

GenomeDx™ Prenatal: Clinical Genome Sequencing for Fetal Anomalies

Description

GenomeDx™ Prenatal uses clinical genome sequencing (GS) to identify the underlying molecular basis of a genetic disorder in a pregnancy with fetal anomalies. Genome sequencing has been shown to identify a causal variant in 19–36% of fetuses with abnormal findings on ultrasound (PMID: 33800913, 35089622, 36360323). Additional large-scale studies of genome sequencing for fetal anomalies are needed to further refine the overall diagnostic yield. Several organizations have published guidance on the use of diagnostic genomic sequencing prenatally (PMID: 35583085, 31911674, 30134429).

This test simultaneously evaluates both the protein-coding and non-coding regions of the human nuclear genome, allowing for the potential detection of characterized/pathogenic variants in regions that are not assessed by exome sequencing (ES). The protein-coding regions represent ~20,000 genes and account for approximately 2% of all human genetic material (PMID: 21946919). The non-coding regions include promoter, intronic, and untranslated regions. While much of the data generated from sequencing the genome is not well understood at this time, genome sequencing may provide more reliable coverage of the exonic regions (PMID: 25973577, 25827230).

For this test, the fetus' nuclear genome sequence is filtered against published reference sequences, and compared to other sequenced family members, population databases, control sequences, and the GeneDx internal database to reveal potentially reportable variants. The affected fetus' sequence is then evaluated using gene-phenotype associations, in conjunction with multiple resources including gnomAD, HGMD, OMIM, PubMed, and ClinVar (PMID: 26633542). Sequence changes of interest are interpreted according to the American College of Medical Genetics and Genomics (ACMG) guidelines (PMID: 25741868). A report is issued with clinically relevant variants, which may include sequencing nucleotide variants (SNV), most copy number variants (CNV) greater than 1 kb, uniparental disomy (when parents are submitted), homozygous loss of exon 8 in the *SMN1* gene, and repeat expansions in select genes that are associated with the fetus' reported phenotype and/or may present in childhood. Reported repeat expansions may correspond to disorders with variable age of onset, ranging from prenatal to childhood to adulthood. Therefore, it is possible that a reported repeat expansion may not result in symptom development until adulthood, as the repeat length alone is not a definitive predictor for age of onset. If a repeat expansion is reported in a proband, the repeat length will also be evaluated and reported in biological relatives included as part of the proband's test (if submitted); therefore, it is possible that a relative may learn about a presymptomatic diagnosis.

The clinical records and results of previous fetal screening, fetal imaging and/or genetic testing submitted at the time of test activation 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 comprehensive analysis of a very large number of genes; therefore, reported results are focused on pathogenic and likely pathogenic variants, along with clinically relevant

variants of uncertain significance, in genes related to the provided clinical information. Rarely, pathogenic, likely pathogenic, and molecularly strong variants of uncertain significance in genes known to cause pediatric morbidity or mortality may be reported.

Result Reporting

Nuclear genome 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 report may contain variations in genes previously implicated in a human disease similar to the clinical presentation of the affected fetus or in genes hypothesized to be related to the cause of the disease (candidate genes, also known as genes of unknown significance). Variants in candidate genes may be reported based upon limited published evidence (including case report(s)) and/or internal data, such as observations of previous cases with similar phenotypes and types of variants in the same gene. In addition, other evidence such as gene function, tissue of expression, and phenotype of model organisms with alterations in the gene may also be used as evidence to support reporting a variant in a candidate gene. Pathogenic and likely pathogenic variants in genes known to cause childhood morbidity or mortality may be reported.

If results are non-diagnostic, or do not fully explain new clinical features presenting in the proband after delivery a reanalysis of the nuclear genome sequencing data can be ordered by the healthcare provider. For fetuses with new ultrasound findings identified after a report has been issued, ordering providers can contact prenatal@genedx.com to determine if a targeted reanalysis can be conducted. GeneDx may contact the ordering healthcare provider to recommend a reanalysis if a proband's genome data suggests the presence of a clinically relevant variant in a newly identified gene implicated in human disease.

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

Genomic DNA from the submitted specimen is sequenced with paired-end reads on an Illumina platform. Average mean sequencing coverage for the proband is at least 40x across the genome, with a minimum threshold of 30x for any individual sample. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19.

Data from next-generation sequencing with CNV calling (NGS-CNV) are filtered and analyzed to identify sequence variants, repeat expansions of select genes, homozygous loss of *SMN1* exon 8 (Spinal Muscular Atrophy), and most deletions and duplications greater than 1 kb in size. Screening for disease associated repeat expansions is included for the following genes: *ARX* (PA1 only), *ATNI*, *ATXN2*, *ATXN3*, *ATXN7*, *DMPK*, *FMRI* (with methylation status for alleles with >150 repeats), *FXN*, *HOXD13*, and *PHOX2B* (see table below for more details). Unless otherwise reported, the proband is considered screen negative for these non-sequencing variants; however, if a high clinical suspicion of any of these disorders is present, gene/disease specific diagnostic testing should be considered.

Reportable variants include pathogenic, likely pathogenic, and variants of uncertain significance; likely benign and benign variants, if present, are not routinely reported. Carrier status for the proband is not reported unless associated with the provided phenotype. Reported variants are confirmed by an appropriate orthogonal method in the proband 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. Repeat expansions are reported with an accuracy of ± 2 repeats for alleles that contain up to 150 total nucleotides (e.g., 50 triplet repeats or 25 hexanucleotide repeats) and ± 5 repeats for alleles that contain >150 nucleotides. The exact number of repeats cannot be determined for very large alleles, which will be reported as "greater than" a gene-specific threshold. Available evidence for variant classification may change over time and reported variant(s) may be reclassified according to the ACMG/AMP Standards and Guidelines (PMID: 25741868), which may lead to issuing a revised report.

Maternal cell contamination (MCC) studies are performed on the fetal and maternal (when submitted) DNA concurrent with genome sequencing. Complete MCC cannot be excluded in a sample from a female fetus unless a maternal sample is included for analysis.

Limitations

This test attempts to evaluate the complete coding and non-coding regions of the genome. However, it is not technically possible to uniquely resolve and align the entire genome at present due to homology and other structural complexities in some regions. This test is limited in the types of variants that are detected and reported including nucleotide repeat expansion (with the exception of those noted in this document), and some structural variants. It is anticipated that approximately 97% of the coding region of an affected fetus' genome (i.e., the exome) will be assessed with this test at 15x coverage.

Genome sequencing for fetal anomalies is a phenotype-informed 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 these tests may identify the presence of a genetic variant in the genome 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 nuclear genome data is available upon request by the healthcare provider to incorporate updated clinical information and/or newly emerging gene and variant information. Updates to the classification of a sequence variant may be accessed through ClinVar (www.clinvar.com). Even if this 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.

Genes Screened for Repeat Expansions by GenomeDx™ Prenatal:

Reference Ranges of Repeat Expansions for Genes Evaluated by GenomeDx™ Prenatal						
Gene	Positive	Incomplete Penetrance	Premutation	Intermediate	Uncertain Significance	Normal
ARX (PA1)*	23 or greater				19-22	18 or less
ATN1**	48 or greater	36-47	21-35			20 or less
ATXN2**	35 or greater (AD)	33-34			31-32	30 or less
ATXN3**	60 or greater				45-59	44 or less
ATXN7**	37 or greater	34-36	28-33		20-27	19 or less
DMPK**	50 or greater		35-49			34 or less
FMRI*	201 or greater		55-200	45-54		44 or less
FXN*	66 or greater	44-65	34-43			33 or less
HOXD13*	22 or greater				16-21	9-15
PHOX2B**	26 or greater (AD)	24-25			21-33	20 or less

AD=autosomal dominant

*=typically congenital or childhood onset

**=age of onset can be in childhood or adulthood with the repeat length having limited predictive value on the age of onset

Not Applicable