50 Genetic Testing

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Individuals cared for in the Heart Center from fetal life through adulthood may have heart disease arising secondary to an underlying genetic disorder. Identification of genotype has the potential to transform how we care for cardiac patients by defining optimal surveillance and therapeutic strategies, and improved accuracy in prognostication and evaluation of recurrence risk. Currently, there are clear guidelines outlining the role of genetic testing in cardiomyopathy, inherited arrhythmias, and aortopathy. Genetic testing is also indicated in those with multiple congenital anomalies and intellectual disability, and is increasingly being offered to patients with apparently isolated structural heart disease. Broadly speaking, approximately 20-30% of individuals with CHD have a syndromic phenotype, although additional features and extracardiac manifestations may not initially be apparent in the neonatal period. Accurate clinical assessment and meaningful interpretation of genetic testing results is dependent upon accurate cardiac phenotyping, identification of extracardiac manifestations and dysmorphic features, and family history.

Genetic Test Overview

Chromosomal Microarray (CMA)

CMA is a technology that compares patient DNA to reference DNA to detect copy number variations, i.e., deletions and duplications. There are different types of CMA studies, each with specific advantages and limitations. For most indications, a "Comprehensive CMA" is ordered, which includes a combination of aCGH (array comparative genomic hybridization) and SNP (single nucleotide polymorphism) techniques to assess the whole genome.

Turnaround time: 2 weeks

Best for:

- When a microdeletion or microduplication disorder is clinically suspected
- Multiple congenital anomalies
- Intellectual disability
- Autism spectrum disorders

CMA is now routinely offered for syndromic and apparently nonsyndromic CHD (although yield in the latter is lower and has not been fully delineated). Comprehensive CMA will detect aneuploidy, unbalanced translocations, regions of absence of heterozygosity indicating consanguinity or uniparental disomy, and triploidy. Mosaicism can be detected depending on the fraction of abnormal cells in the blood. Very low level mosaicism can be missed by this study.

If the comprehensive CMA is *negative*, microdeletion and microduplication syndromes are excluded. Consideration should be given to single-gene disorders, imprinting disorders, and trinucleotide-repeat conditions (e.g., Fragile X, congenital myotonic dystrophy).

Fluoresecent In-Situ Hybridization (FISH) and Multiplex Ligation Probe-Dependent Analysis (MLPA)

FISH and MLPA are targeted tests to detect copy-number variations at specific regions. These tests have been largely replaced by CMA as the first-line test. The exception to this is the use of rapid FISH for trisomy 13, 18, 21, and sex chromosomes in a newborn/unstable patient in whom identification of one of these diagnoses may affect urgent management.

Turnaround time: Rapid FISH 2-3 days

Chromosomal Karyotype

Chromosomal Karyotype is visual inspection of the chromosomes to assess number, large structural abnormalities, and banding patterns.

Turnaround time: 3 weeks

Best for:

- Aneuploidy
- Large translocations, balanced translocations
- Complex chromosomal rearrangements
- Mosaicism

Karyotype is no longer recommended as the first-line test for multiple congenital anomalies. If the karyotype is *negative*, aneuploidy and large translocations are excluded. Consider submicroscopic copy-number variations and single-gene disorders.

Single-Gene Sequencing

Single-gene sequencing determines the nucleotide sequence of individual genes to assess for mutations causing single-gene disorders.

Turnaround time: 3-4 weeks

Best for:

- When a single-gene disorder with a single underlying gene is suspected
- Laboratory confirmation of mutations identified through alternate sequencing modalities (whole-exome sequencing, panel testing)

If single-gene sequencing is *negative*, a mutation in that gene is excluded, however small deletions or duplications within the gene may not be detected. If gene sequencing with deletion/duplication testing is negative, then consider other genes, copy-number variations and chromosomal rearrangements.

Whole-Exome Sequencing (WES)

WES is a large-scale (next-generation) sequencing technology to simultaneously determine the nucleotide sequences of all the exons (protein-coding regions) of all the genes (the whole exome). Analysis of WES data relies heavily on the clinical information provided in order to focus the report to relevant findings. Detection of incidental findings and variants of uncertain significance require significant pretest and posttest counseling with the family.

Turnaround time: 3-4 months

Best for:

- Assessment of multiple single-gene disorders simultaneously in the setting of complex phenotypes or numerous discordant phenotypes

Table 50-1. Recommended genetic testing by lesion.

Left-sided lesions	
HLHS	Male: CMA Female: CMA and rapid FISH for sex chromosomes to screen for Turner syndrome (if outside the newborn period or non-urgent, do only CMA) If CMA negative, with any of the following: renal anomalies, hypotonia, ear anomalies: consider Kabuki panel (may also do WES)
Coarctation of the aorta, moderate-to-severe aortic stenosis	CMA If CMA negative, with any of the following: renal anomalies, hypotonia, ear anomalies: consider Kabuki panel (may also do WES)
BAV in female with any of the following: coarctation, PAPVR, LSVC, absent ductus arteriosus, cystic hygroma, lymphedema, shield chest, short stature, webbed neck	Karyotype
Supravalvar aortic stenosis	CMA If CMA negative, elastin gene (*ELN*) sequencing with deletion/duplication testing
Right-sided lesions	
Pulmonary stenosis with any of the following: hypertrophic cardiomyopathy, conduction abnormalities, fetal chylous effusions, short stature, webbed neck, shield chest, developmental delay, cryptorchidism, dysmorphic features	Noonan panel
Conotruncal defects	
TOF (PS or PA-VSD)	CMA If seen with bile duct paucity, cholestasis, butterfly vertebrae, ocular anomalies, growth delay, hearing loss, horseshoe kidney: also do *JAG1* and *NOTCH2* gene sequencing (or CHD panel that includes these)
Truncus arteriosus, DORV, IAA with VSD	CMA
Right aortic arch (even if isolated)	CMA

Aortic disease	
Aortic root dilation without CHD and features concerning for Marfan-like disorder	Aortopathy panel
Ascending aortic dilation with lung disease and /or pulmonary hypertension	Aortopathy panel
Others	
AVSD	Features suggestive of T21: karyotype (unless diagnosis critical, then rapid FISH for chromosome 21) No concerning facial features, or features not consistent with T21: CMA (will also detect T21) With short stature, conduction abnormalities, hypertrophic cardiomyopathy, webbed neck, shield chest, developmental delay, cryptorchidism, abnormal facies: consider Noonan panel
Heterotaxy	CMA and heterotaxy panel
All other CHD with the exception of isolated muscular VSDs, nonsyndromic isolated ASDs, isolated BAV, isolated LSVC	CMA

ASD: atrial septal defect, AVSD: atrioventricular septal defect, BAV: bicuspid aortic valve, CHD: congenital heart disease, CMA: chromosomal microarray, DORV: double-outlet right ventricle, FISH: fluorescent in-situ hybridization, HLHS: hypoplastic left heart syndrome, IAA: interrupted aortic arch, LSVC: left superior vena cava, PA: pulmonary atresia, PAPVR: partial anomalous pulmonary venous return, PS: pulmonary stenosis, T21: trisomy 21, TOF: tetralogy of Fallot, VSD: ventricular septal defect, WES: whole-exome sequencing.

- Identification of new genes causing inherited disorders

If WES is *negative*, then pathogenic mutations in protein-coding regions of genes related to the specific phenotype reported on the test requisition are not detected. However, single-gene disorders cannot be completely excluded as there are numerous technological limitations and as of yet undiscovered genes. Copy number variations and aneuploidy are also not completely excluded. Data from WES can be periodically reinterpreted as the patient's phenotype evolves and knowledge surrounding genetic disorders continues to expand.

Next-Generation Sequencing Panels

These studies are an adaptation of WES technology to simultaneously determine the nucleotide sequence of a specific set of genes.

Turnaround time: 2 weeks to 2 months (lab dependent)

Best for:

- Suspected Mendelian disorder known to be caused by multiple genes (e.g., Noonan syndrome, Loeys-Dietz syndrome)

If a panel is **negative**, the disorder tested for may not be completely excluded

(technological limitations, other genes implicated that are not on the panel). Consider also other single-gene disorders, copy-number variants, large chromosomal rearrangements.

Test Selection Considerations

1. **What diagnosis is suspected?**
   - Consider the cardiac phenotype, extracardiac phenotype, dysmorphic features and family history. Genetic-test interpretation relies heavily on accurate phenotyping.
   - Select the best test for the suspected diagnosis. The exception is use of rapid-FISH for trisomy 13,18, 21, and sex chromosomes in order to rapidly determine an aneuploidy diagnosis in a critically ill/newborn patient when the diagnosis may affect prognosis and/or management.
   - If a specific diagnosis is not clinically suspected, CMA is the first-line test for multiple congenital anomalies/dysmorphic features, intellectual disability, autism spectrum disorder, and increasingly for syndromic and nonsyndromic heart disease.
2. **What is the test yield?**
   - What percentage of patients with the phenotype will have a positive test?
   - Consider pretest probability. There is a higher yield with extreme phenotypes. If multiple family members are affected, the individual with the most severe phenotype should be tested as the proband.
3. **Test interpretation**
   - What does a positive, negative, or uncertain test result mean?
   - What are the technical limitations of the test?
4. **What are the implications of a positive or negative test for the family?**
   - Can the disorder be diagnosed or excluded with certainty?
   - Cascade screening in family members, inheritance, recurrence risk, and surveillance
   - Risk of detecting variants of uncertain significance and incidental findings
5. **Follow-up and counseling**
   - Pretest counseling and consent for genetic testing must be performed
   - All genetic tests require insurance preapproval in the outpatient setting but this may not be required in the inpatient setting. Not all tests are covered by all insurance plans. This can result in a large out-of-pocket cost to families.
   - Testing results may be returned weeks or months after the testing is performed and frequently after the patient has been discharged from the hospital. It is essential that results are obtained, reviewed, and communicated to the family.

Genetic Testing Algorithms

Positive Fetal Genetic Testing

Biomarker screening (i.e., maternal serum screening, integrated prenatal screening) is performed routinely for most pregnancies. A "screen positive" result is given when

Table 50-2. Recommended genetic testing by suspected diagnosis.

Suspected diagnosis	Testing
Down syndrome	Karyotype If the diagnosis is critical in the newborn period, rapid FISH for chromosome 21 Will also be detected by CMA, but won't identify rearrangement, low-level mosaicism
Trisomy 13, 18	Newborn rapid FISH for chromosome 13, 18 and karyotype Will also be detected by CMA, but won't identify rearrangement, low-level mosaicism
Turner syndrome	With HLHS: rapid FISH for sex chromosomes If strongly suspect or to confirm diagnosis: karyotype with FISH for Y centromere Will also be detected by CMA, but won't identify rearrangement, low-level mosaicism
Williams syndrome	Best: CMA Will also be detected with FISH for Williams region If CMA negative or if family history of SVAS, strongly consider elastin gene (*ELN*) sequencing with deletion/duplication testing
22q11.2 deletion syndrome (DiGeorge syndrome, velocardiofacial syndrome)	Best: CMA Will also be detected with FISH for DiGeorge (usually includes chromosomes 22 and 10)
Noonan syndrome	Best: Noonan syndrome panel (adding deletion/duplication will diagnose an additional 5%) Will also be detected on WES
Marfan syndrome	Best: Aortopathy panel Will also be detected on WES
Loeys-Dietz syndrome	Best: Aortopathy panel Will also be detected on WES
Holt-Oram syndrome	TBX5 gene sequencing Will also be detected on WES and most CHD gene panels
Ehlers-Danlos syndrome	
- Hypermobile type (type 5)	There is no current diagnostic test (but there are clinical diagnostic criteria). If personal history of easy bruising/bleeding, or abnormal skin (atrophic scars, poor wound healing, highly elastic), or family history of vascular/organ rupture, must exclude other types of EDS with EDS or aortopathy panel
- Vascular EDS (type 4)	Best: EDS panel (unless there is root dilation, then do aortopathy panel) Will also be detected on aortopathy panel or WES
- Classical EDS/Classical-like EDS/Cardiac-valvular EDS (types 1-3)	Best: EDS panel (unless there is root dilation, then do aortopathy panel) Will also be detected on aortopathy panel or WES
CHARGE syndrome	Best: CHD7 sequencing with deletion/duplication Will also be detected on WES

CHD: congenital heart disease, CMA: chromosomal microarray, EDS: Ehlers-Danlos syndrome, FISH: fluorescent in-situ hybridization, HLHS: hypoplastic left heart syndrome, SVAS: supravalvar aortic stenosis, WES: whole-exome sequencing.

the risk of aneuploidy based on biomarker testing is higher than maternal age-related risk. This is not a specific genetic test and does not yield a specific diagnosis. Biomarker screen results should not influence the decision to evaluate and perform genetic testing postnatally.

Non-Invasive Prenatal Testing (NIPT) is analysis of cell-free fetal DNA in the maternal blood stream to assess for aneuploidy (most commonly 13, 18, 21, and sex chromosomes). Some companies also offer testing for select microdeletion/duplications; however this technology has yet to be validated in population studies. NIPT is a screening test for specific aneuploidy conditions and requires confirmation with a diagnostic test (i.e., via amniocentesis or postnatal testing):

- **Positive NIPT without prenatal confirmatory testing:** complete evaluation and postnatal confirmatory testing of the NIPT screen-positive result
- **Negative NIPT:** Should not influence a postnatal decision to perform genetic testing when clinically indicated

If amniocentesis or chorionic villus sampling (CVS) was performed to obtain a sample for genetic testing, then genetic counseling, karyotyping, CMA and/or FISH may have been performed. The reports detailing the type of testing performed and the results should be obtained and scanned to the patient's medical record.

- **Positive genetic test result (karyotype, CMA, FISH) on sample obtained by amniocentesis/CVS:** Consult genetics to determine if the patient's phenotype is consistent with the prenatal diagnosis and if confirmatory/additional testing is required
- **Negative genetic test result on sample obtained by amniocentesis/CVS and genetic disorder is suspected:** Confirm what type of testing was performed (e.g., CMA by amniocentesis will not rule out Noonan syndrome). Consult genetics for additional evaluation and testing.

CHD and Aortopathy

Table 50-1 lists the recommended genetic testing by CHD lesion. Table 50-2 lists the recommended genetic testing by suspected diagnosis.

Cardiomyopathy

Genetic testing in cardiomyopathy is recommended for patients who have undergone a comprehensive evaluation (including clinical history, detailed 3-generation family history, examination, ECG, echocardiography) and have been given a clinical diagnosis of cardiomyopathy. Testing is not recommended for individuals with non-diagnostic clinical features (e.g., athlete's heart). Accurate genetic test interpretation relies upon accurate clinical phenotyping, therefore evaluation of a patient with cardiomyopathy must include consideration of:

- Syndromic features (dysmorphic features, multisystem organ involvement, developmental delay, developmental regression)
- Arrhythmia and conduction system involvement
- Skeletal myopathy
- Family history and inheritance pattern

Table 50-3. Considerations and genetic testing in patients with cardiomyopathies.

Cardiomyopathy	Considerations	Testing
Hypertrophic cardiomyopathy (HCM)	Highest yield of all cardiomyopathies; highest in familial HCM	Comprehensive or HCM-targeted panel testing Also consider Rasopathy panel if features of Noonan-spectrum disorders are present
Dilated cardiomyopathy (DCM)	Highest yield in those with DCM and conduction system disease, family history of sudden death and familial DCM	Comprehensive or dilated-targeted panel Also consider muscular dystrophy, metabolic cardiomyopathy, mitochondrial disorders
Restrictive cardiomyopathy	Numerous modes of inheritance, rare diagnosis	Comprehensive panel testing Also consider skeletal myopathy, storage disorders, Noonan-spectrum disorders
Arrhythmogenic cardiomyopathy (ARC)	Numerous modes of inheritance and complex genetics (e.g., compound heterozygosity) make test interpretation challenging High incidence of false positives is suspected	Comprehensive or ARC-targeted panel testing may be recommended but should be undertaken by Cardiomyopathy Team/Electrophysiology Team
LV non-compaction cardiomyopathy	Numerous modes of inheritance, numerous implicated genes, broad phenotypic spectrum, and considerable overlap with other forms of cardiomyopathy Lower yield than for other forms of cardiomyopathy	Comprehensive or LVNC-targeted panel testing may be recommended but should be undertaken by Cardiomyopathy Team/Electrophysiology team Also consider Barth syndrome, muscular dystrophy, mitochondrial disease, ARC

ARC: arrhythmogenic cardiomyopathy, DCM: dilated cardiomtyopathy, HCM: hypertrophic cardiomyopathy, LVNC: left-ventricular non-compaction.

Evaluation and genetic testing should be guided by the Cardiomyopathy Team. The role of genetic testing in cardiomyopathy includes:

- Identification of a specific disorder with altered management:
  - Hypertrophic cardiomyopathy in Fabry disease, Danon disease
  - Dilated cardiomyopathy in muscular dystrophy
  - Metabolic cardiomyopathy for which enzyme-replacement is available
- Permits mutation-specific testing of family members who might otherwise require long-term clinical surveillance

Next Generation Comprehensive Cardiomyopathy Panel Testing ("Pancardiomyopathy Panel") is a panel that encompasses genes known to cause hypertrophic, dilated, restrictive, non-compaction, and arrhythmogenic cardiomyopathy. It is often used due to the overlap in clinical cardiomyopathy phenotypes within families and evolution of cardiac phenotype over the lifespan. This approach also helps identify specific genetic

disorders with altered management (e.g., identification of Fabry disease in hypertrophic cardiomyopathy or muscular dystrophy in dilated cardiomyopathy). However, some data suggest that pancardiomyopathy testing is not more effective than phenotype-targeted testing and may increase the risk of detecting a variant of uncertain significance. Additionally, this approach will not necessarily identify the cause of cardiomyopathy associated with syndromic disorders (e.g., Noonan syndrome), skeletal myopathies, or metabolic and mitochondrial disorders. Therefore, a thorough and thoughtful approach to test selection must be employed.

Table 50-3 lists the recommended genetic testing and important considerations for patients with cardiomyopathies. For details on diagnosis and management of the different cardiomyopathies, see Chapter 32.

Inherited Arrhythmias

Details on genetic testing for inherited arrhythmia syndromes and workup of cardiac arrest can be found in Chapter 35.