Test Information Sheet - Wales Infants' and ChildreN's Genome Service - WINGS

The Rapid Whole Genome (WGS) Sequencing Service for acutely unwell children with a likely monogenic disorder **(R14)** is available from April 2020.

Description

AWMGS Genetic Consultants, Bioinformaticians and Clinical Scientists have been working in partnership with specialist Paediatric Consultants and Cardiff University to develop the UK's first diagnostic rapid Whole Genome Sequencing (WGS) service for paediatric patients. This service is available for patients being cared for in the neonatal or paediatric intensive care units who have a combination of serious health problems. The AWMGS WINGS test is designed to reduce the child's diagnostic journey by searching through their whole genome data to find a genetic cause of their clinical features.

Research studies to examine the application of WGS in a routine clinical setting have shown that it is highly effective in improving outcomes for this group of patients by reducing the need for multiple diagnostic tests, facilitating earlier treatment decisions and preventing repeat inpatient admissions (Kingsmore et al., 2019, PMID: 31564432; French et al. 2019, PMID: 30847515; Mestek-Boukhibar et al. 2018, PMID: 30049826).

Referral Criteria

Rapid whole genome sequencing (WGS) is available for acutely unwell children with a likely underlying genetic cause (R14). This test is only available on a trio basis and the child and BOTH parents must be available. Alternative rapid genetic testing is available if only one parent is present.

For full details, please see the referral criteria leaflet for this service (MI-GEN-RWGSRefCrit).

Result Reporting and Risk of Incidental findings

It is possible that as part of this test, incidental or secondary findings that are not of direct relevance to the clinical features in the patient may be identified. These will not be reported in the patient's initial result.

If the patient or family wish to receive information about additional, clinically actionable findings unrelated to the patient's clinical features, they can discuss this during a follow-up appointment with Clinical Genetics.

Trio whole genome sequencing will reveal possible non-paternity (or non-maternity) and this result would be discussed with the referring clinician.

If a diagnosis is not found, the variant data from genome sequencing tests will be stored long-term in order that further analysis can be undertaken in the future. The results of re-analyses may be issued as a supplementary report to the referring clinician.

Test Methods

Genomic DNA extracted from the child's and parent's blood samples is sequenced simultaneously using next generation sequencing (NGS) with the Illumina NovaSeq 6000 system. Sequences are aligned to human genome assembly GRCh38 (hg38), and variants identified with the Illumina DRAGEN (Dynamic Read Analysis for GENomics) Bio-IT Platform.

Analysis includes evaluation of variants that are identified to be de novo, compound heterozygous, homozygous and X-linked using in-house bioinformatic pipelines. Variants are interpreted and reported following the latest ACGS/ACMG guidelines (Ellard et al., 2020; Richards et al., 2015).

The WINGS analysis pathway utilises a two-phase approach for analysis of small variants (SNVs and Indels), copy number variants (CNVs), and short tandem repeats (STRs). In the first phase, a virtual panel of genes based on the PanelApp Paediatric Disorders panel (https://panelapp.genomicsengland.co.uk/panels/486/) is used to only look at variants in genes included on the gene panel. If no variant of interest is found in the first phase analysis moves to the second phase. The second phase uses a "gene agnostic" approach to look for variants in genes not included in the first phase panel of genes and that are ranked highly by Exomiser based on the patient's phenotype. The top 8 Exomiser variants and all de novo, compound heterozygous and homozygous variants will be looked at. This means that nearly all genes in the human genome, related to the patient's phenotype, are inspected for variants that pass the bioinformatic filtering pipeline. It should be noted that only STRs within STR defined regions on the panel are analysed, and those outside of the panel are not assessed.

From 01/04/2022, we will adopt a policy for WINGS where single nucleotide variants (SNVs) that meet the specified quality thresholds will be reported as an actionable final result without Sanger sequencing confirmation. For complex variants; multi nucleotide insertions or deletions, large indels, and any variant which fails the internal QC parameters, Sanger confirmation will still be required prior to the release of results from the laboratory. For short tandem repeats, PCR confirmation will be required.

Following a normal result, requests are often made about whether a specific gene of interest has been included in the WINGS analysis. If a normal result has been issued *both* phases of analysis have been completed and so nearly all genes have been accounted for. However, if you would like to check if a gene is included on the initial panel analysis, please refer to the PanelApp Paediatric Disorders panel (https://panelapp.genomicsengland.co.uk/panels/486/) in the first instance.

Limitations

Currently this test is validated for the detection of small variants (SNV and Indels), Copy Number Variants (CNVs) and Short Tandem Repeats (STRs) from whole genome sequencing data only. Other Structural Variants (SVs) may also be detected, but the sensitivity of detection is unknown. This analysis does not include complex structural genetic variants such as inversions or translocations, mitochondrial variants, or UPD. Small CNVs <5KB in size, whilst commonly detected, have reduced sensitivity and may not always be discovered. This assay may not always detect multi-nucleotide variants. SNP Array testing will be carried out concurrently for an interim period for CNV detection.

The WGS analysis is primarily looking for *de novo, recessive, compound heterozygous, and X linked* variation. Therefore, variants inherited from an affected parent, or from a parent with milder clinical features or causing disorders with a later age of onset may not be detected. These variants may be missed if they are present in genes with low or variable penetrance. Please see above for incomplete penetrance analysis, where applicable.

This test will not detect mosaicism for SNV or Indel variants.

Intronic variants: only conserved splice site variants (defined as variants located within 1–3 bases of an exon or 1–8 bases of an intron are analysed. Other non-coding variants are not currently analysed unless they were predicted to impact splicing by SpliceAI (Jaganathan et al., 2019) or previously reported in the ClinVar database as pathogenic.

CNVs detected via WGS will be included in analysis if they intersect a protein coding region or overlap significantly with an ISCA region on the virtual panel. Therefore, non-coding CNVs are unlikely to be detected.

Expansions of short tandem repeats detected via WGS will be included in analysis if they correspond to an STR defined region on the panel. Expansion is determined by the upper confidence interval of the repeat count being greater than the normal number of repeats for that gene. Expansions in GLS and XYLT1 are excluded from analysis due to lack of validation and an inability to confirm expansions by an orthologous method, however sequence variants and/or CNVs resulting in loss of function of these genes that are detected will be assessed against the patient phenotype and may be reported if relevance is suspected.

On average 97% of coding region exons +-20 base pairs will be covered at greater than 20x, whilst 89% of the entire genome will be covered at a minimum of 20x.

Given typical sequencing conditions, the lower confidence interval of the sensitivity of variant calling for Single Nucleotide Variant (SNV) detection is 99.9%. The lower confidence interval of the sensitivity of variant calling for Indel detection is 99.6%. The sensitivity was calculated over a set of high confidence genome regions which are accessible by NGS. The sensitivity of variant calling outside of these regions is unknown. The lower confidence interval of the sensitivity of variant calling for Copy Number Variant (SNV) detection is 95.5%. The lower confidence interval of the sensitivity of expansion detection for Short Tandem Repeats (STRs) on WGS is >99.9%.

It is well established that some regions of the genome are inherently difficult to sequence e.g. pseudogene and GC rich regions, and result in low coverage and other issues that may compromise the sensitivity of variant calling. In the event of a negative result, please contact the laboratory to discuss the coverage of any genes that may be considered a likely cause of the patient’s phenotypic features.

It is possible that there is a pathogenic variant in the genome which has not currently been detected by this primary analysis. Variants may be identified but not recognised as causative due to insufficient scientific knowledge about the genomic region and its function at the time.

Reanalysis of the data to incorporate updated clinical information and/or newly emerging gene and variant information may be performed and reported at a later time.

Recessive carrier status will not be disclosed.

Samples required

Please send samples in EDTA to the laboratory. We require at least 1ml from the proband and 3-4ml from the parents.

Reporting times

The reporting time for this test is 14 calendar days.

References

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11. Jaganathan et al. Predicting Splicing from Primary Sequence with Deep Learning. Cell. 2019 Cell, Jan 176(3):414-416