AWMGS
All Wales Medical Genomics Service

Test Information Sheet – Fetal Anomaly Gene (FAGP) testing

The Fetal Anomaly Gene Panel (FAGP) Service for Fetal anomalies with a likely genetic cause **(R21)** is available from the All Wales Medical Genomics Service from May 2022.

Description

The ongoing Prenatal Assessment of Genomes and Exomes (PAGE) study in the UK published findings in 2019 following the whole exome sequencing of 596 trios and 14 duos with fetal structural anomalies. A virtual gene panel was applied so that only genes associated with a developmental or prenatal phenotype were investigated. This study reported a diagnostic yield of 8.5%, with the highest diagnostic yields obtained from cases with multisystem anomalies (15.4%) or skeletal anomalies (15.4%) (Lord et al., 2019 PMID: 30712880).

The PAGE study, along with others, provided evidence that whole exome sequencing should be available following the identification of fetal anomalies with a likely genetic cause.

Referral Criteria

The Fetal Anomaly Gene Panel (FAGP) Service (R21) is available for cases where multiple major structural abnormalities have been detected on fetal ultrasound, where a monogenic malformation disorder is considered likely following multidisciplinary review. This service is only relevant for ongoing pregnancies where a genetic diagnosis may influence management of the ongoing pregnancy.

Clinical indications include the following:

- Fetuses with multiple anomalies, suspected skeletal dysplasias (IUGR should be excluded), large echogenic kidneys with a normal bladder, major CNS abnormalities (excluding neural tube defects), multiple contractures (excluding isolated bilateral talipes), small for gestational age, persistent megacystis.
- Nuchal translucency measured between 11 and 14 weeks gestation of greater than 6.5mm plus another anomaly (that can include a minor finding) with a normal SNP array
- Isolated non-immune fetal hydrops (detected at or after the routine 18-20-week scan in the second or third trimesters), defined as fluid/oedema in at least two compartments (e.g. skin, pleural, pericardial or ascites) with a normal SNP array
- Persistent nuchal translucency (>3.5mm) can only be considered in the presence of other structural abnormalities in two or more systems.
- Minor ‘markers of aneuploidy’ – choroid plexus cysts, echogenic foci, mild renal pelvis dilation, small nasal bone, long bones on 3rd centile etc are excluded.
- Mild ventriculomegaly should only be considered as an abnormality if the posterior horn is persistently >11mm on two or more scans. Under these circumstances it is not considered a major CNS abnormality in isolation
- Abnormality of the corpus callosum, either partial or complete agenesis – either in isolation or with other anomalies
- Pregnancies of consanguineous couples that do not strictly fulfil the above criteria, but where a monogenic disorder is considered likely.

- Recurrences of particular fetal anomalies in pregnancies of the same couple that do not strictly fulfil the above criteria, but where a monogenic disorder is considered likely due to the recurrence. Neural tube defects excluded

The two criteria below can be considered as eligibility criteria alone or in association with other major abnormalities. Requests will not be actioned without having doppler evidence.

- Small for gestational age can be considered as eligible for R21 under the following circumstances; all measurements $<3^{rd}$ percentile with a confirmed early ultrasound estimated date of delivery (EDD) scan, including abdominal circumference (AC) and head circumference (HC), and no evidence of placental insufficiency including normal fetal and maternal dopplers, no history of previous FGR, PAPP-A (if measured) not low, no maternal history of SLE etc and no past obstetric history of FGR or still birth.
- Isolated short long bones can be considered as an abnormality and eligible for R21 under the following circumstances; all long bones $<3^{rd}$ percentile with a confirmed early ultrasound EDD, and HC and AC within normal limits, and no evidence of placental insufficiency including normal fetal and maternal dopplers, no history of previous IUGR, PAPP-A (if measured) not low, no maternal history of SLE etc and no past obstetric history of FGR or still birth

Exclusion criteria:

- Confirmed aneuploidy or pathogenic copy number variant consistent with fetal anomalies detected by microarray
- Fetuses with confirmed thanatophoric dysplasia, achondroplasia or Apert syndrome on other relevant rapid tests (R23, R24, R25, R306 or R309)
- Cases where familial causative variant(s) are known -targeted testing should be performed
- For cases where sonographic findings indicate a specific monogenic disorder, targeted testing should be applied where appropriate

This test will be available on a trio basis where samples from the fetus and both biological parents will be tested. Should only one biological parent be available for testing, alternative testing may be available – this will be discussed by Clinical Genetics.

This service will not be available in cases where there is imminent fetal loss or termination of the pregnancy, or miscarriage has already occurred. If you have suspicion of a monogenic cause in such cases, please send a fetal tissue sample to the laboratory for DNA storage and refer to clinical genetics to discuss alternative testing options.

Result Reporting and Risk of Incidental findings

As part of pre-test counselling and taking of consent it is important that the family are aware of the following;

- Incidental or secondary findings that are not of direct relevance to the clinical features in the fetus may be identified.
- Trio whole exome sequencing may reveal possible non-paternity, although non-paternity will only be reported if disclosure is deemed to be of clinical relevance.
- 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, should it be requested.
- A normal FAGP result does not exclude a genetic cause of the fetal anomalies.

Test Methods

Genomic DNA extracted from the prenatal sample and the parent's blood samples are sequenced simultaneously using next generation sequencing (NGS) with either the llumina NovaSeq 6000 or NextSeq systems. 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 (small nucleotide variants and Indels) 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).

FAGP testing applies a virtual panel of genes based on the Panel App Fetal anomalies (Version: 4.0) panel. This means that only "green genes" on this panel will be analysed, genes outside of this panel will not be looked at. The full gene list can be viewed at https://panelapp.genomicsengland.co.uk/panels/478/.

Limitations

Currently this test is 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, mitochondrial variants, triplet repeats or UPD. This assay may not detect multi-nucleotide variants. Array testing will be carried out concurrently.

The FAGP 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, if the laboratory is aware of the parental phenotype the bioinformatics filtering pipeline can be modified to minimise this risk. It is important, therefore, to provide full details of any clinical features in either parent on the request form.

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. Non-coding variants between 8 and 20 bases of the exon are not currently analysed, unless they were predicted to impact splicing by SpliceAI (Jaganathan et al., 2019) or previously reported in the literature as pathogenic. All other non-coding variation will not be analysed.

On average 95% of coding region exons +-5 base pairs will be covered at greater than 20x. Given typical sequencing conditions the lower confidence interval of the sensitivity of variant calling for Single Nucleotide Variant (SNV) detection is 99.8%. The lower confidence interval of the sensitivity of variant calling for Indel detection is 96.2%. The sensitivity was calculated over a set of high confidence genome regions which are accessible via 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 on the panel which are known to have low coverage across the majority of samples 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.

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.

Reporting times

The reporting time for this test is 14 calendar days.

References

Lord J, McMullan DJ, Eberhardt RY, Rinck G, Hamilton SJ, Quinlan-Jones E, Prigmore E, Keelagher R, Best SK, Carey GK, Mellis R, Robart S, Berry IR, Chandler KE, Cilliers D, Cresswell L, Edwards SL, Gardiner C, Henderson A, Holden ST, Homfray T, Lester T, Lewis RA, Newbury-Ecob R, Prescott K, Quarrell OW, Ramsden SC, Roberts E, Tapon D, Tooley MJ, Vasudevan PC, Weber AP, Wellesley DG, Westwood P, White H, Parker M, Williams D, Jenkins L, Scott RH, Kilby MD, Chitty LS, Hurles ME, Maher ER; Prenatal Assessment of Genomes and Exomes Consortium. Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study. Lancet. 2019 Feb 23;393(10173):747-757. doi: 10.1016/S0140-6736(18)31940-8. Epub 2019 Jan 31. PMID: 30712880; PMCID: PMC6386638.

Appendix

Gene	Median Percentage of Exonic Bases (+-5bp) covered at greater than 20x
SMN1	0.0
CFC1	19.3
CYP21A2	45.9
IKBKG	47.9
EXOC3L2	53.3
HBA2	55.3
ANAPC1	61.0
OCLN	64.8
TUBB2A	71.5
MSTO1	74.0
DPM2	74.0
TUBB2B	75.3
HMGA2	77.3
HBA1	78.4
AP1S2	79.9

SNORD118	80.4
ACAN	86.2
USP18	86.5
NEB	86.7
GBA	87.5
NDUFAF8	90.6
PAX2	91.1
SNX10	91.2
B3GAT3	91.4
WDPCP	91.7
B3GALNT2	92.8
FUT8	92.9
IDS	93.2
ODAD2	93.8
SHANK3	94.2
SHOX	94.6
CWC27	94.6
FBLN5	94.9

Table 1. Genes with known coverage issues within Fetal Anomalies panel when analysed on Whole Exome Sequencing. Genes where the median sample had less than 95% of exonic bases +- 5bp covered at 20x are shown.