mutation of the genes in a large cohort of cancer patients. However, due to the heterogeneity of tumours, more attention is now being paid to identify co-occurring variants in which the corresponding genes usually function in different pathways. It has been recognised that multiple pathways with mutations are generally required for cancer, and that pathways often function together in carcinogenesis (1). The importance of identifying co-occurring driver variants centres on the potential of molecularly targeted therapy in cancer treatment. Matching patients with targeted therapies based on identification of single genomic drivers leads to large variability in response to targeted treatment. The identification of co-occurring drivers can function as more precise predictive biomarkers of therapeutic response, as well as enable development of highly personalised therapeutic approaches (2). It has also been observed that there is variation in the success of RNA sequencing from FFPE samples between pathology centres. Samples may fail either pre-test QC (following DNA and RNA extraction) or post-test QC. Samples may fail pre-test QC due to variation in tissue collection and preservation methods, cellularity, or extraction method. Post-test QC fails can occur due to the choice of library preparation protocol. However, anecdotally we have observed that a significant proportion of post-test QC fail cases are isolated to specific centres, which suggests that tissue processing also plays a role (3). It is also important that we can reflect inwardly on how internal processes could be improved or optimised to ensure that we deliver a sufficiently robust service. A component of this audit will therefore aim to understand where we can improve our processes from sample receipt to the reporting of results.

Therefore, the aims of this audit are to: target performance including turnaround times and process mapping and NGS failure rate; understand the causes of RNA sequencing success variation across the main referral centres; review the prevalence and nature of co-occurring driver variants (rarely reported prior to the implementation of medium/large cancer panels).

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