Clinical Exome Sequencing for Fetal Anomalies: XomeDx® *Prenatal* Targeted, XomeDx® *Prenatal* Comprehensive, XomeDx® *Fetal*

Description

XomeDx® clinical exome sequencing (ES) is utilized to identify the underlying molecular basis of a genetic disorder in a pregnancy with fetal anomalies. Recent studies have shown a positive diagnostic result in 9% to 32% of fetuses with abnormal ultrasound anomalies.[1-5] At GeneDx, ES identified a definitive molecular diagnosis in approximately 25% of fetuses presenting with anomalies. Several organizations have recently published guidance on the use of prenatal clinical exome sequencing.[6-7] Clinical ES at GeneDx can be performed on multiple fetal specimens including direct or cultured chorionic villi, amniocytes, cord blood, products of conception, or extracted fetal DNA.

The XomeDx test is different from other types of genetic diagnostic tests in terms of the number of genes that are analyzed simultaneously. XomeDx targets the protein-coding regions of the human genome, which represents ~20,000 genes and accounts for approximately ~2% of all human genetic material. [8] These targeted gene regions, called exons, are captured and sequenced using massively parallel sequencing. The fetal sequence is then compared to (1) a published reference sequence, (2) other family members submitted for testing (e.g., biological parents), and (3) control individuals. Phenotype-driven gene lists, generated using Human Phenotype Ontology and HGMD gene-phenotype terms corresponding to the reported fetal anomalies, are used to select phenotypically relevant variants to report. Additional resources such as gnomAD, NHLBI Exome Sequencing Project, OMIM, PubMed, and ClinVar are used to evaluate genes and sequence changes of interest, which are then interpreted according to the American College of Medical Genetics and Genomics (ACMG) recommendations.[8-9] Clinical exome sequencing is most effective when other family members (both biological parents, if available) are included in the analysis of the affected fetal exome.

Results Reporting

While ES analysis is performed on DNA from the fetus and both biological parents, only a single report will be issued for the fetus. A separate report will not be issued for parents or other family members who submitted a specimen for the purpose of allowing better interpretation of the fetal results. If additional reports are requested for other family members, additional fees will apply.

Expedited Turn-Around-Time Reporting Options

XomeDx *Prenatal* Targeted

The XomeDx *Prenatal* Targeted fetal report will include medically relevant pathogenic or likely pathogenic variants in genes expected to be related to the reported fetal phenotype. Variants of uncertain significance (VUS) may be reported when there is compelling evidence to suggest clinical significance. Pathogenic and likely pathogenic variants in genes known to cause significant childhood morbidity and mortality may be reported. Unless the family opts-out, secondary findings as recommended by the ACMG will also be reported. Due to the expedited turn-around-time of this option, maternal cell contamination studies are performed simultaneously

XomeDx *Prenatal* Comprehensive

The XomeDx *Prenatal* Comprehensive fetal report will include all of the same information as the XomeDx Prenatal Targeted report. In addition, variants in novel candidate genes may also be

reported. Candidate genes are not currently implicated in human disease; however, there may be animal models or other data to suggest a causative relationship. Due to the expedited turn-around-time of this option, maternal cell contamination studies are performed simultaneously.

Expedited Turn-Around-Time Reporting Options

XomeDx *Fetal*
The XomeDx *Fetal* report will include medically relevant pathogenic or likely pathogenic variants in genes expected to be related to the reported fetal phenotype. Variants of uncertain significance (VUS) may be reported when there is compelling evidence to suggest clinical significance. In addition, variants in novel candidate genes may also be reported. Pathogenic and likely pathogenic variants in genes known to cause significant childhood morbidity and mortality may be reported. Unless the family opts-out, secondary findings as recommended by ACMG will also be reported.

Exome Sequencing for Fetal Anomalies: Comparison of Testing Options

	Test Code(s)	Turn Around Time	Candidate Genes Reported	MCC Studies	Payment Options
XomeDx *Prenatal* Targeted	959	Expedited	No	Included	Institutional and Self-Pay
XomeDx *Prenatal* Comprehensive	J499	Expedited	Yes	Included	Institutional and Self-Pay
XomeDx *Fetal* (Ongoing Pregnancy and Deceased Fetus)	TK89a (Trio), TK89e (Duo), TK89b (Proband Only)	Standard	Yes	Can Order Separately	Insurance, Institutional and Self-Pay

Please email WESPrenatal@GeneDx.com with any questions or to inform us of an incoming case.

ACMG Secondary Findings
The American College of Medical Genetics and Genomics (ACMG) recommends that secondary findings identified in a specific subset of genes associated with medically actionable, inherited disorders be reported for all probands undergoing exome or genome sequencing. Please refer to the latest version of the ACMG recommendations for reporting of secondary findings in clinical exome and genome sequencing for complete details of the genes and associated genetic disorders. Secondary findings will be included for all exome or genome sequencing reports, unless a family opts-out of receiving this information on the Informed Consent as part of the test requisition form. The status of any secondary finding(s) reported for the affected individual will be provided for all relatives included as part of the proband's test; **GeneDx does not conduct an independent evaluation of secondary findings in relatives**. Relatives have the ability to opt-out of receiving secondary findings. Secondary findings will be confirmed by an alternate test method when needed.

Test Methods
The clinical records and results of prior fetal screening, fetal imaging and/or genetic testing will be reviewed prior to analysis. Using genomic DNA from the submitted specimen(s), DNA is enriched for the complete coding regions and splice site junctions for most genes of the human genome using a proprietary capture system developed by GeneDx for next-generation sequencing. The enriched targets are simultaneously sequenced with

paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. Using a custom-developed analysis tool, data are filtered and analyzed to identify sequence and copy number variants.8 Reported clinically significant variants are confirmed by an appropriate orthogonal method in the fetus and, if submitted, in selected relatives as necessary. Sequence and copy number variants are reported according to the Human Genome Variation Society (HGVS). Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported.

Limitations

The XomeDx test attempts to evaluate the most important regions of the majority of the ~20,000 genes in the human genome. However, it is not technically possible to capture and sequence the entire exome at present. It is anticipated that approximately 98% of the targeted region of an affected fetus' exome will be assessed with the XomeDx test at a minimum of 10x coverage, the minimum read depth necessary to detect a variant. Across the exome, the average depth of coverage is 100- 120x. The test report will include case-specific exome coverage. There may be some genes or portions of genes that are not amenable to capture, sequencing, and alignment. Additionally, certain types of sequence variations are difficult to identify using ES, such as repeat expansions. Average read depth statistics for the XomeDx test are as follows:

Read Depth	10x	20x	30x	40x	50x
Mean Percent of Target Covered	98%	97%	94%	90%	83%

Exome Sequencing for Fetal Anomalies is a phenotype-driven analysis; specimens will not be accepted in the absence of abnormal ultrasound findings. The test is not a substitute for fetal cytogenetic analysis, newborn screening, or carrier screening. Carrier status in the fetus or parents is not reported unless associated with the presenting phenotype of the fetus.

REFERENCES:

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6. Joint Position Statement from the International Society for Prenatal Diagnosis (ISPD), the Society for Maternal Fetal Medicine (SMFM), and the Perinatal Quality Foundation (PQF) (2018) Prenat. Diagn. 38 (1):6-9 (PMID: 29315690)
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8. Retterer et al. (2016) Genet. Med. 18 (7):696-704 (PMID: 26633542)
9. Richards et al. (2015) Genetics In Medicine 17 (5):405-24 (PMID: 25741868)