

***Xpanded*[®] Congenital Heart Defects (CHD) Panel:** **A targeted test for monogenic causes of isolated and syndromic structural heart defects using a trio approach**

CLINICAL FEATURES

A congenital heart defect (CHD) is a structural anomaly of the heart that occurs during fetal development and is present at birth. CHD is the most common type of birth defect, occurring in approximately 1% of births. Different anatomic structures of the heart (chambers, valves, arteries, veins) can be involved and severity varies from mild to critical. While CHDs are often diagnosed early in life, some may not be identified until adulthood. CHD may be isolated (non-syndromic) or present with other congenital anomalies or extra-cardiac features (syndromic). Organ laterality defects, ranging from situs inversus to various forms of heterotaxy, are often seen in patients with CHD.

Due to the heterogeneous nature of CHD, it can be challenging to determine a specific diagnosis or predict the disease-causing gene based on clinical features alone. Therefore, it is often necessary to perform testing of multiple genes, either concurrently or as reflex tests, to identify the underlying genetic cause in an individual. Moreover, new genes associated with CHD are being discovered regularly, making it challenging for clinical laboratories to keep traditional testing panels updated. Lastly, interpretation of the clinical significance of variants in these newly discovered genes is often difficult in the absence of parental testing to clarify which variants are de novo or inherited.

The *Xpanded*[®] CHD Panel is based on whole exome capture, next-generation sequencing (NGS), and targeted analysis of a comprehensive list of genes currently reported in association with isolated and syndromic CHD. The design of the panel allows for a comprehensive, dynamic gene list that is updated regularly to ensure inclusion of genes recently associated with CHD. The *Xpanded*[®] CHD Panel uses a trio-based approach that includes concurrent analysis of the affected proband and both parents, which may increase the likelihood of identifying a definitive genetic explanation for the CHD. Depending on the family structure, family history, and the availability of both parents, other family members of the affected individual may be evaluated in conjunction with the proband. Please contact GeneDx for prior approval when both parents are not available to submit samples for the *Xpanded*[®] CHD Panel.

GENETICS

The etiology of CHD is complex, including multiple genetic, epigenetic, and environmental factors. Known causes of CHD include chromosomal abnormalities, single gene disorders, environmental exposures, and teratogens. While the cause for the largest majority of CHDs remains unknown, approximately 30% of individuals with CHD have been reported to have an underlying genetic etiology.¹ Nearly 25% of CHD cases have been associated with aneuploidy or copy number variants and 3–5% with single gene disorder.^{1,2} The inheritance patterns can be autosomal dominant, recessive, or X-linked. In some cases, confirmation of the molecular genetic cause of CHD may have implications for surveillance for associated complications or other organ systems involvement and recurrence in the family.

TEST METHODS

Genomic DNA is extracted from the submitted specimen. For skin punch biopsies, fibroblasts are cultured, or used directly, for DNA extraction. The DNA is enriched for the complete coding regions and splice junctions of most genes of the human genome using a proprietary capture system developed by GeneDx for next-generation sequencing with CNV calling (NGS-CNV). 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. Smaller deletions or duplications may not be reliably identified. Reported variants are confirmed, if necessary, by an appropriate orthogonal method in the proband and, if submitted, in selected relatives. 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. Reportable variants include pathogenic variants and likely pathogenic variants. Variants of uncertain significance, likely benign and benign variants, if present, are not routinely reported. A list of additional variants not included in this report is available upon request.

Please note that while the *Xpanded*[®] CHD Panel captures and sequences the whole exome, analysis is targeted to the specific phenotype-driven gene list for this panel. The *Xpanded*[®] CHD Panel gene list includes more than 350 genes. The list was developed by searching for genes associated with isolated and syndromic CHD in multiple sources, including OMIM, HGMD, and Human Phenotype Ontology (HPO) terms. This list undergoes continual review and curation by GeneDx experts and is regularly updated/improved using evidence from the literature and from GeneDx data. Specifically, genes are added to the list using GeneDx data from clinical exome sequencing (ES) done on patients with CHD. Additionally, genes may be removed from the panel if they are found to be weakly or questionably associated with CHD. In rare situations, genes are removed from the panel if they are expected to be low yield for this phenotype but contain an inherent high risk for incidental findings.

RESULT REPORTING

The *Xpanded*[®] CHD Panel is performed on the proband and the parental samples (and/or additional relatives) when submitted together for analysis. A single report will be issued on the affected proband in the family. A separate report will not be issued for unaffected parents or other family members who may also have submitted a specimen for the purpose of allowing better interpretation of the results from the affected individual. If additional reports are requested for other affected family members, extra fees will apply.

The report that is issued for the affected individual will include reportable variants in genes that have been previously associated with CHD in the published or emerging literature. Pathogenic/likely pathogenic variants in genes responsible for the phenotype of the patient will be reported; however, because this is a phenotype-driven test of a large number of genes, variants of uncertain significance (VUS) are not routinely reported, only at our discretion. Variants that are considered to be benign or likely benign will not be reported. As the *Xpanded*[®] CHD Panel includes 350+ genes, the report will not include a comprehensive list of all observed variants. If desired, clinicians can request a separate file containing a list of identified variants that will be provided upon completion of testing.

In rare instances, this test may reveal a pathogenic variant that is not directly related to the test indication. For example, pathogenic variants in genes associated with an increased risk for cancer or metabolic disease could be identified. In the event that an incidental finding is identified, this information will be disclosed to the ordering health care provider if it is likely to impact medical care.³ The absence of reportable incidental findings for any particular gene does not rule out the possibility of pathogenic variants in that gene.

TEST SENSITIVITY

The clinical sensitivity of the *Xpanded*[®] CHD Panel depends in part on the patient's clinical phenotype. It has been demonstrated that the yield of ES testing is higher with a Trio approach compared to a Proband-only approach.⁴ The sensitivity of this test is expected to be comparable to trio-based exome sequencing since it uses a trio approach to test a comprehensive list of genes previously associated with CHD. The clinical sensitivity is expected to be significantly lower for singleton testing when only the affected proband is tested.

The average coverage of all genes on the panel is greater than 99% at 10X (with a depth of 10 or more reads). Some genes with a relatively high clinical sensitivity have an average coverage of less than 90% at 10X, including *CDKN1C*, *CFC1*, *HYDIN*, *RPL15* and *TTC25*. Note that these numbers represent the average coverage for the genes on the panel, derived by combining data from a large number of patients. The coverage of each gene on the panel for a specific patient may vary, and the actual coverage for each gene is included in the final report.

LIMITATIONS

Some types of genetic disorders, such as those due to nucleotide repeat expansion/contraction, abnormal DNA methylation, large-scale chromosomal aneuploidies, and other mechanisms may not be detectable with this test. Additionally, small segments of a few individual genes have inherent sequence properties that yield suboptimal data and variants in those regions may not be reliably detected. For example, variants derived from genes such as *CFC1*, *HYDIN* and *RPL* may not be detectable, due to the presence of highly homologous sequences in the human genome. This test only analyzes genes included on the gene list; therefore, the ability to detect large contiguous gene deletions/duplications is limited.

The scientific knowledge available about the function of all genes in the human genome is incomplete at this time. It is possible that the *Xpanded*[®] CHD Panel may identify the presence of a variant in the sequence of an affected individual, but that we will not recognize it as the cause of their disease due to insufficient knowledge about the gene and its function. Even if the *Xpanded*[®] CHD Panel identifies the underlying genetic cause of a disorder in an affected individual, 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.

References

1. Zaidi et al. (2017) *Circ Res.* 2017 Mar 17;120(6):923–940 (PMID: 28302740)
2. Pierpont et al. (2018) *Circulation* 138 (21):e653–e711 (PMID: 30571578)
3. Kalia et al. (2017) *Genet. Med.* 19 (2):249–255 (PMID: 2785436)
4. Retterer et al. (2015) *Genet. Med.*: (PMID: 26633542)