Prenatal ExomeSeq

Test Description:

Prenatal ExomeSeq is a rapid clinical exome sequencing service indicated for pregnancies where the fetuses have structural anomalies and a genetic cause is suspected. Fetal structural abnormalities are identified in approximately 2–4% of pregnancies, of which more than half do not achieve a genetic diagnosis by chromosome karyotyping, fluorescence in situ hybridization (FISH) and high-resolution microarray analyses (Monaghan et al. 2020). Furthermore, clinical diagnoses of prenatal abnormalities are often challenged by limited clinical information obtained during the prenatal stage, and ruling out multiple differential diagnoses may be necessary. Exome sequencing (ES) examines the majority of exons and exon/intron boundaries of most of the genes at one time. This test is different from most genetic tests that only analyze one gene or a set of genes at a time. Studies have demonstrated that among structurally abnormal and chromosomally normal fetuses, ES resulted in an overall diagnostic yield of about 8-10% and rose to 15-35% in fetuses with more than one anomaly (Lord et al. 2019, Petrovski et al. 2019). Results of prenatal ES may guide clinical management decisions, such as delivery planning and neonatal care (Tolusso et al. 2021).

Indications:

Pregnancies with a baby having structural anomalies and a genetic cause is suspected, but other testing strategies have not achieved a genetic diagnosis.

What is Reported:

- Variants that are known to be pathogenic, or for which there is sufficient evidence suggesting pathogenicity, in a gene suspected to cause the baby's signs and symptoms.
- Variants of uncertain significance in genes related to the baby's signs and symptoms may be reported if they could provide a diagnosis should the variant classification be likely pathogenic or pathogenic.
- Genetic changes found in genes not related to the baby's condition, but may have an important impact on health early in life and/or are considered medically actionable (i.e. incidental/secondary findings) will be reported only if patients decide to receive them in the report.

What is Not Reported:

- Variants in genes not currently believed to be associated with any disease or that have an unclear association with disease.
- Variants that predict an increased risk of a disease, but do not cause a disease by themselves.
- Variants in genes that are not known to be associated with the baby's signs and symptoms, including those that confer carrier status for an autosomal recessive or X-linked disorder with the exception of incidental and secondary findings (see below).
- Variants of uncertain significance in genes that cause autosomal recessive or X-linked recessive (in a female fetus) diseases that are associated the baby's signs and symptoms, unless a second variant is also detected in this gene.

Note: On the written report of the proband, parental origin of each variant will be indicated (unless if the variant in the baby occurred de novo); however, parents will not receive separate written reports.

Incidental and Secondary Findings:

Prenatal ES may detect pathogenic variants in genes deemed medically actionable by the American

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College of Medical Genetics and Genomics (Miller et al. 2023), known as secondary findings. Additionally, prenatal ES may detect pathogenic variants in genes not related to the baby's condition and/or do not have prenatally detectable features, but may have an important impact on health early in life, so-called incidental findings. For families who choose to receive secondary and/or incidental findings, information about these disease-causing variants and familial segregation, when known, will be included in the baby's report. Family members will not receive separate reports. Secondary and incidental findings will not be sought or reported if the family chooses not to receive them.

Submission Requirements:

Prenatal ES results can be difficult to interpret because clinical information obtained during the prenatal stage is often limited to imaging results. It is important to have reliable clinical information and an accurate family history in order to interpret the prenatal ES results. ES testing is most likely to find the baby's genetic diagnosis when samples from biological parents are analyzed at the same time. These items must be included in order to begin the ES process:

- Baby's sample from amniocentesis or cultured cells
- Maternal sample (for maternal cell contamination exclusion and duo or trio testing)
- Paternal sample (for trio testing)
- Additional family members' samples (following discussion with laboratory)
- Test requisition (all billing and clinical information must be completed)
- Incidental and secondary findings consent documentation
- Family history and pedigree
- Detailed clinical summary of the baby, including fetal imaging result reports
- Summary of previous genetic test results, if available

Methodology:

Procedure: Prenatal exome analysis is performed off a genome sequencing (GS) backbone. GS is performed on genomic DNA using a PCR-free library preparation and sequenced on an Illumina NovaSeq X Plus instrument to an average autosomal sequencing depth of at least 30x. Sequenced reads are aligned to human reference build (GRCh38) and variant calling is performed using the Illumina DragenGermline pipeline in AllCallers mode which produces simple variants (SNVs and small indels). Sample QC and identity are evaluated based on best practices for clinical whole-genome sequencing (Marshall et al. 2020). The sensitivity and specificity for SNVs and small insertions and deletions up to 50 base pairs is greater than 99%.

Data Analysis: Variant annotation and filtration are performed using a bioinformatics pipeline available in Fabric Enterprise. Variants are prioritized for review using GEM (Fabric Genomics) and classified for clinical significance based on the standards and guidelines for the interpretation of sequence variants, recommended by ACMG-AMP (Richards et al. 2015) with ClinGen rule specifications (https://www.clinicalgenome.org/workinggroups/sequence-variant-interpretation/). Reported variants are further limited to clinically relevant variants meeting our inclusion criteria. Carrier status for autosomal recessive conditions is not reported unless there is sufficient overlap with the reported clinical phenotype. Variants occurring in medically actionable genes may be reported for individuals who have elected to receive secondary findings. Reported variants are limited to pathogenic and likely pathogenic SNVs and small indels that meet reportable criteria in genes as defined by the ACMG Secondary Findings Maintenance Working Group (SFWG) (https://search.clinicalgenome.org/kb/genes/acmgsfpage=1&size=25&order=asc&sort=symbol&search=). Variant confirmation by an orthogonal technology is performed on reported variants unless otherwise indicated. Variants that have not been confirmed by an orthogonal methodology could represent technical artifacts.

Technical Limitations:

- ES attempts to examine approximately 20,000 genes in the MANE Select dataset. Each sample is required to achieve at least 20X coverage for 90% or more of the genomic regions occupied by these genes, based on GS best practices (Marshall et al 2020). Regions with inadequate coverage will not be able to support accurate variant calls.

Genes in the MANE Plus Clinical dataset that are not located on canonical chromosomes, unless manually curated (e.g., replacing the original transcript on a contig with a matched transcript on a canonical chromosome), are not supported by Fabric Enterprise software.

- Pathogenic variants may be present in a portion of the gene regions not covered by this test and therefore would not be identified. Thus, the absence of reportable findings for any gene does not mean there are no pathogenic variants in that gene.
- This analysis is limited to the detection of single nucleotide variants (SNVs), small deletions and insertions (indels). Examples of genomic abnormalities not detectable in the current version of this assay include copy number variations, mitochondrial genome variants, nucleotide repeat expansions (such as CGG repeats in FMR1, CAG repeats in HTT, etc.), balanced genomic rearrangements, absence of heterozygosity, and methylation differences. Variants at low-level mosaicism or in challenging regions of the genome (genes with pseudogenes, high GC content, repetitive regions, low sequencing coverage) may escape detection. Regions identified as systematically problematic due to high homology are listed in https://www.ncbi.nlm.nih.gov/books/NBK535152/.
- The clinical utility of ES depends on the accuracy of the clinical information provided by the referring physician. DNA sequencing from family members often improves the interpretation of test results.
- Due to the large number of variants called in each specimen, this assay utilizes the phenotype-driven variant prioritization tool, GEM, by Fabric Enterprise V3 (De La Vega et al. 2021) as an automated first-tier variant filtration strategy. Disease-associated loci may escape review prioritization for several reasons, which include, but are not limited to, incomplete or inaccurate phenotype data, newly or yet to be established disease association, non-Mendelian etiology, poor variant quality metrics, or algorithmic bias. Additionally, GEM prioritization is limited to variants in genes with a MANE transcript ID (Matched Annotation from the NCBI and EMBL-EBI).
- Studies have shown that the chance of receiving a diagnosis from prenatal ES highly depends on the baby's specific health problems. In general, fewer than 50% of prenatal ES identify a diagnosis. A negative or uncertain result does not exclude the possibility that there is a genetic cause for the reported clinical indications in the baby. In some situations, further testing might be indicated.
- Prenatal ES results do not predict how severe a particular condition will be or predict the age of onset of a particular condition.
- Our understanding of the human genome is incomplete at this time.

Genetic Counseling and Interpretation:

- It is highly recommended that patients have genetic counseling before the test is ordered, as they will have an important choice to make regarding which results they wish to know. Understanding the risks and benefits of this testing is important for the patient and his or her family. Genetic counseling after the test is likewise important to aid in the understanding of test results and their implications for the patient and his or her family members.
- It is the ordering physician's responsibility to interpret the results from this test within a clinical context.

Reanalysis of Prenatal ES Results:

One reanalysis of the prenatal ES data after birth of the proband is provided at no additional charge. It is highly recommended that the healthcare provider submit additional postnatal clinical information about the baby with this reanalysis request. If no additional clinical information is available, it is recommended to wait at least 12 months before requesting reanalysis.

Specimen:

- Prenatal sample: 25 mL amniotic fluid or two (2) T25 flasks grown to confluence.
- Maternal sample: At least 3 mL whole blood in a lavender top (EDTA) tube. Alternately, 15 mcg of DNA extracted from peripheral blood in a CLIA lab may be submitted. A maternal sample is required for prenatal ES.
- Paternal or biological family member sample: At least 3 mL whole blood in a lavender top (EDTA) tube. Alternately, 15 mcg of DNA extracted from peripheral blood in a CLIA lab may be submitted.

Note: Label the tube with patient's name, birth date, and date of collection.

*Please call the lab at 513-636-4474 before sending amniotic fluid.

Turnaround Time:

28 days

**Preliminary results without Sanger confirmation will be provided upon request.*

CPT Codes:

- **Prenatal ExomeSeq (Trio) -** proband and two biological family members**:** 81415
- **Additional family member(s):** 81416

Shipping Instructions:

Please enclose test requisition with sample(s). **All information must be completed before sample(s) can be processed.** Place sample(s) in styrofoam mailer and **ship at room temperature by overnight Federal Express** to arrive Monday through Saturday.

Ship To:

Genetics and Genomics Diagnostic Laboratory
3333 Burnet Avenue NRB 1042
Cincinnati, OH 45229
513-636-4474

References:

Monaghan, K. G., Leach, N. T., et al. (2020). "The use of fetal exome sequencing in prenatal diagnosis: a points to consider document of the American College of Medical Genetics and Genomics (ACMG)" Genet Med. 22(4): 675-680.

Petrovski S, et al. (2019). "Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study" Lancet. 393:758–767.

Lord J, et al. (2019) "Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study" Lancet. 393:747–757.

Tolusso LK, et al. (2021). "Beyond diagnostic yield: prenatal exome sequencing results in maternal, neonatal, and familial clinical management changes." Genet Med. 2021 Jan 13:1-9.

Miller, DT, et al. (2023). "ACMG SF v3.2 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG)" Genet Med. 25(8):100866.

Richards, S., et al. (2015). "Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology" Genet Med. 17(5):405–424.