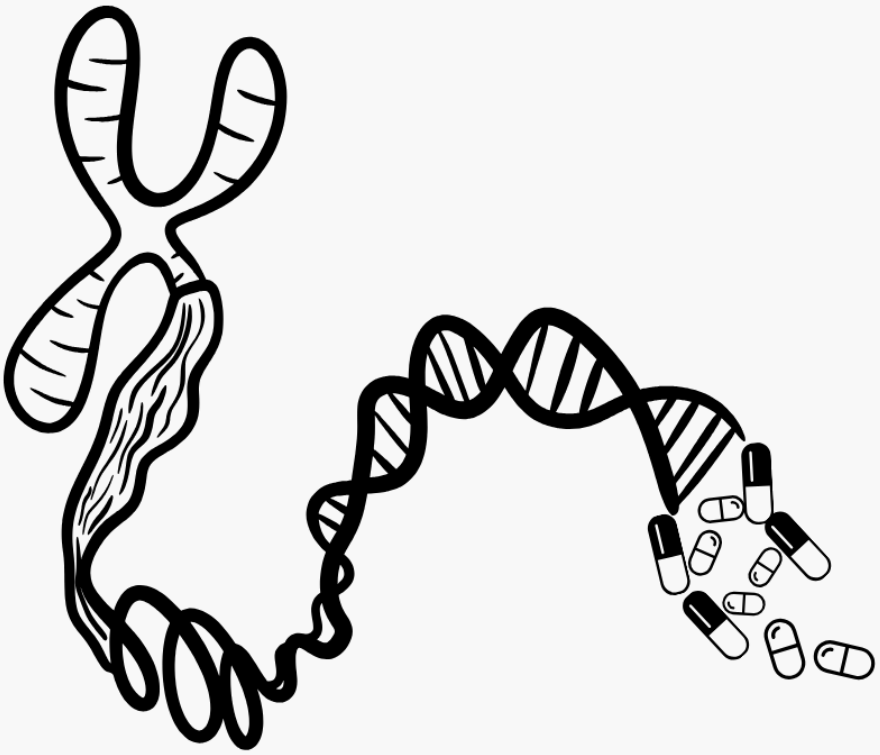
2025 Conference proceedings

WMRGL
STP CONFERENCE



PHARMACOGENOMICS

Contents

Programme

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.	09:00	09:15
Director's welcome and agenda The lab director will welcome everyone, and the conference host will go through the agenda for the day.	09:15	09:30
Overview of the workshop Speaker: Eudmar Marcolino	09:30	09:45
Guest Speaker 1: Introduction to pharmacogenomics. Speaker: Maria Monro	09:45	10:30
Guest Speaker 2: How new genes are added to the test directory. Speaker: Lowri Hughes	10:30	11:00
Mid-morning break (Complementary tea, coffee, cereal bar and fruits served)	11:00	11:15
Guest Speaker 3: Current Pharmacogenomics tests are available in the test directory. Speaker: Caroline Picard	11:15	12:00
Lunch Break Hot and cold buffets are served at the hotel restaurant.	12:00	13:00
Guest Speaker 4: Clinical research in pharmacogenomics - research case: Assessing pharmacogenomic testing to treat mental health conditions in the NHS Speaker: Jessica Woodley	13:00	13:45
Workshop STPs will group to discuss and reflect on the lectures, examining the impact of pharmacogenomics on their specialism. STPs will prepare a 5-minute presentation to share with the other groups the results of their discussions.	13:45	15:00
Selected oral presentations from abstracts **DDX41 Splice Region Variant RNA Studies in Acute Myeloid Leukaemia** Speaker: Georgia Robson	15:00	15:20
Development of a pharmacogenomic pipeline utilising next-generation sequencing data for their implementation into routine patient care at the point of prescription. Speaker: Matthew Lord	15:20	15:40
Poster sessions Twelve posters will be selected from the abstracts submitted. The guests will be encouraged to network. Complementary tea, coffee and a sweet treat served	15:40	17:00

Introduction to Pharmacogenomics

Speaker: Maria Monro

Notes:

How new genes are added to the test directory.

Speaker: Lowri Hughes

Notes:

Current Pharmacogenomics tests are available in the test directory.

Speaker: Caroline Picard

Notes:

Clinical research in pharmacogenomics - research case: Assessing pharmacogenomic testing to treat mental health conditions in the NHS.

Speaker: Jessica Woodley
Notes:

Workshop

S-C1_14: Identify and evaluate an innovation which could impact the future of practice and patient care in your speciality and communicate your findings to a multidisciplinary audience.

Considerations:

Reflect on the analysis, including:

- Critical appraisal
- Horizon scanning
- Evidence-based practice
- Effective communication
- Patient-centred care and support
- Access to healthcare and health inequalities
- Continued professional development
- Continuous improvement
- Drivers and barriers to change, including political, economic, environmental, social and technological
- Leading change

Trainers: Eudmar Marcolino **One File Assessor:** Anita Luharia and Jennifer Whitfield

This training activity will draw on the information you have acquired throughout this conference, such as through your lectures on:

- Introduction to pharmacogenomics.
- How new genes are added to the test directory.
- Current Pharmacogenomics tests are available in the test directory.
- Clinical research in pharmacogenomics - research case: Assessing pharmacogenomic testing to treat mental health conditions in the NHS
- Recordings of the Pharmacogenomics Network of Excellence supplied via the conference website: National Pharmacogenomic Network of Excellence events:: North West Genomics Medicine Service Alliance
    - Please watch:
        - Progress update and Network of Excellence overview (from 16 min)
        - Using Genetics with Health-related Data in Drug Discovery & Development

If you have any specialism-specific questions, please direct them to the appropriate person.

Part 1: Reflecting

Individual reflection: Please complete at the end of the lectures	
What policies or resources do you think would be necessary to make pharmacogenomic care accessible to all?	
Do you know of any new gene-drug pair that is nearly ready academically and could be added to the list of tests offered?	
What metrics could be used to evaluate the impact of pharmacogenomics on our practice?	
Would you suggest different approaches to implementing pharmacogenomics in our healthcare system? What could your speciality contribute to the process that scientists in other specialities may have missed?	
How can pharmacogenomics personalise treatment for patients in our speciality?	

Group Reflection

Amongst the group of STPs who are also on rotation with yourself or other first-year students, you will need to compare your learning and highlight the similarities and differences between your experiences. This can then be added to an overall reflection, which is below.

Group Reflection: Please complete the following reflective discussion	
What are the main political, economic, environmental, social, and technological drivers and barriers that could impact the adoption of pharmacogenomics?	
When do you think patients should be offered pharmacogenomics tests?	
Should we give priority to terminal patients?	
How might pharmacogenomics create or reduce health disparities within our patient population?	
What elements of pharmacogenomics and its implementation have surprised you or caused you to think differently?	

What will you take away from this reflection on the rest of your STP journey?	
Any other reflections?	

Part 2 Present your group's discussions and conclusions

Using the laptops provided, please prepare a short 5-minute presentation where you summarise your group reflection, highlighting any consensus that you may have reached.

Presentation assessment

Your presentation will be assessed using a multi-feedback model. Your assessors, your peers, and you will evaluate you.

Please fill in a form for yourself and one for each colleague in your group.

https://forms.office.com/e/ZCnzK2J2Ug

Criteria

1. Slides

(24-33.3)	Clear, high impact, eye-catching
(20-23)	Clear, visually attractive
(17-19)	Clear, Easy to Read
(13-16)	Readable
(<13)	Unclear, e.g. Print too small, poorly set out and difficult to follow

2. Presentation

(24-33.3)	Exceptional Delivery and Engagement
(20-23)	Strong Delivery with Minor Gaps
(17-19)	Good Delivery but Lacking Consistency
(13-16)	Basic Delivery with Significant Issues
(<13)	Poor Delivery with Major Shortcomings

3. Content

(24-33.3)	Reflection is Comprehensive, Insightful, and Well-Communicated
(20-23)	Reflection is Detailed, Relevant, and Well-Delivered
(17-19)	Reflection is Adequate, With Minor Gaps
(13-16)	Reflection is General, With Significant Omissions
(<13)	Reflection is Incomplete or Lacking Critical Understanding.

List of Abstracts

Evaluation of pharmacogenomic targets and laboratory platforms for the delivery of cost effective and high throughput pre-emptive testing within the NHS Genomic Medicine service

Aisling Massey[1,2;] Lydia Percival[2]

[1] The University of Manchester; [2]North West Genomic Laboratory Hub

Adverse drug reactions (ADR) account for 6.5% of UK hospital admissions and have been estimated to cost the NHS £2.21 billion. Notably, 40% of ADRs are thought to be preventable. To increase the efficacy and safety of therapeutics, the benefit of integrating pharmacogenomics (PGx) testing into the National Health Service (NHS) is of current interest.

Pharmacogenomic analysis involves genotyping haplotypes within PGx-associated regions to predict an individual's drug metaboliser phenotype. Commonly, the star (*) allele nomenclature system is applied for describing PGx haplotypes. Increasing evidence supporting the role of specific star alleles in ADRs has led to the publication of pharmacotherapeutic recommendations that can be utilised by prescribers for optimal patient treatment. So far, recommendations have been produced for actionable allele-drug associations for over 90 drugs.

The UK Government has recognized the potential of taking a pre-emptive, panel-based approach to pharmacogenomic testing, including it in their 2020 10 –year plan for healthcare. This has already been trialed in the Netherlands where, in 2019, the Dutch Pharmacogenetics Working Group (DPWG) guidelines were utilised to design a standardised pre-emptive gene panel. It has been estimated that, if the UK population utilised this panel, an estimated 8.6–9.2% of initial prescriptions would require a change in prescription according to Clinical Pharmacogenetics Implementation Consortium (CPIC) and/or DPWG guidelines

The aim of this project is to review evidence in current literature to design a pre-emptive pharmacogenomic panel for use within the NHS Genomic Medicine Service. This will involve the evaluation of haplotypes to target on the panel and choice of methodology/technology to deliver the service. I will then critically appraise the feasibility of its use in the NHS.

Keywords: star alleles, pharmacogenomic recommendations, pre-emptive gene panel

R21 Prenatal Exome Sequencing – Further Development of the Service

Bethany Jordan[1,2]

[1]West Midlands Regional Genetics Laboratory, Birmingham Women's and Children's NHS Foundation Trust, Edgbaston, Birmingham, UK; [2]Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK

The R21 prenatal exome sequencing service, introduced by NHS England in 2020, aims to improve prenatal diagnosis for pregnancies with fetal anomalies of likely monogenic cause. The R21 pathway involves trio whole exome sequencing and analysis of 1,417 genes on the fetal anomalies panel (Panel App, v5.0). This service development project aimed to improve diagnostic yield and clinical utility of R21 testing. The project included 5 components: 1) an audit of R21 cases (October 2020-November 2024) to determine diagnostic yield and evaluate inheritance modes, variant consequences, and causative genes in positive cases; 2) a review of the fetal anomalies panel to identify new genes for inclusion in the next panel update; 3) optimisation of Congenica's variant filtering presets to improve diagnostic sensitivity and specificity; 4) gene-agnostic reanalysis of R21 cases with no clinically relevant findings; and 5) a clinical audit of isolated short long bones (SLB) and small for gestational age (SGA) cases following their inclusion in the R21 eligibility criteria in April 2023.

The R21 audit revealed a diagnostic yield of 30.3%, with autosomal de novo variants accounting for most diagnoses (46.6%). Missense, frameshift and stop-gained variants were the most prevalent, and causative variants were identified in 176 unique genes. The fetal anomalies panel review added 85 new green genes, including 24% upgraded from amber, 5% from red, and 72% newly included, broadening the panel's diagnostic scope. A review of Congenica's variant filters proposed changes to capture splice region and non-PASS QC variants, refine inheritance filters, and introduce a knowledgebase-only filter, improving sensitivity while maintaining specificity and reducing incidental findings. Gene-agnostic reanalysis of 9 cases identified potential causative variants in 3 cases pending MDT discussion and reportable incidental findings in 2 cases, highlighting the benefit of gene-agnostic analysis to identify diagnoses in genes outside the panel. The SLB/SGA audit demonstrated a higher diagnostic yield for isolated SLB cases (36.4%) than isolated SGA (5.9%), supporting the clinical utility of exome sequencing for isolated SLB. This project highlights key strategies to optimise the R21 service, improving diagnostic yield to support prenatal diagnosis and inform clinical management for pregnancies with fetal anomalies.

Keywords: prenatal exome sequencing, prenatal diagnosis, fetal anomalies

Research environment – Long Read Sequencing for Rare Disease

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

In 2018, the Genomic Medicine Service (GMS) was created to ensure nationwide access to genetic and genomic testing[1]. The NHS employs various testing strategies for these genetic conditions, with WGS being the most extensive, it uses a "short read" sequencing (SRS) technique[2]. However, challenges arise when assembling "short reads," particularly for identifying variants in repetitive regions, pseudogenes and complex structural variants[2,3]. Additionally, WGS is limited as it uses virtual panels for analysis, meaning that although the whole genome is sequenced, only exonic regions of genes known to be associated with the patient's features are analysed[1]. These limitations leave ~65% of patients without a genetic diagnosis.

To increase diagnostic yield, a new testing pathway is needed. We will be utilizing Oxford Nanopore (ONT) long-read sequencing (LRS) to identify previously undetectable variants, as it sequences larger genomic segments (theoretically it is only limited by the length of DNA or RNA molecules) in one go[2,3]. Compared to SRS, LRS uses a nanopore and electric current to read DNA without fragmentation, enabling the detection of the mentioned complex variants[2]. We will also be utilising a gene-agnostic approach meaning that we will be analysing all of a patients' genomic data without the use of virtual panels and with no preconceptions about which genes are associated with a patients' condition.

A proof-of-principle project demonstrated LRS's effectiveness, identifying diagnostic results in at least 10% of previously undiagnosed patients. My project aims to build on the success of this, by taking a new, larger cohort of patients and sequencing their genome using ONT, then analysing the data produced using Geneyx to identify Pathogenic/ Likely Pathogenic variants, thereby providing families with a diagnosis. Confirmation studies or RNA studies may also be required to confirm the pathogenicity of some variants. The project will also review these patients to determine why LRS was able to identify these variants when other technologies could not, and which referrals reasons were linked to these newly identified variants.

1. Friedrich B, Vindrola-Padros C, Lucassen AM, Patch C, Clarke A, Lakhanpaul M, et al. "A very big challenge": a qualitative study to explore the early barriers and enablers to implementing a national genomic medicine service in England. Frontiers in Genetics. 2024, Jan, 4;14.
2. Hu T, Chitnis N, Monos D, Dinh A. Next-generation sequencing technologies: An overview. Human Immunology. 2021, Mar, 19;82(11): 801-811.
3. Warburton PE, Sebra RP. Long-Read DNA Sequencing: Recent Advances and Remaining Challenges. Annual Review of Genomics and Human Genetics. 2023 Apr 19; 24:109-132.

Keywords: Long Read Sequencing, Novel testing pathway, Increased diagnostic yield.

Evaluation of CNVs in New Inherited Cancer Panel

Deanna Wood[1]; Samantha Butler[1]

[1]West Midlands Regional Genetics Laboratory, Birmingham Women's Hospital, UK

Copy Number Variants (CNVs) arise due to losses or gains of DNA and are caused by deletions and duplications. They are observed in healthy individuals and contribute significantly to genetic diversity. However, CNVs can cause changes in gene expression that increase susceptibility to several diseases, including inherited cancers. Therefore, CNV testing for inherited cancers is included in the National Genomic Test Directory (NGTD).

WMRGL (West Midlands Regional Genetics Laboratory) used the TSHC (TruSight Hereditary Cancer) NGS Panel for SNV (Single Nucleotide Variant) detection, and MLPA (Multiplex Ligation-dependent Probe Amplification) testing for CNVs in inherited cancer.

The NGTD includes guidance on which methods of testing are funded. Recently, MLPA was removed as a funded method for upfront CNV testing in inherited cancer for most genes. MLPA kits are not available for all genes, and it is costly to set up for new genes, therefore NGS is the preferred method of testing.

Previous work has been done at WMRGL to validate the TSHC panel for CNV detection, however, limitations with gene coverage meant there was a need for a new panel, capable of both CNV and SNV detection.

The aim of this project was to compare CNV detection in two different panels, Nonacus GALEAS Hereditary Plus, and SOPHiA Genetics Hereditary Cancer Solution.

Stored patient samples with known CNVs were tested using each panel and analysis involved comparing observed results with expected results. CNVs that are known to be challenging to detect by MLPA, such as those in GC rich regions, were included to compare performance.

Another important factor was ease of analysis. For Nonacus GALEAS Hereditary Plus, Congenica was used to analyse the results in house, whilst Nonacus carried out their own analysis using a different method. SOPHiA Genetics have a web platform for analysis called SOPHiA DDM (Data Driven Medicine), and this was used by SOPHiA Genetics and WMRGL for analysis.

Whilst analysis and interpretation of the results from these validation runs are ongoing, initial findings have shown that SOPHiA DDM is a more suitable tool for CNV analysis compared to analysing Nonacus data on Congenica.

Keywords: service development, CNV detection, evaluation

Long Read Sequencing for Prenatal Diagnosis – Proof of Principle

Ehlam Leyla Ahmed-Ali; Stephanie Allen; Lorraine-Hartles Spencer

West Midlands Regional Genomics Laboratory, Birmingham Women's and Children's NHS Foundation Trust

Prenatal diagnosis of genetic disorders has evolved significantly, from traditional karyotyping to advanced whole exome sequencing (WES) and chromosomal microarray analysis (CMA). Despite these advances, existing technologies have limitations in detecting structural variants, repetitive regions, and accurately phasing variants. Long-read sequencing (LRS), also known as third-generation sequencing, offers a promising solution by generating significantly longer DNA sequence reads, thereby overcoming many of these challenges. This proof-of-principle study investigates the feasibility and clinical utility of LRS in prenatal diagnostics.

The study focuses on optimising DNA extraction and sequencing protocols to accommodate the unique challenges of fetal samples such as amniotic fluid and chorionic villus sampling (CVS), which often yield limited and degraded DNA. Approximately 20 samples will be processed to optimise DNA extraction protocols, and 5–8 confirmed prenatal cases will undergo LRS using Oxford Nanopore Technology. Data analysis will be conducted using Geneyx software, and the results will be compared to those obtained through short-read sequencing analysed via Congenica.

The objectives of this study include exploring the benefits of LRS over traditional methods in identifying fetal genetic anomalies, evaluating sample preparation requirements, and assessing the quality and diagnostic yield of LRS data. By leveraging LRS's ability to detect structural variants, repetitive elements, and phase variants with higher accuracy, this research aims to demonstrate its potential as a transformative tool in prenatal diagnostics.

This project will not only address the technical barriers of implementing LRS in a clinical prenatal setting but also provide insights into its diagnostic advantages over short-read sequencing. If successful, this proof-of-principle study could pave the way for the routine adoption of LRS in prenatal care where traditional methods failed to yield a diagnosis.

Keywords: Long read sequencing, prenatal diagnostics, Oxford Nanopore

Title: *DDX41* Splice Region Variant RNA Studies in Acute Myeloid Leukaemia

Georgia Robson[1,2], Rachel Price[1]

[1]West Midlands Regional Genetics Laboratory, Birmingham Women's and Children's NHS Foundation Trust, Birmingham, United Kingdom; [2] Department of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom.

Germline mutations in the DEAD-box helicase 41 (*DDX41*) gene define an adult familial AML syndrome characterised by long-latency, advanced disease, and a normal karyotype. *DDX41* encodes a 75kDa DEAD-box-type RNA helicase that is involved in RNA metabolism. Through defective pre-mRNA splicing and RNA processing, *DDX41* variants result in a loss of tumour suppressor function. Several splice region variants in *DDX41* have been identified at our institution at variant allele frequencies consistent with being germline. Despite being one of the most common genes associated with a myeloid neoplasia predisposition, there is limited data available in the literature to support the pathogenicity of splice region variants. The ClinGen SVI Splicing Subgroup published recommendations for applying splicing evidence following the American College of Medical Genetics and Genomics (ACMG) and the Association of Molecular Pathology (AMP) 2015 framework for classifying variants. Variants that have a variant-specific impact on RNA splicing can be weighted pathogenic very strong (PVS1) according to these recommendations. Identification and characterisation of a pathogenic germline variant in *DDX41* is necessary for appropriate patient clinical management and haematopoietic stem cell transplantation (HSCT) donor assessment. *DDX41*-related disease shows long-latency, reduced penetrance, and manifestation at an advanced age often with no concerning family history, making identification of hereditary disease a challenge. Stem cell transplants from *DDX41* mutation carriers may promote donor cell leukaemia so in the context of stem cell donor selection, excluding candidates with a germline pathogenic variant appears necessary, and can improve family management of asymptomatic carriers. This research will provide functional evidence of the effect of these splice region variants on *DDX41* transcription. Our approach uses RT-PCR to investigate the variants detected within genomic DNA. RNA extracted from whole blood or bone marrow samples referred to the West Midlands Regional Genetics Laboratory for diagnostic acute myeloid leukaemia genomic testing, converted into cDNA was interrogated by PCR amplification, followed by Sanger sequencing. Preliminary results suggest concordance between predicted splicing effect by in silico tools and observed RNA splicing effect in acute myeloid leukaemia.

Keywords: acute myeloid leukaemia, splice region variants, RNA studies

Missing Heritability in Inherited Cancer Genes

Leanne Herbert, Jennie Dring, Samantha Butler

West Midlands Regional Genomics Laboratory, Birmingham Women's and Children's NHS Foundation Trust, Birmingham, United Kingdom

The loss of the tumour suppressor gene *adenomatous polyposis coli* (APC) has been well characterised for its role in familial adenomatous polyposis (FAP). FAP is an autosomal dominant inherited condition which results in the development of an extensive number of colonic polyps. In the absence of intervention, FAP patients have up to a 100% risk of developing colorectal cancer (CRC) in their lifetime. There is currently a subset of patients which represent a diagnostic odyssey in the inherited cancer context. There are patients exhibiting clear phenotypes associated with FAP and may even have a clinical diagnosis, however current sequencing panels have been unable to identify and likely pathogenic/pathogenic variants. This is due to current sequencing panels targeting the exons and only capture a few bps into the introns. A previous in-house study was able to identify a deeply intronic APC variant which was clearly pathogenic in a patient affected with FAP. This was done by whole genome sequencing (WGS) using oxford nanopore technology (ONT).

This project aims to identify a sequencing method, that is feasible in a clinical setting, which can be offered to patients with a clinical diagnosis of FAP or a strong family history where current NGS panels did not identify any variants in the APC gene. A review of the literature will help to identify sequencing methods that have been used previously and select a method that could be implemented into the service. Previous studies have attempted to tackle missing heritability in both APC and other inherited cancer conditions by using methods such as paired DNA-RNA multigene panel testing or WGS. Other studies have investigated the use of adaptive long read sequencing, which is another potential option, both in CRC and other inherited conditions.

This project may have direct relevance to routine clinical service and will not only benefit the patient but also family members as presymptomatic testing can then be offered. This will mean that unaffected individuals will not have to undergo invasive screening procedures when there is no clinical need and affected individuals can be monitored appropriately.

Keywords: missing heritability, sequencing, colorectal cancer

Evaluation of the First 3 Years of Solid Cancer WGS at WMRGL.

Lynda Speake[1]; Lowri Hughes[1]; Natasha Vafadar[1]

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

Discovery of recurrent driver variants in a range of cancer-associated genes and the development of targeted cancer treatments has led to an increased demand for solid tumour genetic testing. This demand is currently met using NGS panels which only interrogate certain genes, depending on tumour type. Whole genome sequencing (WGS) provides the opportunity to sequence the whole genome, allowing detection of small nucleotide variants, copy number variants and mutational signatures in a single test. The use of paired germline and tumour samples also allows identification of both somatic and germline variants, providing information on cancer susceptibility alongside tumour profiling. The 100,000 Genomes Project pioneered the use of WGS in routine care, offering the potential benefits of WGS to certain NHS patients.

This project had two aims: to review the WMRGL solid cancer WGS pathway to identify potential areas for improvement and to evaluate the utility of solid cancer WGS.

Process mapping of the solid cancer WGS pathway identified that the average number of days from sample receipt to report authorization decreased from 209 days in 2021/2022 to 103 in 2024. Analysis and reporting was identified as a key bottleneck, taking an average of 109 days across all years of the service. A range of external and internal aspects were identified for potential improvements, such as clinician engagement, workload prioritization, funding and IT infrastructure. Review of 375 WGS reports showed that 'consistent with diagnosis' and 'future trials' were the most common type of reported outcome. Actionable findings were identified in 39% cases across all tumour types, except for paediatric. When compared with standard of care (SoC) testing, WGS was concordant with SoC in 52% of cases and provided additional information in 46% of cases.

WGS is a significant investment of time and resources, and this project highlights that WGS currently has limited utility, however; patients currently receiving WGS testing have often exhausted other options, so any new avenues provided by WGS are beneficial. Currently, WGS results are not reported within a clinically relevant timeframe; however, this project identifies key areas which need to be changed to help reduce turnaround times.

Keywords: whole genome sequencing, tumour profiling, quality improvement.

Development of a pharmacogenomic pipeline utilising next generation sequencing data for their implementation into routine patient care at the point of prescription

Matthew Lord – 3rd Year Genomics STP (selected for oral presentation)

Manchester NHS Foundation Trust

As pharmacogenomic (PGx) testing becomes more available and PGx guidelines continue to be developed by groups such as NICE, CPIC and the FDA, implementation options must be explored. PGx testing for genes such as DYPD for the use of fluoropyrimidines and CYP2C19 for the use of Mavacamten exemplify the benefits of PGx testing but do so via targeted testing separate to their respective diagnostic tests. The inclusion of PGx targets in existing diagnostic exome sequencing panels presents the opportunity for PGx testing to be implemented into existing pathways, reducing the need for secondary targeted tests and therefore turnaround times for patients. To investigate this, a bioinformatic pipeline has been developed to analyse exome sequencing data at PGx target locations and a new exome sequencing build has been developed by Agilent Technologies including a curated list of PGx targets. The pipeline was used to analyse data from the existing CCP19 rare disease exome used at MFT, and the newly developed Agilent exome. Results demonstrate a functional bioinformatic pipeline able to determine PGx allele status across a range of PGx targets. The current CCP19 exome sequencing panel however shows limited coverage of important PGx alleles such as the CYP2C19*2 allele, present in 30%,17% and 15% in Chinese, African American and Caucasian populations respectively. The exome build developed with Agilent Technologies however was developed with a curated list of PGx targets, and demonstrated good coverage across all PGx targets predictive of phenotypic presentation. This project demonstrates a possible method by which PGx targets could be analysed using current exome sequencing technologies which, if implemented into service, could allow for PGx genotyping alongside diagnostic tests, or retroactively from exome sequencing data at the request of prescribers for a number of indications.

Keyword: Pharmacogenomics, next generation sequencing, implementation

A Web-Based Competency Tracking Application for NHS Workforce Development

Mishaal Amir

ePortfolios have become a vital tool in the NHS, transforming the management and assessment of competency, training, and learning. Fundamentally, an ePortfolio is a thorough digital archive that centralises a healthcare professional's career. ePortfolios enable a comprehensive perspective of a person's professional story, encompassing everything from academic achievements and continued professional development (CPD) to reflective practices and evidence of competencies. This is essential in a field where continuous education and training are critical for improving patient care and operational effectiveness in addition to career advancement.

This research introduces an integrated digital application designed to centralise and streamline these essential components into a single, user-friendly platform. The tool facilitates seamless collaboration between management and healthcare professionals by providing real-time access to all training materials. Its incorporation demonstrates a dedication to modernising training procedures with safe, effective and sustainable digital solutions. It also aligns workforce development with NHS standards. The goal being to reduce administrative burdens, increase staff efficiency, and ultimately enhance clinical outcomes across the department.

Main features include dynamic dashboards for measuring performance in real time, integrated analytics that offer useful information at the departmental and individual levels, clear audit trails and automatic alert tools that draw attention to competency gaps. The application will be developed as a web-based platform using Python, leveraging existing SQL Server databases for backend development and JavaScript libraries for frontend functionality. This architecture ensures scalability, flexibility, and seamless integration with existing NHS systems.

Key words: Web-based application, Workforce development, Analytics

Preliminary Investigation of Feasibility of Copy Number Variant Testing using Genexus

Nancy Atieno

School of Biological Sciences, University of Manchester

Evaluation of copy number variations (CNVs) is important for disease diagnosis, prognosis, and targeted therapy. CNVs in genes with known clinical utility and actionability such as *MET*, *EGFR*, and *HER2* have implications for cancer progression and targeted therapy in solid tumours. Although In Situ Hybridization (ISH) remains the gold standard for CNV detection, it is limited by labour-intensive workflows and potential for subjective interpretation.

This study aims to investigate the feasibility of CNV testing in the laboratory using the Oncomine Precision Assay (OPA) on the Ion Torrent Genexus platform. The Genexus is an automated turnkey next-generation sequencing (NGS) system that offers rapid, high-throughput CNV analysis. Moreover, it aims to assess the likelihood of NGS replacing or complementing ISH in routine clinical diagnostics.

A cohort of 55 formalin-fixed, paraffin-embedded (FFPE) tumour samples previously analysed for *MET*, *EGFR*, and *HER2* amplifications was collected. The samples had a range of CNV types (i.e., no amplification, loss or multiple copies) or increased gene expression. DNA was extracted using a magnetic bead-based protocol and its quality and quantity measured by a Qubit fluorometer. The samples underwent CNV analysis on the Genexus platform, and the correlation between phenotypic ISH data and genotypic NGS data will be assessed.

Concordance between ISH and NGS CNV results is expected, with any discrepancies being explored further. Additionally, the Genexus platform exhibited greater workflow efficiency, multiplex analysis capability, as well as faster turnaround times (TATs). If the resulting values show high concordance, the laboratory will continue to verification/validation for clinical use and extension to scope with the United Kingdom Accreditation Service (UKAS). The laboratory can therefore introduce CNV testing as part of its horizon scanning initiative and pre-emptively position itself for appropriate assessment of novel actionable diagnostic and therapeutic targets.

Keywords: CNV analysis, Genexus platform, In Situ Hybridization (ISH)

Adaptive Sampling with Oxford Nanopore Long-Read Sequencing for Variant Phasing in Rare Disease Singleton Patients

Nicola Jones[1,2]

[1]West Midlands Regional Genetics Laboratory, Birmingham Women's Hospital and Children's NHS Foundation Trust, Edgbaston, Birmingham, UK; [2]Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK

The laboratory's gene panel testing currently relies on whole genome sequencing or whole exome sequencing. However, these short-read methodologies are limited in their ability to phase variants in recessive diseases and often fail to accurately detect variants in genes with pseudogenes. Long-read sequencing (LRS), capable of producing reads over 1 Mb in length, overcomes these challenges by enabling variant phasing in singleton patients without requiring parental testing. Additionally, it ensures precise sequencing of genes, in cases involving pseudogenes. The Rare and Inherited Disease Genomic Test Directory now incorporates semi-rapid testing for various clinical indications, including likely inborn errors of metabolism (R98). LRS will help streamline upfront testing, facilitating rapid turnaround times and timely diagnoses for these indications, with the potential to significantly enhance patient care and management. Additionally, many metabolic conditions arise from recessive diseases, where LRS provides a critical advantage by enabling the phasing of two variants within the same gene. This capability is essential for assessing pathogenicity and classifying variants, applying PM3 evidence in accordance with the ACGS 2024 variant interpretation guidelines for rare diseases.

This project is a feasibility study exploring the use of Oxford Nanopore (ONT) LRS with adaptive sampling to target genes of interest in rare disease singleton patients and address the challenges of phasing variants in recessive diseases. It will focus on participants who have previously undergone clinical testing and have been identified as having two compound heterozygous variants, where the phasing has already been determined. The PromethION instrument, provided by the University of Birmingham, has been used for ONT adaptive sampling, following the ONT ligation sequencing kit (SQK-LSK114) protocol. The project has involved extensive troubleshooting, including the use of ONT's Short Fragment Eliminator Buffer and Long Fragment Wash Buffer to enrich for longer DNA fragments. Moving forward, the project will expand adaptive sampling to a larger gene panel, aiming to increase the number of long reads produced per run and the N50, to try and optimise the quality of data and enable variants to be phased using this method.

Keywords: Long read sequencing, adaptive sampling, phasing variants

Development of a Standardised Long-Read Sequencing Pipeline for Whole Genome Sequencing in Rare Disease Diagnostics

Nour Mahfel[1]; Lorraine Hartles-Spencer[2]; Chipo Mashayamombe-Wolfgarten[3]

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

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This research addresses the need for a standardised bioinformatics pipeline for long-read sequencing in whole genome sequencing (WGS) to improve rare disease diagnostics. While short-read sequencing technologies have been the mainstay in genomics, their limitations in detecting complex structural variants (SVs) and long-range genomic features pose challenges in diagnosing rare diseases. Oxford Nanopore Technology (ONT) offers a promising alternative with the ability to detect SVs, copy number variants (CNVs), single nucleotide variants (SNVs), and methylation patterns.

Our project aims to develop a robust pipeline, focusing on processing ONT data from POD5 format to standardised VCF outputs for clinical interpretation. The pipeline incorporates tools for base calling, variant calling, and methylation analysis while integrating platforms like EPI2ME for data processing and Geneyx for variant interpretation. Workflow management tools, such as Nextflow, are considered to enhance reproducibility and scalability.

The pipeline will be validated using sequencing data from 27 families with suspected rare genetic disorders, where 16 have undergone preliminary analysis, and three have received diagnoses. Positive control samples with known pathogenic variants will further ensure accuracy and clinical relevance.

This work will standardise long-read sequencing diagnostics, enabling reliable detection of clinically significant variants and improving diagnostic outcomes for undiagnosed cases.

Keywords: long-read sequencing, rare disease diagnostics, structural variants

Comparison of EPIC array and GSA array as assays to deliver test code M7.10 from the National Genomic Test Directory

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The accessibility of genomic testing for patients in England is dictated by NHS England (NHSE) and is described in The National Genomic Test Directory (NGTD), which contains available tests, the methodologies through which they are delivered and their associated eligibility criteria. Tests are subdivided by methodology/ genomic targets and are assigned a unique code to simplify requests. Patients referred with a suspicion of melanoma are entitled to various genomic tests as per the NGTD, including those under the codes M7.5 through M7.10. These test codes describe the detection of copy number variations (CNVs) in the following genes: MYB, RREB1, CCND1, MYC, CDKN2A, by means of either Fluorescence in Situ Hybridisation (FISH) or microarray techniques. These genes have been identified as targets, since imbalances in their copy number are known mechanisms for the development of melanoma. It is the expectation of NHSE that all tests listed on the NGTD are made available to patients in England, either as a core service offered by all Genomic Laboratory Hubs, or as a specialist service provided by select centres only. Currently the M7.10 test is not available to patients in the central and south regions of England and therefore a need exists for this service to be implemented. The West Midlands Regional Genetics Laboratory (WMRGL) intends to compare two assays to determine which could provide a suitable method for the future implementation of this service. The first assay is the Global Screening Array (GSA) from Illumina, which detects gene copy numbers directly using target-specific probes. The second assay is the EPIC Methylation array, also from Illumina, which indirectly determines gene copy number by detecting epigenetic changes called methylation signatures. A cohort of samples from a range of solid cancers including melanoma, will be analysed by both methods to assess each one's proficiency in detecting CNVs and reaching agreeance with prior clinical diagnoses. Following this process, a primary method for the implementation of this testing service at WMRGL will be selected. In addition, a potential secondary method will hopefully be identified, to serve as a contingency in the event of a break in routine service.

Keywords: melanoma, National Genomic Test Directory, microarray

Development of a Copy Number Variation confirmation methodology

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The human genome contains genetic variations, ranging from small single nucleotide variants (SNVs) to structural changes known as copy number variants (CNVs). CNVs, which encompass deletions, duplications, and insertions of at least 50 base pairs, are estimated to span 4.8–9.5% of the genome. These variations arise from errors in DNA recombination, replication, and repair, often due to repetitive sequences in the genome. While CNVs contribute to normal genomic diversity, they are also associated with health conditions and increased susceptibility to diseases. Therefore, reliably detecting CNVs is crucial.

Various methods for CNV detection have been developed and evolved over time. Historically, karyotyping was considered the gold standard, but microarray analysis has since become the primary method in clinical practice. More recently, Next Generation Sequencing (NGS) including Whole Exome Sequencing (WES) and Whole Genome Sequencing (WGS) are increasingly being used as they can offer superior resolution compared to microarrays and their ability to streamline CNV testing. Despite the benefits of NGS and its integration into routine practice as part of the NHS Genomic Medicine Service (GMS), it is necessary that some CNVs identified through NGS are confirmed by an orthogonal method due to its limitations, following recommendations by the NHS England guidelines for rare disease genomic testing, interpretation and reporting. These limitations include the variable accuracy of NGS variant calls, limited coverage, the increased cost, labour and the turnaround time of testing where cascade family testing is required.

CNVs can often be confirmed via microarray testing, however, some findings, especially smaller CNVs where microarray does not have sufficient resolution, are not well covered by the current microarray platform in service. To address this limitation, this project is geared towards the development and implementation of a new assay beyond the resolution of microarray for confirming CNVs in our laboratory. The new assay will be either droplet digital or quantitative polymerase chain reaction (PCR), both of which are sensitive, cost-effective, rapid, and do not require complex bioinformatics pipelines. This study will involve options appraisal for the most appropriate method, primer design for a range of CNVs and validation of the selected assay.

Keywords: Copy number variant, Next Generation Sequencing, Polymerase chain reaction

Validation of RNASeq Using IonTorrent Chemistry for Service Continuity in Delivering Solid Cancer Services

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RNA sequencing (RNASeq) is used to identify fusion genes and structural variants. These variants often have oncogenic or loss of function effects on the genes involved and are implicated in many cancer types. In our laboratory, RNASeq is used to inform diagnosis, prognosis, and identify therapeutic options for a wide range of tumour types. The reliable and consistent delivery of RNASeq services is therefore vital for any diagnostic genomic laboratory.

Our solid cancer team use the Archer FusionPlex panel, a comprehensive next-generation sequencing (NGS) platform designed to detect a wide array of structural variants across 105 genes. It employs Anchored Multiplex PCR (AMP), which enables the identification of gene fusions without prior knowledge of fusion partners. By using unidirectional primers that anchor to known exonic regions, the panel allows amplification of unknown fusion partners located down or upstream. The generated libraries are then sequenced. This method is particularly effective for identifying rare or novel gene fusions, which are often essential for accurate cancer diagnosis and guiding targeted therapies.

Libraries generated by this panel are currently sequenced using Illumina's NextSeq550 platform. To provide service contingency and to minimise service disruptions, it is important to have an alternative platform to pivot to if needed. My project will therefore focus on validating the Genexus Integrated Sequencer (IonTorrent sequencing technology) as an alternative sequencing platform to support the generation of results from the Archer FusionPlex panel. The Genexus sequencers are already established and are currently used routinely by the solid cancer team for the tumour profiling workflow.

The project will look to compare the RNASeq results and the sequencing metrics of samples sequenced on the Genexus instruments with those produced by the NextSeq, including previous positive and negative samples which will serve as controls. We will assess the concordance of the Genexus sequencing instruments and the sequencing QC metrics in order to validate this process.

Key words: RNASeq, Solid Cancer, Validation