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Unraveling the mystery of oligogenic inheritance under way?

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Complex genetic combinations are thought to be responsible for multiple human diseases, but identifying responsible mutations or polymorphisms and experimentally demonstrating the causality has been difficult (Boyle et al., 2017; Khera et al., 2018). In a notable report, Gifford and coauthors (2019) described dissecting genetics of congenital heart disease (CHD) inherited in an oligogenic fashion which means that a number of mutated genes interact genetically to produce the phenotype. Notably, the authors point out that monogenic aberrations and copy number variations account for only 10% and 25% of CHD respectively. Their investigation started with a 2-month-old patient suffering from left ventricular noncompaction (LVNC). The immediate family members included a disease-free mother, an asymptomatic but radiologically positive father, a previously undiagnosed but affected sibling, and a deceased sibling with LVNC. The involvement of multiple family members clearly indicated that inheritance of mutated gene or genes rather than de novo mutation is responsible for the disease. The initial diagnosis was followed by whole exome sequencing of the family members. As expected, multiple heterozygous loci were isolated, and a number of them were projected to represent damaging or loss-of-function polymorphisms. Genes among them that are expressed highly in the heart tissue represented prime candidates.

The first gene of interest was myosin heavy chain 7. Single nucleotide variation of this gene has been implicated in cardiomyopathy and LVNC (McNally et al., 2015). All affected family members were heterozygous with a leucine-to-phenylalanine (L387F) missense mutation. L387 is a highly conserved amino acid residue across species and resides within the adenosine triphosphatase domain of the protein. The mutation was also predicted to be damaging by multiple algorithms. The second missense variation of potential interest was found in the transcription factor MKL2 (Trembley et al., 2015). A glutamine-to-histidine (Q670H) heterozygous mutation was found only in the affected family members again. Q670, found adjacent to the leucine zipper domain, is also a highly conserved residue, and the substitution was predicted to be damaging as well. Given the increased severity among the children compared with the father, the investigators hypothesized that a maternally originating mutation functioned as a genetic modifier for LVNC. This led to the identification of alanine-to-serine missense mutation at position 119 (A119S) in the transcription factor NKX2-5 which is one of the core transcriptional regulators of cardiac development. Heterozygous mutations in NKX2-5 have been associated with CHD (Chung and Rajakumar, 2016). A119 is not as tightly conserved as the other residues described above. For example, rhesus macaque has valine substitution in this position. A119 is not within the DNA binding domain, nor is A119S mutation expected to be damaging, but A119S mutation has been reported to reduce DNA binding in vitro (Dentice et al., 2006).

With the candidate combination of mutations at hand, the investigators first resorted to transgenic mice for confirmation (Fig. 1). Indeed, triple heterozygotes but not the other mice with less mutational burdens showed phenotypes of LVNC including deep trabeculations in the left ventricular wall. Under increased pressure in the left ventricle induced by transverse aortic constriction, the triple mutant also showed a significant reduction in cardiac function again consistent with LVNC. Next, the investigators used induced pluripotent stem cells (iPSC) derived from the family members to generate cardiomyocytes. While the initial differentiation efficiency was similar, cardiomyocytes derived from symptomatic family members showed a deficiency in cell adhesion. Triple mutant mice and patient-derived cells also showed similar alterations in gene expression patterns most notably with elevated levels of genes associated with cell cycle and downregulation of genes associated with cell adhesion and extracellular matrix deposition. These data together strongly support the oligogenic basis of LVNC involving mutations of myosin heavy chain 7, MKL2, and NKX2-5.

It should be emphasized above all that multiple technological advances made this study possible. The first is the advent of deep sequencing techniques which replaced gene mapping and can provide a list of polymorphisms and mutations with high accuracy and efficiency (Salk et al., 2018). Combined with the accumulation of information on gene expression patterns, deep sequencing can readily lead to potential causal mutations. The second is the advance in algorithms that allow the prediction of functional changes in proteins in association with specific mutations or polymorphisms (Yazar and Ozbek, 2021). This allows for filtering out numerous silent polymorphisms present in individual genomes. Another obvious crucial advance was the vastly improved efficiency of transgenesis owing to the adoption of the CRISPR-Cas9 system and the use of fertilized eggs rather than embryonic stem cells for gene targeting (Hall et al., 2018). Replicating intended human mutations in mice has thus become readily achievable. Finally, iPSC with its potency for

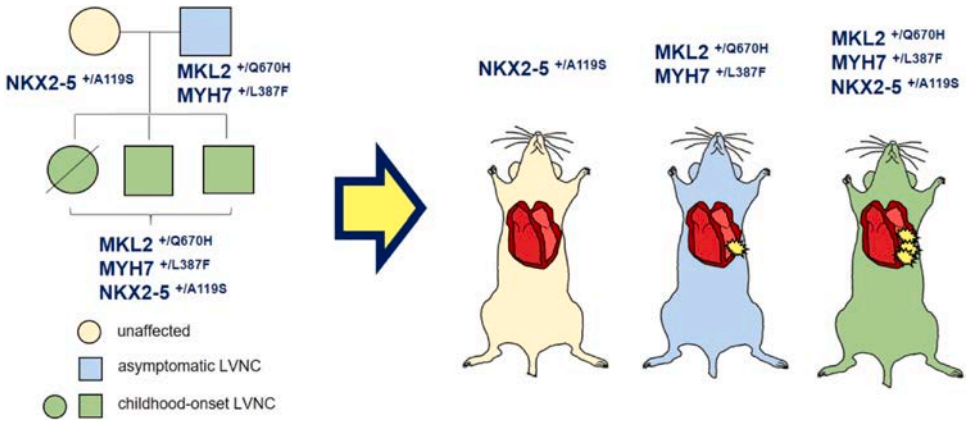

Fig. 1. Left ventricular noncompaction (LVNC) is a genetic disorder that is believed to result from oligogenic combinations of multiple mutations. In the figure above, the pedigree of family members and their phenotypes are matched with results from genotypic analyses. A study conducted on transgenic mice harboring identical combinations of mutations found that the 3 missense mutations together produced the severe cases seen among the children thus supporting the oligogenic origin of the disease. MYH7, myosin heavy chain 7.

multiple lineages can be used to mimic human development and pathogenesis (Sharma et al., 2020). Transgenic mice and patient-derived iPSC together represent the most relevant genotype-specific disease models that can also serve as platforms for the development of therapeutic interventions down the road.

This study, taking advantage of the latest advances in experimental techniques that can identify and validate significant polymorphisms, may serve as a prototype for analyzing oligogenic human diseases. Accumulation of genomic sequences in combination with phenotypic description is taking place at a rapidly growing pace. This will lead to candidate combinations of genetic variations responsible for specific pathologies. Once a daunting task, dissecting the complexity of oligogenic human disease may finally be within our grasp.

AUTHOR CONTRIBUTIONS

Y.L. and J.K. wrote the manuscript.

DECLARATION OF COMPETING INTERESTS

The authors have no potential conflicts of interest to disclose.

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