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), which simultaneously examines most of the genes in the body in one test, is thought to be a more efficient diagnostic method compared to genetic tests that analyze only one gene or a limited number of genes at a time. Recently 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:

- Pathogenic or likely pathogenic variants that may have caused 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 on the report.

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.

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 (see Incidental/Secondary Findings section for exceptions).
- 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.

Incidental and Secondary Findings:

Prenatal ES may detect pathogenic variants deemed to be of medical value in genes recommended by the American College of Medical Genetics and Genomics (Miller et al. 2021) or 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. Secondary and incidental findings will not be sought or reported if the family chooses not to receive them. For families who choose to receive secondary findings, information on these disease causing variants will be included on the baby's report. In addition, the report will note if these variants were found in family members who submitted samples.

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

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Genetics and Genomics Diagnostic Laboratory
CLIA#: 36D0656333
Phone: (513) 636-4474
Fax: (513) 636-4373
Email: LabGeneticCounselors@cchmc.org
www.cincinnatichildrens.org/diagnosticlabs

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)
- Other biological family member sample (for trio or quad testing)
- Test requisition (all billing and clinical information must be completed)
- Signed informed consent form
- Family history and pedigree
- Detailed clinical summary of the baby including fetal imaging result reports
- Summary of previous genetic test results and reports, if available
- A list of genes/conditions highly suspected to be baby's clinical differential diagnosis (for preliminary report)

Methodology:

Procedure: Prenatal ES is performed on genomic DNA using the Human Comprehensive Exome kit from Twist Bioscience to enrich the whole exome. The exome is sequenced using an Illumina sequencing system with paired-end reads at a minimum coverage of 20X of 95% of the target regions. The exome DNA sequences are aligned to the human reference genome (build UCSC hg19) with BWA-MEM. Variants are called using GATK and quality controls are performed as part of an in-house developed bioinformatics pipeline based on GATK best practices. Data Analysis: Variants are annotated, analyzed, and those identified as potentially clinically relevant are interpreted for clinical significance based on the standards and guidelines for the interpretation of sequence variants, recommended by ACMG-AMP (Richards et al. 2015). Sanger confirmation is performed on all variants included in the final report.

Technical Limitations:

- This test attempts to examine the important coding regions of approximately 20,000 genes in the genome, known as the exome. However, the technical ability to capture and sequence the exome is limited, and not every exon can be evaluated.
- Pathogenic variants may be present in a portion of the genes 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.
- ES finds only single base pair changes or small insertions or deletions of DNA. This test does not detect other types of disease-causing variants including large deletions, duplications, or rearrangements, mitochondrial genome mutations, nucleotide repeat expansions, genes with pseudogenes, and many epigenetic defects. This test may not detect DNA changes that are not present in all cells (mosaic changes).
- 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 exome 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, saliva in an Oragene saliva kit or 10 mcg of DNA 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, saliva in an Oragene saliva kit or 10 mcg of DNA 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 or for a free saliva collection kit.

Turnaround Time:

Fifteen (15) days for preliminary report (verbal or written) of variants in the set of provided genes of differential diagnoses without Sanger confirmation; 30 days for final report for a full ES analysis with Sanger confirmations.

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, D.T., Lee, K., Chung, W.K. et al. (2021). "ACMG SF v3.0 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. 2021 May 20. Online ahead of print.

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.