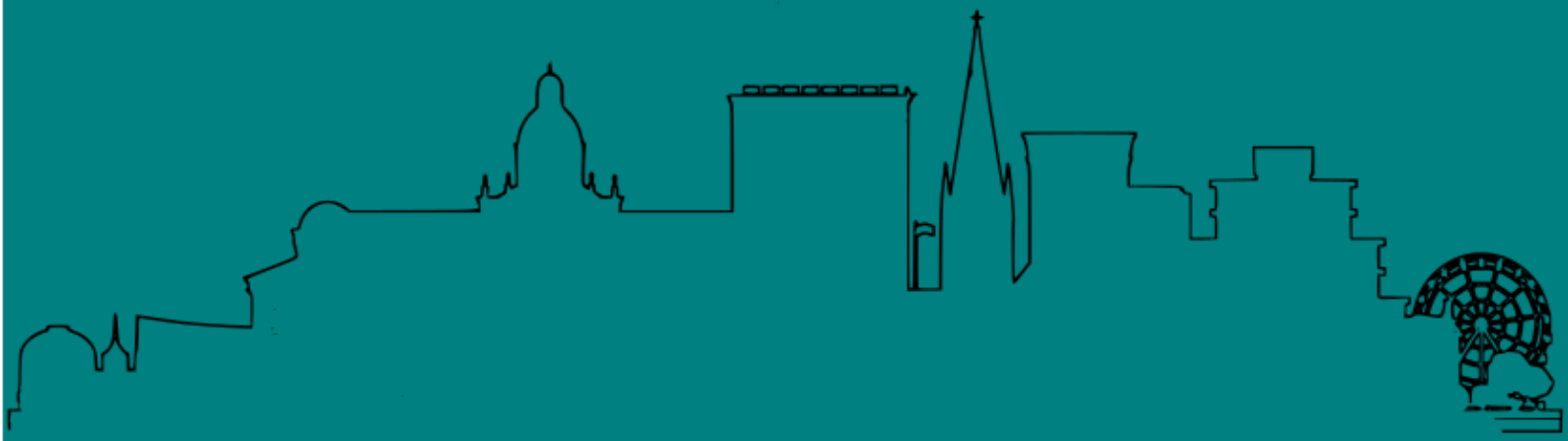
WMRGL STP Conference 2022



Tuesday, 23rd August 2022

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

Hosted by:

NHS
Birmingham Women's and Children's
NHS Foundation Trust

WMRGL STP Conference

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

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.	9:30	10:00
Getting to know the team During this time our Training Officers will share a welcome message to the trainees. • **The present and future of laboratory Genetics** Speaker: **Jennie Bell** • **The importance of peer networking on learning and well-being** Speaker: **Sarah Clinton**	10:00	11:00
Mid-Morning break (Complementary tea, coffee, cereal bar, and fruits served)	11:00	11:15
Professional practice training session 1 Comp. 9 - **Comply with relevant guidance and laws.** Speaker: **Lorraine Hartles-Spencer**	11:20	12:20
Lunch Break Hot and cold buffet served at the hotel restaurant	12:30	13:30
Workshop - Stress Awareness and Management Speaker: Paris Lalousis and Frankie Lewns	13:30	15:00
Selected oral presentation 1 - Development of a CD3+ T-Cell Fraction Purity Assessment Assay for Post-Stem Cell Transplant Lineage-Specific Chimaerism Analysis Speaker: Thomas Evans	15:00	15:30
Selected oral presentation 2 - An audit and evaluation of the R21 prenatal exome sequencing service. Speaker: Megan Horton-Bell	15:30	16:00
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	16:00	16:30

Comp. 9 - Comply with relevant guidance and laws.

Lorraine Hartles-Spencer

Notes:

Workshop - Stress Awareness and Management

Paris Lalousis and Frankie Lewns

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List of abstracts

Embryo-endometrial synchrony: is slow blastulation a fertility phenotype?

Nicolle Suttie

Infertility affects 1 in 7 couples in the UK with many requiring intervention to create healthy embryos. Embryos develop in culture for 6 days and can be replaced to the patients' uterus in a fresh embryo transfer (ET) using cleavage embryos (2- or 3-days post egg collection) or blastocysts (5 days post egg collection). Blastocyst formation (blastulation) typically occurs 5 days post egg collection, with blastocysts being developmentally advanced embryos consisting of an inner cell mass (which forms the baby) and trophectoderm cells (which form the placenta). Typically, blastocyst ET is desired as there is strong correlation with increased implantation, clinical pregnancy and live birth rates compared to cleavage embryos. Cleavage stage embryos can still achieve successful clinical outcomes and are typically opted for if embryo quality is poor and/or if the embryo yield was low and there is concern embryos will not blastulate.

Successful ETs are dependent on embryo quality and receptivity of the endometrial lining, with the endometrium being transiently receptive in a period known as the window of implantation (WOI) – in IVF, this is typically exogenously controlled to occur 5 days after egg collection. Since embryos continue to develop following ET, cleavage embryos still coincide with the WOI. From retrospective analysis of time-lapse data (Embryoscope), it has been observed that blastulation can be variable – even within the same embryo cohort - with rates ranging between 5 days and up to 7 days. Therefore, slowly blastulating and so underdeveloped embryos are asynchronous with the endometrium for fresh ET.

One way to mediate this is to not do a fresh ET, instead freezing all suitable blastocysts to undergo a future frozen ET – this involves thawing the frozen blastocyst prior to transferring back to the endometrium. This allows for resynchronisation of the blastocyst with the endometrium, coinciding with the WOI. It should be noted that irrespective of stage at fresh ET, any surplus blastocysts are cryopreserved and can then be used in frozen ET.

Ultimately, the aim of this project is to identify if slow blastulation is an observable infertility phenotype. By freezing all blastocysts and having frozen ET, this may improve patient outcomes, leading to synchronisation of the endometrium and embryo and subsequently resulting in fewer ETs. Data from the time-lapse system, as well as blastocyst freeze records, will be analysed in line with implantation rate. This data could potentially elucidate a developmental timeline for slow blastulation which can be referenced against current known blastulation timings and be used to inform clinical decisions for patient treatments in future.

Clinical audit to determine the range and frequency of copy number variants detected using microarray technology across different clinical cohorts

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

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

Introduction: Microarray technologies are the frontline tests for patients referred for a wide range of phenotypes including autism spectrum disorders, intellectual disability and congenital anomalies. It is a powerful, high throughput tool, which enables copy number variant (CNV) detection across the genome. There are currently no large published datasets of CNVs observed across all clinical cohorts in neonates using microarrays. The West Midlands Regional Genetics Laboratory (WMRGL) has completed 1,000s of neonatal microarray analyses using array comparative genomic hybridisation (aCGH) and single nucleotide polymorphism (SNP) array from Oxford Gene Technology (OGT) CytoSure and Illumina Global Screening Array (GSA) platforms respectively. The overarching aim of this retrospective audit is to determine the range and frequency of pathogenic/likely pathogenic CNVs observed across different clinical cohorts, such as developmental delay, autistic behaviour, cardiac abnormalities, using data held within the patient database (Shire) and array databases (OGTCytoSure and NxClinical).

Methods: Initially, collate all microarray and patient clinical data in an easily accessible and readable format with the help of bioinformatics support, potentially in an excel spreadsheet format. Define clinical cohorts according to Human Phenotype Ontology (HPO) terms/clinical features. Next, critically review each case to ensure CNV classification is correct and updated according to the 2020 ACMG microarray interpretation technical standards. Finally, use descriptive statistics to calculate the diagnostic yield, the frequency and type of CNV detected in each clinical cohort.

Results: The project has not commenced yet. We aim to gather the data by April 2022 and subsequent to CNV review, obtain audit results by end of 2022.

Conclusion: With the dawn of whole genome sequencing (WGS) which enables both single nucleotide variant (SNV) and CNV detection, the data from this audit will be invaluable in informing the requirements of WGS with respect to CNV detection and sensitivity requirements for detection of mosaicism. The requirements may differ across different clinical cohorts and would ensure appropriate testing pathways are put in place during the transition of moving away from array technology to WGS only. Conversely, this data will inform what variation may or will not be detected if using WGS only.

Detection of Novel Diagnoses Using Exomiser Platform, and Identification of Gene-Disease Associations in PanelApp Panels

Conor Pallatt

Background: The huge amount of variants produced during large-scale sequencing is somewhat overcome by thorough filtering strategies and the application of curated gene panels. Patients remained undiagnosed and challenges in timely gene-panel curation and ineffective variant filtering persists. Exomiser variant prioritisation tools has demonstrated its ability to rank diagnostic variants. More information is needed to determine its effectiveness across different clinical pictures and how it might be applied to investigations of variants that fall outside of the gene panels applied.

Methods: Using previously identified causative variants from the 100,000 genome project (100kGP), this study looked at the relationship between Exomiser's performance and different clinical variables. Exomiser was then used to prioritise variants in undiagnosed 100kGP patients. To further curate gene panels and look for new gene-disease associations, a cohort of undiagnosed, in-house patients were analysed using of a list PanelApp amber genes for select clinical indications.

Results: Retrospective analysis showed Exomiser was able to rank the previously identified diagnostic variant as top 68% of the time. Exomiser performed poorly for metabolic disorders (41% of variants ranked outside the top 10). Trio referrals performed best (variant ranked top 75% of the time), however duo and singleton referrals still performed well (variant ranked top 64% and 61% of the time respectively). Referrals with 1 HPO term showed the largest range and the lowest phenotype score. Variants with higher phenotype scores had higher Exomiser combined scores and ranked higher suggesting phenotype score is the largest contributor to rank. When using Exomiser rank and combined score to filter for diagnostic variants, this study shows that top 4 rank and Exomiser Combined score >0.7 captured 85% of diagnostic variants. Applying an Exomiser filtering strategy on undiagnosed patients provided a diagnostic rate of 5%, revealing 5 new diagnoses that were missed during the first analysis of 100kGP. Analysis of amber genes for in-house undiagnosed patients did not reveal any new gene-disease associations.

Conclusion: The Exomiser variant prioritising tool is effective at identifying diagnostic variants, including those outside of panels applied, and relies on sufficient and accurate descriptions of patient phenotypes.

Comparison of two next generation sequencing technologies for testing of ctDNA as part of the solid cancer salvage pathway

Francesca Buxton

Background: The National Genomic Test Directory lists the tests that are commissioned by the NHS in England for solid cancer. A large proportion is next-generation sequencing (NGS) panel testing, of which there are several limitations. An alternative testing strategy, termed the 'salvage pathway' is currently being developed, to deliver results to patients with solid cancer in cases where an NGS panel test is not possible, has failed, or there is clinical urgency. This dissertation will focus on the potential use of circulating tumour DNA (ctDNA) as part of the salvage pathway. The aim was to assess andcompare two different NGS technologies.

Materials and methods: ctDNA controls were purchased, that cover several hotspots frequently mutated in solid cancer, at different variant allele frequencies (VAFs) (0.1%, 0.5% and 1%). The two technologies used were the Oncomine Precision Assay (OPA) on the Genexus Integrated Sequencer and the Archer LIQUIDPlex ctDNA 28 kit on an Illumina NextSeq Sequencer. Comparisons of quality of data, sensitivity, accuracy, intra-run and inter-run variability and limit of detection (LOD) were made. Cost per sample, hands-on time, processing time, and ease of use were also assessed.

Results: The Archer technology outperformed the Genexus technology in terms of sensitivity, accuracy, inter-run and intra-run variability and LOD, although both meet the requirements needed for a ctDNA testing service within the NHS. Cost per sample is less expensive when using the Archer technology, however the total processing time is considerably longer and requires much more hands-on time in the laboratory (staff time not included in the pricing).

Discussion and conclusion: Although further validation is required, this project demonstrates that both technologies would be capable of detecting low level variants in ctDNA as part of the solid cancer salvage pathway testing strategy. This has the potential to provide genomic results to patients for whom all other testing options have been exhausted.

Variant viewer – Web application development.

Joseph Larkman

The advent of Next Generation Sequencing (NGS) has resulted in a cosmic shift in the testing capabilities of clinical laboratories, enabling highly accurate, cost-effective and massive throughput genetic testing. Consequently, a vast quantity of data is now being generated on a routine basis and it is vital to consider the best methods of data storage, retrieval and visualisation. Presently, the NGS pipelines in operation at West Midlands Regional Genetics Laboratory (WMRGL) conclude with the automatic generation of a series of excel workbooks, containing sequencing coverage statistics, quality control metrics and details of all identified variants that pertain to a particular sample. The presentation of data in this way has historically limited the ability of scientists to detect recurrent variants across patients or integrate data for a single patient across multiple assays. Excel files are also difficult to audit, and the absence of sophisticated permission controls can result in poor data integrity and significant lost time due to the need for error checking or file consolidation.

Here we introduce Variant Viewer, a bespoke web application and accompanying SQL database providing an interactive and streamlined results portal for NGS data; built using the Django web development framework. An appealing user interface provides powerful run and sample management functions; whilst comprehensive details for each identified genetic variant, alongside metrics of gene- or exon-based coverage, facilitate classification in accordance with ACGS criteria. Modern CSS and JavaScript components provide an enhanced user experience, including on-the-fly curation of filter presets based on variant characteristics; an embedded genome browser; evidence upload and preview tools; PDF report generation; and automated detection of previously classified variants. NHS.net-associated user accounts are mandated for enhanced security and auditability. Variant Viewer offers an efficient workflow that is more secure, flexible and extensible than existing approaches at WMRGL. Going forward, it stands to offer automated analysis for recurrent variants, improve departmental turnaround times and assist with the integration of patient data from orthogonal assays.

The Development of RNA Sequencing Protocol to Support Transcriptomic Analysis for the Classification of Solid Cancers using Bladder Cancer as the Paradigm

Lauren Kettle, Yvonne Wallis and Samuel Clokie

Non-muscle invasive bladder cancer (NMIBC) accounts for 70-75% of bladder cancer cases (Kamat, Bagcioglu and Huri, 2017). In these cases, the tumour is confined to the bladder's epithelial lining leaving the deeper smooth muscle layer unaffected (CRUK, 2022). Despite these cases having a >90% 5-year survival, many of these patients have a high risk of disease recurrence (50-70%) or disease progression to muscle invasive cancer (20%) after this time point (Babjuk et al., 2017). Patients must therefore receive lifelong surveillance as well as multiple rounds of treatment, leaving both an emotional and financial burden for patients and the NHS. Recent publications have reported the utility of transcriptomic analysis to classify molecular subtypes of NMIBC which can predict a patient's risk of recurrence to a higher accuracy than current methods (CRUK, 2022; Mo et al., 2020). This method identified four molecular classes (1, 2a, 2b and 3) with class 2a tumours exhibiting the worst progression free survival and class 1 the best (Mo et al., 2020). Classifying patients into these different subgroups will allow appropriate treatment stratification and/or entry into a clinical trial for those at high risk. It also has the potential to reduce the number of patients receiving lifelong surveillance by targeting high risk patients, reducing the financial burden on the NHS.

Currently, 2021/2022 National Genomic Test Directory only specifies that variants in FGFR2/3 and NTRK1/2/3 should be investigated for patients with bladder cancer (NHS England, 2021). While this provides essential prognostic and treatment information, many high-risk patients are still not identified, leaving a gap in the test directory (Casadei et al., 2019). This project therefore aims to assess the utility of transcriptomic analysis and expression profiling of NMIBC in a clinical setting and assess its suitability for the test directory. A small number of NMIBC samples will be taken through an end to end workflow from RNA extraction to bioinformatics analysis and classification. This will act as a proof of principle study for the clinical utility of this technology with the hope of it becoming a standard of care for NMIBC patients, improving the capabilities of the test directory and patient management.

An audit and evaluation of the R21 prenatal exome sequencing service. (Selected for oral presentation.)

Megan Horton-Bell

Background: Foetal anomalies are detected in ~2-5% of pregnancies and are responsible for approximately 20% of deaths in utero or within the 1st year of life. These can be due to a variety of aetiologies, including genetic abnormalities. Conventional genetic tests such as microarray and karyotyping only detect causative genomic abnormalities in ~40% of cases. These techniques detect larger structural changes and copy number variants and therefore do not identify smaller sequence variants. Prenatal exome sequencing (ES) provides the potential to increase diagnostic rates through the identification of these pathogenic variants. This would provide additional families with crucial information to allow them to make informed choices about their pregnancies.

Prenatal ES has previously been used in research settings, with diagnostic rates varying significantly between studies. Those with smaller cohorts and more specific inclusion criteria often had higher diagnostic yields, whereas those that recruited more patients with increased variability of scan findings identified fewer clinically significant variants. The larger studies have shown that prenatal ES can result in an additional diagnostic yield of ~10%. However, prenatal ES has not yet been adopted into any national healthcare service and therefore, the clinical utility and feasibility need to be assessed.

The West Midlands Regional Genetics Laboratory (WMRGL) is one of two genomic laboratories providing prenatal exome sequencing (R21) testing, accepting referrals from the Central and South, North West, and North East and Yorkshire Genomic Laboratory Hub regions. WMGRL began offering R21 testing in October 2020 for foetuses displaying multiple scan anomalies (e.g. skeletal dysplasias or central nervous system abnormalities). This project will evaluate the results from the first years' service, to assess the service delivery to date, the equity of access and the clinical utility of prenatal exome sequencing.

Aims and Objectives:

- Assess referral stats (total, accepted, declined, location)
- Parental characteristics (ethnicity, consanguinity, maternal age, paternal age)
- Diagnoses
    - Rate per month
    - HPO phenotype groups and top terms
    - Genes involved, disease pathways, recurring diagnoses
- New phenotype-genotype links?
- VUS' and reclassifications
- Impact on pregnancy management
- Pregnancy outcomes

Improved genetic testing and characterisation for imprinting disorders

Luke Redford

Historically, laboratories only had the capability to test for one imprinting disease loci at a time. It was difficult to determine which disease specific loci to choose to test as many imprinting disorders have an overlapping phenotype of intrauterine growth restriction and neonatal hypotonia. We now have the technology to test multiple known imprinting locations using one test which is much more efficient and can identify multi loci imprinting disturbances as well, a condition where multi imprinting loci lose methylation.

This study performed a systematic review of previous patients who were referred for Prader Willi but tested negative for this imprinting disorder and received no further imprinting testing. There was a special interest in those patients who were referred for slow growth during pregnancy and newborns who show features such as low muscle tone (known as a floppy baby) as this phenotype overlaps with several imprinting disorders. This study has found that patients in the past ,who have been referred for Prader Willi, have had another imprinting disorder which has had overlapping features and not been referred for additional testing. This proves that imprinting disorder referrals are missing correct testing and poses the question whether in receipt of a negative prader Willi referral should a multi loci imprinting test should be used?

Development of a CD3+ T-Cell Fraction Purity Assessment Assay for Post-Stem Cell Transplant Lineage-Specific Chimaerism Analysis. (Selected for oral presentation.)

Thomas Evans

Background: Reduced intensity conditioning (RIC) has become a common non-myeloablative approach to stem cell transplantation. RIC utilises the graft vs leukaemia (GvL) effect to eradicate leukaemic cells. The goal is complete donor engraftment, with T-cell engraftment critical in RIC to inducing the GvL effect. Chimaerism monitoring of CD3+ fractions, attained using magnetic antigen cell separation (MACS), from both peripheral blood and bone marrow aspirates (BMA), can therefore aid in measuring engraftment, including detection of relapse. Guidelines now require fractions for chimaerism be purity-assessed to ensure results validity. Flow Cytometry (FC) is currently the accepted approach, while a PCR-based assay is available from Accumol. The aim of this project was to develop a cost-effective, PCR-based method of CD3+ fraction purity assessment, integrated into current workflows.

Methodology: PCR primers were designed to target the Dδ2- Dδ3 region of the TCRD gene locus, known to be excised during early T-cell maturation, to amplify as a marker of non-T cell contamination. Primers for ALB were designed as controls. Primer performance was assessed on existing chimaerism PCR and fragment analysis conditions. CD33+ MACS fractions, cultured fibroblasts, and plasmids containing a copy of each target region were run to identify a suitable control sample, with cultured fibroblasts performing best. From these a skew coefficient was determined. Ten patients were selected, post-standard of care, for purity assessment by FC, Accumol, and in-house assays.

Results: Results in blood samples were consistent across assays. In BMA samples, results were discordant in specific samples, with FC predicting high purity in all samples, but the two PCR-based assays highlighting lower purity in some, and the in-house assay predicting a larger range.

Discussion: The project aim was demonstrated in peripheral blood samples, which was however a small sample size with only high scores. Discussions with Accumol suggested BMAs contain damaged and fused cells, which could provide a source of contaminating non-T DNA even post-MACS. This could explain the results disparity, with contaminating DNA detected in BMAs by the PCR assays, but their source fused and/or damaged cells gated out by FC. Discussions are ongoing with the FC providers to identify whether gating is observable within their data.

Recent studies have shown that the quality of cells within aspirates can vary greatly, with one suggesting that the quality of the procedure has decreased over time, due to less frequent application of the technique. Density gradient-based techniques to separate the mononuclear cell population in the BMAs were suggested by the manufacturer, and were found to be a commonly adopted pre-processing step for fractioning BMAs in literature.

These preliminary findings demonstrate a PCR-based purity analysis assay which could offer more sensitive detection of contamination in MACS fractions for chimaerism analysis than FC. However, a larger sample size is needed. Follow-up studies should aim to identify the cause of the discordant and inconsistent BMA results, and establish whether low-purity BMA MACS fractions are prevalent, skewing BMA chimaerism scores, and potentially impacting patient results and care.