Malformations of the Left Ventricle
What Comes First: Form or Function?

Sabine Klaassen, MD

Both left ventricular (LV) noncompaction of the myocardium (LVNC) and hypoplastic left heart (HLH) are thought to be congenital developmental defects of the LV musculature. Mutations in genes encoding sarcomere proteins have been identified in a significant proportion of LVNC patients,1,2 as well as in association with congenital heart defects (CHDs).3-5 The presence of sarcomere mutations suggest an underlying cohesiveness of cardiomypathy and structural CHDs, supporting the essential role for normal sarcomere function during cardiac development. In this issue of Circulation Cardiovascular Genetics, 2 papers6,7 report on the results of whole exome sequencing and whole genome sequencing (WGS) in patients with LVNC and HLH, respectively. By next-generation sequencing of multiple affected and unaffected family members, they demonstrate (1) that heterozygous MYH7 mutations are common in LVNC and identify NNT, encoding a nuclear-encoded mitochondrial protein, as a novel LVNC gene (2) compound heterozygosity for recessive MYH6 mutations in patients with HLH and reduced right ventricular ejection fraction.

Left Ventricle Noncompaction Cardiomyopathy

LVNC has been recognized as a distinct primary cardiomyopathy with a genetic pathogenesis by the American Heart Association and is still considered an unclassified cardiomyopathy according to the European Society of Cardiology. LVNC is characterized by a unique myocardial morphology: hypertrophic segments that consist of a thin compacted epicardial layer and a thick noncompacted endocardial layer which may be hypokinetic. The noncompacted layer contains numerous prominent trabeculations and deep intertrabecular recesses.8 During heart development, the myocardium is initially trabeculated during a period before coronary artery development. Between embryonic weeks 5 and 8, the trabeculae regress as the compact myocardium develops from base to apex. Therefore, LVNC is considered to be an abnormality reflecting arrested early cardiac morphogenesis.9 LVNC may be an isolated finding in the absence of any coexisting cardiac anomaly (isolated LVNC) or may be associated with other congenital heart anomalies, such as complex congenital heart disease (nonisolated LVNC). Variable clinical features include both asymptomatic and symptomatic patients with the triad of congestive heart failure, thrombembolic events, and arrhythmias, including sudden cardiac death. Based on the variation in clinical assessment of family members, familial cases were found with a frequency between 18% and 64%.10 Syndromal patients with facial dysmorphism and developmental retardation associated with isolated LVNC have first been described by Chin et al in 1990.11 LVNC has been associated with neuromuscular disorders, such as dystrophinopathies, and with mitochondrial disease. TAZ was the first gene shown to be associated with isolated LVNC.12 TAZ encodes for Taffazin, a protein involved in the biosynthesis of cardiolipin, an essential component of the inner mitochondrial membrane. Mutations in genes encoding sarcomere proteins were identified in about one third of LVNC patients. Therefore, LVNC has been put into the context of other cardiomyopathies, including hypertrophic cardiomyopathy and dilated cardiomyopathy.

In the paper by Bainbridge et al,8 whole exome sequencing from 5 families with LVNC provided causative mutations in sarcomere genes in 4/5. Subsequent sequencing of MYH7 in a larger LVNC cohort identified mutations in 18%, confirming MYH7 mutations as the single most common cause of LVNC. Interestingly, 50% of the discovered MYH7 mutations have been reported previously in the European population. In the fifth family, they identified a frameshift mutation in NNT, encoding nicotinamide nucleotide transhydrogenase, a nuclear-encoded mitochondrial protein, not implicated previously in human cardiomyopathies. Multiple lines of evidence from in vivo functional testing in zebrafish indicate that nnt suppression results in malformation of the ventricle, likely resulting in contractility defects and bradycardia. Their data also suggest that altered cell proliferation contributes to the development of this malformation. This confirms what has been seen in a previous study by Arndt et al13 in zebrafish modeling of both PRDM16 haploinsufficiency and a human truncation mutant leading to LVNC. Interestingly, PRDM16 has a dominant positive effect on cardiomyocyte proliferation in zebrafish, where either activated or repressed PRDM16 levels impair cardiomyocyte proliferation. Whether impaired proliferative capacity links ventricular dysfunction in LVNC because of NNT mutation causing deficient mitochondrial respiration and PRDM16 mutation remains to be established.
Hypoplastic Left Heart

In 1958, Noonan and Nadas published a series of 101 patients with hypoplasia of the left heart structures and proposed the term HLH syndrome. Currently, HLH syndrome/HLH is defined as hypoplasia of the LV chamber, stenosis or atresia of the mitral and aortic valves, and hypoplasia of the aorta. Remarkable progress has been made in the clinical care of HLH, but the genetic pathogenesis of the disorder is largely unresolved. HLH has one of the strongest genetic components of any CHD. In particular, there is a strong association with other LV outflow tract obstructive lesions, including bicuspid aortic valve. Cytogenetic analysis identified aneuploidy as rare causes for HLH associated with extracardiac features. De novo or recessive mutations, as well as dominant mutations with incomplete penetrance and variable expression in one or more genes, are the most likely modes of inheritance assumed at present. Rare HLH-associated mutations were found in ZIC3, NOTCH, and NKX2.5. To date, there are no genetically engineered animal models for HLH.

In the paper by Theis et al., 5 individuals with HLH had post-operative impaired right ventricular ejection fraction after Fontan palliation, and their first-degree relatives underwent WGS. Functional variants were restricted to those that affected a protein sequence, canonical splice site, microRNA coding sequence/binding site, enhancer region, or transcription factor binding site within a promoter validated by Encyclopedia of DNA Elements (ENCODE) chromatin immunoprecipitation experiments. Using parental and sibling WGS data, rare, functional variants in each proband were then filtered for de novo, homozygous recessive, compound heterozygous, and X-linked recessive (males) modes of inheritance. Family-based filtering assumed monogenic bases of HLH and ventricular dysfunction and focused on genes linked to a cardiac phenotype. Their study revealed compound heterozygosity for recessive MYH6 mutations in 2/5 probands. In silico protein modeling indicated that the identified mutations could impact the dynamics of the myosin filament.

Sarcomere gene mutations were shown to produce cardiac developmental anomalies. Dominant heterozygous MYH6 mutations with variable penetrance were described in atrial septal defect and other CHD. In addition, other rare heterozygous MYH6 variants were identified in dilated and hypertrophic cardiomyopathies. It seems possible that the effects of some mutations in cardiac muscle sarcomeric proteins manifest during development, causing CHD, whereas others cause cardiomyopathy during postnatal life. Theis et al. postulate that compound heterozygous MYH6 mutations implicate a developmental anomaly and cardiomyopathy of left and right ventricles, respectively. Their suggestion that compound heterozygosity for MYH6 mutations may be sufficient for arrested left heart development is intriguing, but deserves further genetic and functional evaluation in the context of what is known for HLH. Post hoc trio-based WGS analysis of 21 additional HLH probands with a Fontan circulation and normal ejection fraction did not identify any instances of recessive MYH6 mutations (data not shown). There are no reports of cardiomyopathy in patients with CHD carrying a heterozygous MYH6 mutation, or more likely, ventricular impairment/failure has not been noticed as such. Assessment of 24 heterozygous MYH6 mutations associated with cardiomyopathy or CHD revealed that 64% of the reported heterozygous MYH6 mutations, although at low frequencies (0.00001–0.001), were present in the Exome Aggregation Consortium (ExAC) database and could account for occult cardiomyopathy. The same assumption could apply for the family members of the 2 HLH individuals with heterozygous MYH6 mutations, anticipating the need for clinical follow-up. The findings of Bainbridge et al support the idea that missense mutations in MYH7 are common causes for LVNC and they are sufficient to cause disease onset in a heterozygous state. The patients being homozygote and double-heterozygote for MYH7 mutations in some reports have a clinical course characterized by either severe LV hypertrophy or progressive LV systolic dysfunction. Findings in hypertrophic cardiomyopathy suggest earlier disease onset and much more severe progression for homozygous patients compared with heterozygous individuals, suggesting a gene–dose effect.

Developmental Aspects and Mechanisms

Horn et al. examined the relatively unexplored role of mitochondrial biology in the embryonic heart. Their data have clinical implications by suggesting that some cardiomyopathies and CHD may be caused by disruption of myocyte differentiation secondary to defects in mitochondrial maturation and redox biology. Mouse Nnt mutants revealed a direct role of mitochondria for cardiac development and function. The forward reaction of NNT, a nuclear-encoded mitochondrial inner membrane protein, couples the generation of NADPH to proton transport and provides NADPH for the mitochondrial regeneration of the antioxidant compounds, glutathione and thioredoxin. Nnt null mice have a mild cardiovascular background phenotype, including lower LV shortening and increased LV weight. Tafazzin knockdown mice provide the first mammalian model system for Barth syndrome in which the pathophysiological relationships between altered content of mitochondrial phospholipids, ultrastructural abnormalities, myocardial and mitochondrial dysfunction, and clinical features can be completely investigated. This model implicates that continuous cardiolipin deficiency has a progressive and cumulative detrimental effect on mitochondrial structures in sarcomeric tissues.

In zebrafish, Auman and colleagues demonstrated that chamber morphology develops via changes in cell morphology. Their model suggests that even subtle changes of circulation or contractility caused by mutated sarcomere genes could lead to abnormalities in cell and chamber morphology. MYH6 has a critical role in cardiac development and function. Although MYH7 is the dominant cardiac myosin heavy chain isoform in humans—and a major disease gene in various cardiomyopathies—MYH6 expression in the human heart persists into adulthood, where it functions in the ventricular myocardium. Time-course analysis of the developing murine LV versus right ventricle revealed no discernible differences in Myh6 expression, which would explain why MYH6 mutations lead to LV-restricted development. MYH6 mutations found in patients with CHD have been shown to disrupt myofibril formation in vitro.

In the chick animal model of HLH, it was demonstrated that ligation of the left atrium, which decreased blood prograde flow to the LV, caused LV hypoplasia. They also showed that
increasing flow to the LV resulted in a rescue of the HLH phenotype; decreased myocyte proliferation in the hypoplastic LV was rescued by increased hemodynamic loading. Increased proliferation resulted in significantly increased ventricular myocardial volume and myocyte number. In the majority of patients, HLH may be caused by a primary defect of valve development, leading to hypoplasia of left-sided structures. Alternatively, HLH may arise from a primary defect in LV development because in some patients relief of aortic valve obstruction does not prevent the development of LV hypoplasia.25,26 In some cases of nonresponders, a thick endocardial layer of cellular fibroelastic tissue, termed endocardial fibroelastosis (EFE), forms, which restricts growth of the LV. EFE develops in the fetus and in association with reduced blood flow in a rodent model. In a corresponding mouse model, cells of the EFE tissue were shown to be of endothelial origin and show aberrant endothelial to mesenchymal transition as causative for EFE.27 Transcriptional suppression of bone morphogenetic proteins 7 through promoter methylation contributed to endothelial to mesenchymal transition and thus EFE formation. The emerging role of epigenetic factors in refining cardiac development, in modulating cardiomyocyte differentiation, and in establishing definitive cardiac structure, as well as function, stresses the complex interplay of various mechanisms.

Next-Generation Sequencing Technologies: Outlook

Whole exome sequencing provides the opportunity to efficiently scan the coding portions of the human genome for causative mutations. This is extremely useful in such genetically heterogeneous diseases as human cardiomyopathies, in which probably a multitude of disease genes has not yet been identified. Nevertheless, all variants must undergo careful evaluation because the filtering and prioritization strategies are crucial, and eventually functional confirmation is needed. Functional in vitro or in vivo studies will become increasingly important in disorders with extreme heterogeneity and where segregation analysis is not possible. The percentage of detected sarcomere mutations in cardiomyopathies and CHDs could even increase in the future with the application of next-generation sequencing technology, including large genes, such as the giant sarcomere gene titin (TTN). CHDs are mostly sporadic and apparently follow non-Mendelian inheritance. Although family-based filtering for variants that fit a specific inheritance model is a powerful strategy for identifying rare variants with major effect, this approach does not identify common variants with minor effects. Whether or how WGS, that is, adding more noncoding sequence to the known coding sequence, of families will add to our current genetic understanding of CHD remains a challenge.

Disclosures

None.

References


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