AWMGS
All Wales Medical Genomics Service

Test Information Sheet – Whole Exome Sequencing for Rare Diseases

Whole exome sequencing (WES) services are currently available from AWMGS for multiple clinical indications for both trio and singleton testing. For more information please refer to service specific test information sheets, available from our website.

Description

Using WES, it is possible to look for changes across all of the protein-coding regions in the genome to reduce the need for multiple different diagnostic tests and improve the patients' journey to finding a genetic cause of their clinical features.

Research studies have shown that the application of WES in routine and urgent clinical settings is highly effective in improving outcomes for patients by reducing the need for multiple diagnostic tests, facilitating earlier treatment decisions and preventing repeat inpatient admissions (Gubbels 2020, PMID: 31780822; Stark & Ellard 2021, PMID: 34744166; Malinowski et al., 2020, PMID: 32203227; Petrovski 2019, PMID: 30712878; Lord 2019, PMID: 30712880).

Referral Criteria

For full details, please see the referral criteria leaflet for these services which are available on our website.

Reporting of Results and Possible Incidental Findings

It is possible that as part of this test, incidental or secondary findings that do not explain the current clinical features in the patient may be identified. The AWMGS policy is to analyse and report actionable (likely) pathogenic variants from a gene list. The list has been agreed by the AWMGS Clinical Genetics team and is based on the ACMG list for reporting of secondary findings v3.0 (PMID: 34012068). Note that variants in the list of genes will **not** be actively looked for. These will only be detected if the genes on the list are included on the gene panel applied or when performing agnostic analysis. Therefore, in cases where the genes on the list are not on the panel, our analysis has not excluded variants in these genes.

Trio WES will reveal possible non-paternity (or non-maternity) and this result could be discussed with the referring clinician.

If a diagnosis is not found, the variant data from exome 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 proband's and parents' blood samples is sequenced using next Generation Sequencing (NGS) enrichment of the whole exome using Nonacus Cell3™ Target ExomeCG kit and sequenced on Illumina NovaSeq 6000 platform. 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 filtered on criteria depending on the applied panel and whether parental samples are available. Variants are interpreted and reported following the latest ACGS/ACMG guidelines (Ellard et al., 2020; Richards et al., 2015).

The analysis and interpretation of WES data for analysis of small variants (SNVs and Indels) is split into two phases. In the first phase, a virtual panel of genes based on the PanelApp gene panels (https://panelapp.genomicsengland.co.uk/panels/entities/) and related to a specific condition is used to only look at variants in genes included on the gene panel. This is the **only** phase used for singleton cases.

The agnostic **trio** WES analysis pathway has a second phase if no variant of interest is found in the first phase. The second phase uses a "gene agnostic" approach to look for variants in genes not included in the first phase panel of genes. This means that nearly all protein-coding genes in the human genome are inspected for variants that pass the bioinformatic filtering pipeline.

Following a normal result, requests are often made about whether a specific gene of interest has been including in the analysis, please refer to the relevant PanelApp gene panel in the first instance.

As phenotypic based filtering methods are used in both analyses, it is **vital** that clear and detailed phenotypic information for both the proband and parents is included on the request form in order to give accurate results.

Limitations

Currently WES tests are validated for the detection of small variants (SNV and Indels) from whole exome sequencing data only. This analysis does not include structural genetic variants such as inversions or translocations, copy number variants (CNV), mitochondrial variants, triplet repeats or UPD. This assay may not detect multi-nucleotide variants. This test will detect mosaicism down to 20% allele frequency for SNV or Indel variants. SNP Array testing will be carried out concurrently for CNV detection, where appropriate, for an interim period.

The trio WES 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. However, singleton analysis with a gene panel will **not** filter out inherited variants that pass other filtering parameters.

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.

On average 95% of coding region exons ±5 base pairs will be covered at greater than 20x.

Given typical sequencing conditions, the average sensitivity of variant calling for Single Nucleotide Variant (SNV) detection is 99.87%. The average sensitivity of variant calling for Indel detection is 96.23%. 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.

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.

Genes within the exome panels used which are known to have low coverage are described in the appendix. However, this list is not exhaustive.

It is possible that there is a pathogenic variant in the exome 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.

Re-analysis 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. Only compound heterozygous and homozygous variants will be analysed.

As the exome services are new to the laboratory's testing repertoire, they have not currently been assessed by UKAS against the ISO15189 standards and are therefore not within the scope of accreditation held by the laboratory

Samples Required

Please send samples in EDTA to the laboratory. We require at least 1ml from the proband and 3–4ml from the parents (where required).

Reporting Times

The reporting time for rapid WES is 14 calendar days – this is applicable to the Fetal Anomalies (R21) service.

The reporting time for PRRS is 21 calendar days - this WES service is applicable for PRRS referrals on solid tissue samples only.

The reporting time for routine WES and panels is 84 calendar days.

Appendix

Table 1. Genes with known coverage issues within the Paediatric Disorders and Intellectual Disability PanelApp gene panels when analysed on Whole Exome Sequencing. Genes where the mean sample had less than 95% of exonic bases +- 5bp covered at 20x are shown.

B3GALNT2	KCNN2	MSTO1
OCLN	KDM4B	MTX2
TUBB2A	LYRM7	NDUFAF8
TUBB2B	MED25	NEB
IKBKG	POLR2A	OXCT1
TNXB	PRODH	PAX2
ARV1	RFX7	PCBD1
CNPY3	SHANK3	PISD
DHPS	SLC39A14	SCLT1
FUT8	TLK2	SDHD
GABRG2	WDPCP	SHOX
GBA	YIF1B	SNX10
GOSR2	ACAN	SRY
PHACTR1	ADARB1	TBXAS1
PIGU	ANAPC1	TPK1
SLC9A6	ANO10	FBRSL1
SNORD118	AP1S2	TSEN15
UFM1	COA8	HPGD
ZNF142	ARMC4	IDS
C4A	ATAD1	IFT81
C4B	B3GAT3	IL12RB1

CD55	CA5A	RFXANK
CFI	CFC1	TNFRSF1A
CORO1A	HYDIN	COX15
CSF2RA	KIAA0825	DPM2
CXCR4	NCF1	STRC
OTOA	LYRM4	CCDC32
DTYMK	CELF2	HECTD4

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