

## XomeDxPrenatal: Whole Exome Sequencing for Ongoing Pregnancies

### Description:

XomeDxPrenatal is an expedited whole exome sequencing (WES) service specifically designed for ongoing pregnancies. WES is utilized to identify the underlying molecular basis of a genetic disorder in a pregnancy with fetal anomalies. Several small studies have shown a positive diagnostic result in 10% to 25% of deceased fetuses with abnormal ultrasound anomalies.<sup>1-3</sup> In our own laboratory, WES identified a definitive molecular diagnosis in approximately 27% of deceased fetuses presenting with abnormal ultrasound findings.

The XomeDxPrenatal test is different from other types of genetic diagnostic tests in terms of the number of genes that are sequenced simultaneously. The XomeDxPrenatal test targets the protein-coding regions of the human genome, which represents ~20,000 genes and accounts for approximately ~2% of all human genetic material.<sup>4</sup> These targeted regions of genes, called exons, are captured and sequenced using massively parallel sequencing (NextGen sequencing). The fetal sequence is then compared to published reference sequences and the gene sequences of the biological parents to identify causal variants that could explain the fetal ultrasound findings. Specimens from biological parents are required for all XomeDxPrenatal cases to determine de novo occurrence of variants and phase for variants identified in recessive genes.<sup>5</sup> If parental relationships (gamete donor, non-paternity, or non-maternity) are misspecified, or GeneDx does not receive specimens on both biological parents, testing will be canceled.

### Result Reporting:

While WES analysis is performed on DNA from the fetus and both biological parents, only a single XomeDxPrenatal report will be issued on the fetus. 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 fetal results. If additional reports are requested for other affected family members, additional fees will apply.

### Option 1: XomeDxPrenatal Targeted

The XomeDxPrenatal 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 may be reported only if there is compelling evidence to suggest clinical significance. Pathogenic and likely pathogenic variants in genes known to cause significant childhood morbidity and mortality may also be reported. Unless the family opts-out, secondary findings as recommended by the American College of Medical Genetics and Genomics (ACMG) will also be reported.

## Option 2: XomeDxPrenatal Comprehensive

The XomeDxPrenatal Comprehensive fetal report will include all of the same information as the XomeDxPrenatal Targeted report. In addition, variants of uncertain significance (VUS) in genes that may be related to the fetal phenotype and variants in novel candidate genes may also be reported. A candidate gene is a gene that is not currently implicated in human disease; however, there may be animal models or research data to suggest a causative relationship.

## ACMG Secondary Findings:

The American College of Medical Genetics and Genomics (ACMG) recommends that secondary findings, known and/or expected pathogenic variants, identified in a specific subset of genes associated with medically actionable, inherited disorders be reported for all probands undergoing whole exome sequencing. Please refer to the latest version of the [ACMG Recommendations for Reporting of Secondary Findings in Clinical Exome and Genome Sequencing Report](#) for complete details of the genes and associated genetic disorders. Secondary findings will be included for all XomeDxPrenatal reports, unless a family opts-out of receiving this information on the Informed Consent and Authorization Form as part of the XomeDxPrenatal Test Requisition Form. The status for any secondary finding(s) reported for the fetus will be provided for all relatives tested by XomeDxPrenatal; 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.

## 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 fetal specimen and biological parents, the exonic regions and flanking splice junctions of the genome are sequenced by massively parallel (NextGen) sequencing on an Illumina sequencing system with 100bp or greater paired-end reads. Reads are aligned to human genome build GRCh37/UCSC hg19, and analyzed for sequence variants using a custom-developed analysis tool (Xome Analyzer). Capillary sequencing or another appropriate method is used to confirm all potentially pathogenic variants identified in the fetus and parental specimens. Sequence alterations are reported according to the Human Genome Variation Society (HGVS) nomenclature guidelines.

## Limitations:

The XomeDxPrenatal test is designed 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 95% of the targeted region of the fetal exome will be assessed with the XomeDxPrenatal test at 10x coverage, while >98% of the target region will be covered at a minimum of 1x. 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 WES, including repeat expansions and copy number variants. For example, the CGG repeat expansions in the FMR1 gene causative for fragile X syndrome and the copy number variants in the SMN1 gene causative for spinal muscular atrophy (SMA) will **not** be detected by this test. However, upon request, GeneDx can utilize other types of diagnostic tests in conjunction with the *XomeDxPrenatal* test to increase the likelihood of identifying a molecular cause for the fetal abnormalities.

The available scientific knowledge about the function of all genes in the human genome is incomplete at this time. It is possible that the *XomeDxPrenatal* test may identify the presence of a genetic variant in the fetus, but it will not be recognized as causative for the fetus' disorder due to insufficient knowledge about the variant or the gene and its function. Reanalysis of the fetal 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 *XomeDxPrenatal* test identifies the underlying genetic cause of a disorder in a 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.

*XomeDxPrenatal* is a phenotype-driven analysis; specimens will not be accepted in the absence of abnormal ultrasound findings. The *XomeDxPrenatal* 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:

1. Yang et al. (2013) N. Engl. J. Med. 369 (16):1502-11 (PMID: 24088041)
2. Carss et al. (2014) Human Molecular Genetics 23 (12):3269-77 (PMID: 24476948)
3. Drury et al. (2015) Prenat. Diagn. 35 (10):1010-7 (PMID: 26275891)
4. Bamshad et al. (2011) Nature Reviews. Genetics 12 (11):745-55 (PMID: 21946919)
5. Retterer et al. (2016) Genet. Med. 18 (7):696-704 (PMID: 26633542)