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

Description

XomeDx® Prenatal Targeted, *XomeDx*® Prenatal Comprehensive, and *XomeDx*® Fetal tests use clinical exome sequencing (ES) 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 10-38% 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 published guidance on the use of prenatal clinical exome sequencing.[6-7] Clinical ES at GeneDx can be performed on multiple types of fetal specimens including chorionic villi, amniocytes, cord blood, products of conception, cultured fetal cells, or extracted fetal DNA.

The *XomeDx*® test targets the protein-coding regions (exons) of ~20,000 genes, which account for approximately ~2% of the human genome.[8] These targeted regions are captured, sequenced using next-generation sequencing, filtered against published reference sequences, and compared to other sequenced family members, population databases, and control sequences to reveal potentially reportable variants. The fetal sequence is then evaluated using a phenotype-driven approach that includes Human Phenotype Ontology and Human Gene Mutation Database gene-phenotype associations, in conjunction with multiple resources including gnomAD, OMIM, PubMed, and ClinVar.[9] Sequence changes of interest are interpreted according to the American College of Medical Genetics and Genomics guidelines[10] 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.

Result Reporting

Exome sequence analysis is performed on the affected fetus (i.e., the proband) and, when submitted together for analysis, biological parental samples, and/or additional biological relatives as needed. A report will be issued only for the proband. A separate report will not be issued for parents or other family members who may also have submitted a specimen for the purpose of allowing better interpretation of the results for the affected fetus. If additional reports are requested for other affected family members, additional fees will apply.

The *XomeDx*® Prenatal Targeted, *XomeDx*® Prenatal Comprehensive, and *XomeDx*® Fetal reports 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. For *XomeDx*® Prenatal Comprehensive and *XomeDx*® Fetal, variants in novel candidate genes may also be reported.

XomeDx® Prenatal Targeted and *XomeDx*® Prenatal Comprehensive have expedited turnaround times, designed for a current pregnancy with fetal anomalies. *XomeDx*® Fetal has a standard turnaround time and can be ordered for an ongoing pregnancy with fetal anomalies or a deceased fetus/products of conception when ultrasound anomalies were present.

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We accept institutional bill and self-pay for *XomeDx*® Prenatal Targeted, *XomeDx*® Prenatal Comprehensive, and *XomeDx*® Fetal. Insurance billing is only accepted for *XomeDx*® Fetal.

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 fetus will be provided for all relatives included as part of the fetus' 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

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. Data are filtered and analyzed to identify sequence variants and most deletions and duplications involving three or more coding exons. Reported clinically significant variants are confirmed by an appropriate orthogonal method in the fetus and, if submitted, in selected relatives as necessary. Sequence variants are reported according to the Human Genome Variation Society (HGVS) guidelines. Copy number variants are reported based on the probe coordinates, the coordinates of the exons involved, or precise breakpoints when known.

The clinical records and results of previous fetal screening, fetal imaging and/or genetic testing will be reviewed prior to analysis. The fetus' analysis includes evaluation of variants that are identified to be *de novo* (when both biological parents submitted), compound heterozygous (when both biological parents submitted), homozygous, heterozygous and X-linked. In addition, analysis takes into consideration family structure, reported phenotype, and provided clinical and/or differential diagnosis. This is a phenotype-driven test of a very large number of genes; therefore, reported results are focused on pathogenic and likely pathogenic variants in genes related to the clinical information provided. Less frequently, variants of uncertain significance in candidate (*XomeDx*® Prenatal Comprehensive) and differential diagnosis genes (*XomeDx*® Prenatal Targeted and *XomeDx*® Prenatal Comprehensive) are reported.

Traditional maternal cell contamination (MCC) studies are not included in exome-based testing as any significant contamination of the fetal specimen will be identified by evaluation of the next generation sequencing (NGS) data. Complete MCC cannot be excluded in a sample from a female fetus unless a

maternal sample was included for analysis by NGS. If clinically indicated, charged MCC studies can be ordered separately.

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%	98%	97%	95%	91%

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.

The available scientific knowledge about the function of all genes in the human genome is incomplete at this time. It is possible that the *XomeDx*® test may identify the presence of a genetic variant in the exome sequence of an affected fetus, but it will not be recognized as causative for the affected fetus' disorder due to insufficient knowledge about the variant or the gene and its function. Reanalysis of the data is available upon request by the health care provider to incorporate updated clinical information and/or newly emerging gene and variant information. Even if the *XomeDx*® test identifies the underlying genetic cause of a disorder in an affected fetus, it is possible that such a diagnosis will not permit an accurate prediction of the prognosis or severity of the disease. While there is a possibility that identifying the genetic cause may help direct management and treatment of the disease, it is also possible that this knowledge will not change management or treatment.

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