Cardiology Genetics: Heterotaxy Panel

Disorders included: Conditions included in this gene panel involve congenital abnormalities of organ orientation known as heterotaxy, as well as cardiovascular malformations associated with lateralization defects.

Panel Gene List: ZIC3, ACVR2B, LEFTY2, CFC1, NODAL, GDF1, CRELD1, GJA1, NKX2-5, FOXH1

Clinical Features:
Heterotaxy is a clinically and genetically heterogeneous disorder which may include organ isomerism, failure of internal organs to lateralize or failure of paired organ primordia to regress. Situs inversus is the mirror image of normal organ arrangement, while situs ambiguous is any abnormal arrangement of abdominal organs. Also included in the clinical spectrum of heterotaxy are cardiac defects including atrioventricular septal defect (AVSD), common atrial septal defects (ASD), malposition or transposition of the great arteries (TGA), Tetralogy of Fallot (TOF), dextrocardia, and patent ductus arteriosus (PDA). This class of cardiac defects accounts for 3% of all congenital heart disease. Severity of these conditions ranges from asymptomatic to life-threatening. Prevalence is estimated to be 1/10,000 live births with a 2:1 ratio of affected males to females based on a study with strict inclusion criteria for heterotaxy.

Genetics:
Most cases of heterotaxy are sporadic, however familial cases usually display an autosomal recessive inheritance pattern whereby the parents of an affected individual are obligate heterozygotes and there is a 25% chance of transmitting the condition with every pregnancy. X-linked inheritance occurs in ZIC3, whereby male offspring have a 50% chance of being affected, and female offspring have a 50% chance of being a carrier. Autosomal dominant inheritance patterns have been seen in a few families where linkage to a chromosome region has been established but the associated gene is unknown. Heterotaxy is associated with variants in at least 10 genes, most of which encode proteins involved in embryonic left-right patterning. ZIC3 (Zinc family member 3) variants result in abnormal arrangement of visceral organs, asplenia and/or polysplenia, and various congenital cardiac anomalies. The ZIC3 protein product probably functions as a transcription factor in early stages of left-right axis formation. ZIC3 variants have also been identified in females with cardiovascular malformations. At least 75% of familial X-linked heterotaxy and 1% of sporadic cases are due to variants in ZIC3. ACVR2B (Activin receptor type 2B), LEFTY2 (left right determination factor 2), and CFC1 (Cryptic), NODAL, and GDF1 are members of a transforming growth factor-β signal transduction pathway critical for establishing left-right asymmetry during early development.
Variants in these genes have been shown to be associated with congenital cardiac anomalies and/or situs ambiguous.\textsuperscript{3, 5-12} Variants in ACVR2B and LEFTY2 are each responsible for approximately 2\% of heterotaxy cases, while CFC1 variants are responsible for 6-21\%\textsuperscript{5-9}. Variants in NODAL are detected in 5-10\% and variants in GDF1 are detected in approximately 2\% of heterotaxy cases.\textsuperscript{10-12}

\textit{CRELD1} (Cysteine-rich with EGF-like domains 1) variants are associated with AVSD and heterotaxy+AVSD. \textit{CRELD1} mediates interactions between proteins of diverse function; however it is unknown how \textit{CRELD1} is involved in embryonic lateralization. Two variants (T311I, R329C) have been described in one individual each with partial AVSD, and one variant (R107H) was reported in a single individual with partial AVSD+heterotaxy. \textsuperscript{3, 13}

\textit{GJA1} (Gap junction protein, alpha 1, aka Connexin 43) is involved in numerous other conditions in addition to cardiac heterotaxy: oculodentodigital dysplasia (ODDD), deafness, glaucoma, ectodermal & ODDD with skin hyperkeratosis, and Hallermann-Streiff/ODDD syndrome. \textsuperscript{3, 14-16} The encoded protein is the major protein of gap junctions in the heart that are thought to have a crucial role in the synchronized contraction of the heart and in embryonic development. The detection rate for variants in \textit{GJA1} in individuals with heterotaxy is currently unknown.

\textit{NKX2-5} (NK2 transcription factor homeobox 5, aka cardiac specific homeobox) variants are associated with cardiac disease, atrial septal defect (ASD), TOF, and VSD. \textit{NKX2-5} is thought to be involved in cardiac specific spatial development during embryogenesis. \textsuperscript{7, 11, 17}

\textit{FOXH1} (Forkhead box 1) is associated with congenital heart defects. \textit{FOXH1} encodes a forkhead transcription factor. The forkhead protein domain binds DNA and the C-terminal domain interacts with phosphorylated Smad proteins to mediate TGFbeta signaling. The detection rate for variants in \textit{NKX2.5} and \textit{FOXH1} in individuals with heterotaxy is unknown at present.

\textbf{Test Methods:}

Using genomic DNA from the submitted biological material, the entire coding regions of 10 genes (\textit{ZIC3, ACVR2B, LEFTY2, CFC1, NODAL, CRELD1, GJA1, NKX2-5, FOXH1, and GDF1}) and their splice junctions are sequenced using a novel solid-state sequencing-by-synthesis process that allows sequencing a large number of amplicons in parallel (aka. Next Generation Sequencing, Massive Parallel Sequencing) (18). For analysis, DNA sequence is assembled and compared to the published genomic reference sequences. The presence of any potentially disease-associated sequence variant(s) is confirmed by conventional dideoxy DNA sequence analysis. A reference library of up to 800 alleles is used to evaluate the frequency of novel sequence variants if indicated. If appropriate, testing of one affected relative
or, if not available, of both biological parents, is performed to clarify variants of unknown significance at no additional charge.

**Test Sensitivity:**
It is not currently known what percentage of individuals have a pathogenic variant in the 10 genes tested in this panel. Variants in ZIC3 account for approximately 75% of familial cases of X-linked heterotaxy, and 1% of sporadic cases. Disease-causing variants in the remaining 9 genes may be responsible for up to 39% of cases of heterotaxy (3). The technical sensitivity of this testing approach is estimated to be 98%. This sequencing test will not detect large chromosomal aberrations and deletions, insertions, or rearrangements greater than or equal to 5 base pairs.

**References:**
4. Bedard et al., (2011) PLOS one. 6(8):e23755