Test Information

The benefits and risks of the IriSight® Comprehensive Analysis - Prenatal are explained below. It is recommended that you receive genetic counseling from a licensed and/or certified healthcare provider who can answer your questions about genetic testing and provide information about alternatives. Information about genetic counselors in your area is available at https://www.nsgc.org/.

Background

The IriSight® Comprehensive Analysis - Prenatal is a whole genome sequence based test designed to identify genetic variants that correlate with prenatal findings and/or are predicted to result in severe, early onset genetic disorders. This test includes sequence analysis (single nucleotide variants, deletions/insertions, characterized intronic variants); copy number variants, duplications/deletions, regions of homozygosity (ROH), uniparental disomy (UPD), mobile element insertions, selected inversions, unbalanced translocations, and aneuploidy; mitochondrial genome sequence analysis and large deletions; and short tandem repeat expansion analysis in select genes.

The IriSight® CNV Analysis includes copy number variants, duplications/deletions, unbalanced translocations, regions of homozygosity (ROH), possible uniparental disomy (UPD), mobile element insertions, inversions, and aneuploidy.

Additional information about IriSight® Comprehensive Analysis - Prenatal is available on the Variantyx website at https://www.variantyx.com/products-services/reproductive-genetics/irisight-prenatal-analysis/.

Test Process

The referring medical provider will coordinate collection of the fetal sample and the parental blood samples. Fetal DNA will be extracted and undergo quality control including determination of maternal cell contamination (MCC). If elected, the sample will also be processed for fluorescence in-situ hybridization (FISH) for common aneuploidies. If the sample is insufficient to perform all testing, then DNA extraction for sequencing, MCC, and cell cultures will be preferentially performed and FISH testing may be canceled.

IriSight® is offered as a “trio" test, which compares the fetus’s DNA sequence to its biological parents' DNA. Fetal sex is calculated from the sequencing data and displayed in the analysis report.

Variantyx is not responsible for specimen errors (e.g. labeling, extraction) for samples received that may have occurred prior to our receipt. For CVS samples, the quality of the micro-dissection of the villi will affect the MCC levels and overall test performance. Backup cell cultures are retained for two weeks. A report with the test results will be delivered to the referring clinician and it is their responsibility to provide post test genetic counseling and follow-up, if necessary. In certain cases, confirmatory testing may be required to verify the test results obtained, and may take several weeks to complete. Maternal cell contamination (MCC) will be determined and may influence the confidence of the results or the ability to proceed with testing. In the case of a sample failing the MCC evaluation but still passing the QC, testing will proceed but may lead to an inconclusive result. In the event a sample fails QC, testing will not proceed and an additional sample will be requested, extending the turnaround time.

Technical Limitations

A negative result from this analysis does not rule out the possibility that the tested individual carries a rare, unexamined pathogenic variant or a pathogenic variant in an undetectable region. All next generation sequencing (NGS) technologies, including whole genome sequencing analysis, may generate false positive and false negative results. Results are applicable to the tissue type used for this sequence test and may not reflect the variation in other tissue types. The minimum average on-target read depth is 30X. At the variant level, read depths fewer than 8X are not reported, which for any given test is approximately 0.5% of the reference genome (GRCh38). Each individual may have slightly different coverage yield distributions within the genome. While most structural variants are detectable, some genetic aberrations, such as gross genomic rearrangements or variants in portions of genes with highly homologous pseudogenes (including *HBA1/HBA2*), mosaicism (with the exception of full chromosomal mosaic aneuploidy), are identified with a lower efficiency. Deletions and duplications in the range of 50-300 base pairs are detected with a reduced sensitivity (0.19). For short tandem repeat expansions, due to possible somatic expansion in the tissue being tested and/or sampling bias, the median size of the expanded allele may not be representative of the actual event in the biologically relevant tissue. In addition, this test detects direct DNA sequence changes, and not indirect changes and aberrations, such as gene expression, epigenetic modifications, fusion, chromosome conformational changes, and other unknown abnormalities. This test may not detect variants in regions homologous to pseudogenes. Variants are not reported if they are not uniquely mappable, are of low coverage or are otherwise determined to be of low quality. Variantyx is not responsible for specimen errors (e.g. labeling, extraction) of samples received that may have occurred prior to our receipt. This test will not report variants related to infertility, carrier status of autosomal recessive disease, carrier status of X-linked recessive diseases, variants for late-onset conditions (including but not limited to neurological diseases, etc.) and variants associated with low penetrance diseases. Variants in 5’ or 3’ untranslated regions are typically not reported. This test will only report variants that correlate with prenatal findings and/or predicted to result in severe, early onset genetic disorders. Variants are not confirmed unless stated and confirmations are not included in published turnaround times. Additional testing may be recommended to assist in the clinical correlation of results. This test will report the sex of the fetus.

This test requires high-quality DNA. In some cases, an additional sample may be needed if the volume, quality and/or condition of the initial sample is not sufficient. Samples submitted as genomic DNA will only be processed if the extraction was performed in a CLIA/CAP accredited laboratory. Any additional test specific limitations are noted on the individual test information web page indicated. The test sensitivity, specificity, accuracy and PPV for different variant types are available upon request.

Reporting Limitations

This test will only report variants that correlate with prenatal findings, family history, and/or are predicted to result in severe, early onset genetic disorders. This test will not report variants related to infertility, carrier status of autosomal recessive disease, carrier status of X-linked recessive diseases, or variants that increase statistical risk for a disease, variants for late-onset conditions (including but not limited to neurological diseases, etc.) and variants associated with low penetrance diseases. Variant confirmations are not part of the test turn-around time. Additional testing may be recommended to assist in the clinical correlation of results.

If there are abnormal findings in the pregnancy, interpretation will be done with reference to the provided personal medical and family history, therefore, it is important to provide accurate and complete medical notes.

Parental samples are used as reference for the fetus' test interpretation only. Parental inheritance will be listed for variants reported in the fetus, but no specific reports are issued in the parent's name. Findings in parents alone will not be reported, and therefore this test is not intended to identify diseases or carrier status in parents. However, positive findings in the fetus may disclose parental genotype, or reveal a risk to a parent.

Due to the time sensitive nature of the test, in the event that a parent is not a biological parent, mosaicism is discovered in one of the parents, or one of the parent's DNA has low quality, the assay will be processed without the parent and the report will contain only positive or likely positive results (i.e. no variants of uncertain clinical significance).

Possible Test Results

This test will report genetic variants with evidence in the medical literature reported to be disease-causing, or that are computationally predicted to be disease-causing, and are classified as likely pathogenic or pathogenic in accordance with the ACMG (American College of Medical Genetics and Genomics) classification guidelines, in the genes and regions tested. Optionally, variants of uncertain clinical significance (VUS) may be reported with this test in cases of abnormal findings or medical history strongly correlated with the provided clinical symptoms of the fetus, the pregnancy and/or the family history.

Positive Result - A positive result indicates that one or more genetic variants were identified that either explain or partially explain the cause of the clinical symptoms or findings, or indicate an increased risk of developing a genetic disorder in the future. Individuals with positive results may wish to consider further independent testing and/or consultation with their physician or genetic counselor.
Negative Result - A negative result indicates that no genetic variant explaining the clinical symptoms or findings was identified by this test. This reduces the likelihood of, but does not exclude, a genetic cause.
Uncertain Result- A variant of uncertain significance was identified by this test. This means that a genetic variant was identified, but based on available information in the medical literature and research and scientific databases it is not certain whether the variant may cause the disorder. The variant could be a normal genetic difference that does not cause the disorder. Without further information, the effects of the variant cannot be known and an "uncertain/clinically inconclusive" result may be reported. The uncertainty may be resolved over time if additional information becomes available. Periodic reanalysis of the sequence data or further analysis, including testing of additional family members, may be recommended.
Indeterminate Result - An indeterminate result indicates that there were relevant genetic variant(s) identified in the analysis, but that it is uncertain whether they are true variants or artifacts. Furthermore, it is considered that a repeat test will not resolve the technical uncertainty and orthogonal confirmation is necessary to resolve the result.
Inconclusive Result - A technically inconclusive result indicates that there was an issue with the patient sample that resulted in data that the lab cannot interpret. Repeat testing may be recommended to resolve the technical uncertainty.

Reporting Standards

All reportable variants in the clinical report will be categorized as pathogenic, likely pathogenic or a variant of uncertain significance (if selected) utilizing the American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP) guidelines as published by Richards et al 2015 (for more information see: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4544753/). Variants may have a strong phenotypic correlation with the reported fetal phenotype(s) and be considered a strong causal candidate for the disorder or may have some phenotypic overlap with the reason for testing but not be considered the sole genetic cause for the phenotype(s) in the fetus. Both types of variants may be reported. Even if this test finds DNA changes that are responsible for the reported symptoms, the testing may not completely predict the severity of the disorder, possible future problems, or response to treatment. Interpretation of results is limited by the current medical understanding of disease and available scientific information. Variants may not be reported if they are not associated with a disease in the OMIM database.

Variants of uncertain significance (VUS) identified in genes correlated with the reported fetal phenotype and severe early onset disorders, and that meet specific reporting criteria, will only be reported if the ordering clinician opts in to receive these results. Reporting criteria for VUSs include:

A VUS in gene with strong clinical correlation to the reported abnormal ultrasound findings and/or the pregnancy, medical or family history, and is: *de novo* VUS associated with an autosomal dominant disorder, compound heterozygous variants (single VUS in trans with a known pathogenic/likely pathogenic variant) associated with an autosomal recessive disease, single VUS in an autosomal dominant disorder if correlated with reported fetal phenotype, structural variants > 1 Mb if a deletion or >2 Mb if a duplication.

A VUS in gene associated with highly penetrant early onset disease and is: *de novo* VUS associated with an autosomal dominant disorder, compound heterozygous variants (single VUS in trans with a known pathogenic/likely pathogenic variant) associated with an autosomal recessive disease, structural variants > 1 Mb if a deletion or >2 Mb if a duplication.

Pregnancies Without Indication

In pregnancies for which there are no abnormal ultrasound findings or other indications for testing, only pathogenic/likely pathogenic variants sufficient to cause disease are reported in the comprehensive analysis. VUS will not be reported for healthy pregnancies.

ACMG Secondary Findings

Patients for whom the IriSight® Comprehensive Analysis - Prenatal test is ordered have the choice to opt-in to ACMG Secondary Findings. The American College of Medical Genetics and Genomics (ACMG) recommends reporting pathogenic and likely pathogenic variants in a list of genes in both a gene-specific and variant-specific manner. Variantyx evaluates the secondary findings list of genes, the version of which will be listed in the report and can be found on the Variantyx website, www.variantyx.com/acmg-secondary-findings. These variants are not typically reviewed during routine processing of fetal samples, but are actively sought and reported to the patient. The ACMG recommends reviewing variants in the genes in their recommended list because the genes are related to conditions that are considered 'actionable', meaning that there are steps that can be taken to mitigate the onset or severity of the clinical outcome. It is important to understand that it is possible to have a pathogenic variant but to have it not detected by the assay. In addition, variants of uncertain significance are not reported in these genes. If a variant is of uncertain significance, and later is considered pathogenic, it cannot be determined without a reanalysis of the data.

The option to receive Secondary (ACMG) Findings is not available for the IriSight® CNV Analysis. In addition, this option is not available for relatives, with the exception of the reported parental inheritance of the variants identified in the fetus. No specific parental results are issued as a separate report under the family member's name.

Patient Confidentiality

To maintain confidentiality, test results will only be released to the ordering healthcare provider or ordering laboratory, and upon your request, to additional healthcare provider(s) indicated on this test requisition form. Test results will only be disclosed to others by your written consent and/or if demanded by a court of competent jurisdiction. It is your responsibility to consider the possible impact of test results on insurance rates, the ability to obtain disability, life or long-term care insurance and employment. The Genetic Information Non-discrimination Act (GINA), enacted by the US Federal Government, provides some protection against discrimination by health insurance companies and employers based on genetic test results, but does not cover life, disability or long-term care insurance. Information about GINA is available at https://www.genome.gov/10002328.

Anonymized information obtained from the test may be included in variant and allele frequency databases used to help healthcare providers and scientists understand human disease, as well as in scientific publications. Names and personal identifying information will not be revealed. Separate from the above, if there are opportunities to participate in research relevant to your condition, and you have consented for recontact, Variantyx may contact you or your healthcare provider for research purposes.

Turnaround Time

The turnaround time (TAT) of this test can be found on the Variantyx website, which begins at the time of sample receipt. If elected, the turnaround time for FISH testing is 3-5 days. For trio testing, the timing starts when the last sample is received. Please note that the following scenarios will likely result in extension of the turnaround time (1) in the event the direct fetal DNA sample does not pass QC or is determined to be insufficient for testing, requiring extraction from the cell cultures or collection of a new sample; (2) when the test is sent for orthogonal confirmation at an external laboratory. Additionally, turnaround time may rarely be extended beyond the published range for extenuating circumstances including, but not limited to, shipping delays, natural disasters, equipment outages, etc.

Sample Retention

DNA extracted from submitted samples may be stored for at least 3 months following completion of testing and may be discarded thereafter. Extracted DNA is not returned unless requested prior to testing (additional fees apply). After completion of testing, anonymized DNA may be used for test development and improvement, internal validation, quality assurance and training purposes before being discarded. Cultured cells may be maintained for up to two weeks following the completion of testing. During this limited time period, cultures may be made available to the clinician, upon written request, for confirmatory testing such as chromosome analysis and/or fluorescence *in-situ* hybridization (FISH).

New York state residents: No other test shall be performed on this sample except the test ordered by the clinician, unless waived by the patient or authorized individual. In addition, the patient's biological sample will be destroyed within 60 days or upon the completion of testing, unless waived by the patient or authorized individual. Orthogonal confirmation of results at a reference lab cannot be performed unless the patient or authorized individual provides permission to do so.

Single Nucleotide Variants

Genome-wide single nucleotide variants and small deletion/insertions (<50 bp) are reported if they are known pathogenic or likely pathogenic and sufficient to cause severe early onset disease. Variants of uncertain significance (if selected) may be reported if there is strong clinical correlation to the fetus' reported medical findings or family history.

Structural Variants

Structural variants are considered genome-wide and are reported if pathogenic or likely pathogenic and have clinical correlation with pregnancy loss, the reported phenotype or are predicted to result in severe early onset disease. If selected, variants of uncertain significance may be reported if there is strong clinical correlation to the reported medical findings or family history. Structural variants are not orthogonally confirmed. Parental inheritance will be reported for structural variants when both parents are available for testing.

Regions of homozygosity (ROH) and uniparental disomy (UPD) are detectable with this analysis. ROH for non-imprinted autosomal chromosomes and the X chromosome is reported for regions greater than or equal to 10 Mb. ROH is reported for regions greater than or equal to 5 Mb for imprinted chromosomes (6, 7, 11, 14, 15 and 20). Multiple regions of ROH can be indicative of shared common ancestry or consanguinity. Although the results of ROH are not interpreted, variants in genes associated with autosomal or x-linked recessive conditions related to the fetal phenotype or severe early onset disorders will be reported if detected. UPD is determined when testing is run as a trio analysis (i.e. both parental samples are available). When trio is not available in IriSight® CNV, ROH in imprinted regions will be reported as possible UPD. If relevant, additional testing may aid in diagnosis.

Short Tandem Repeats

Short tandem repeat expansions in the *AFF, AR, DMPK, FMR1, FOXL2, GLS, PHOX2B, SOX3, ZIC2* genes will be reported if the repeat is in a range associated with juvenile onset. Methylation status is not included in this analysis. Repeat expansions are reported without reference to interrupting repeat status. Parental inheritance will be identified for reportable repeat expansions, which may reveal a risk to a parent.

Mitochondrial Variants

Mitochondrial variants are reported in the mitochondrial genome if they are pathogenic or likely pathogenic, previously reported in the MITOMAP database, and homoplasmic. Homoplasmic large deletions are reported, however duplications are not. Heteroplasmy can be identified above 2%, however the significance of variants with low level heteroplasmy is uncertain.