FLNC (Filamin-C)
A New(er) Player in the Field of Genetic Cardiomyopathies

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In 1990, the Seidman group identified the first pathogenic cardiomyopathy mutation in a large 4-generation family, where several members were affected by hypertrophic cardiomyopathy.1 Since this first report, the number of genes and mutations associated with different cardiomyopathies is increasing from year to year. Currently, mutations in >170 genes associated with different cardiomyopathies, channelopathies, or syndromes with cardiac involvement are described. The huge number of different genes and mutations involved in cardiomyopathies limited routine genetic diagnostics for a long time. For example, Sanger sequencing of TTN, encoding the giant sarcomere protein titin, was difficult, expensive, and time consuming and limited the routine genetic diagnosis.2,3 Therefore, it was not surprising that the development of efficient next-generation sequencing technology pushed also the genetic diagnostics of cardiovascular diseases. Today, cardiovascular next-generation sequencing techniques are implemented in many diagnostic laboratories.4 The availability of next-generation sequencing technology has in the meantime provided the important insight that cardiomyopathies are remarkable heterogeneous disorders with different expressivity and penetrance. The challenges for the future remain the identification of phenotype–genotype relationships and consequences of genotyping for the development of personalized therapies.

See Article Tucker and McLellan et al

In this context, the contribution of a genetic pathogenesis to restrictive cardiomyopathy (RCM) is incompletely understood. Besides genetic factors, RCM might be a secondary cardiomyopathy and part of a systemic disease like the mineralization disorder pseudoxanthoma elasticum or cardiac amyloidosis. First mutations associated with familial RCM were identified in TNNI3 by the research group of William McKenna in 2003.5 During the last decade, most of the known RCM-associated mutations were identified in genes, encoding sarcomeric or cytoskeletal proteins.6,7 However, the interpretation of broad genetic analyses of cardiovascular diseases is still challenging because many patients present several genetic variants in different genes or because the significance and pathogenic impact of novel genetic variants frequently remain unknown. Especially for RCM, the knowledge about relevant mutations and genes is still limited because it is a rare cardiomyopathy. The application of whole-exome sequencing is, therefore, sometimes like searching for a needle in the haystack, especially if families are small and cosegregation analysis is not or only partially possible.

Even in the second decade after the Human Genome Project has been finished, there is a gap between identification of genetic variants in cardiovascular disease and their interpretation.8 Because of the limited functional knowledge, it is appropriate to combine modern sequencing techniques with adequate functional analysis of specific genetic variants. However, in contrast to the pure sequencing approach, the development and functional characterization of adequate cell and animal models is still time consuming but could significantly improve predictions for particular mutations.

In this context, Tucker et al9 identified and functionally characterized a novel mutation in the gene FLNC in a 4-generation family, which is affected by RCM in combination with atrial fibrillation. FLNC encodes filamin-C—a protein, which is localized at the Z-bands and at the intercalated disc. The exact function of filamin-C is still under debate. However, it is known that filamin-C is involved in the connection of the cells to the extracellular matrix by binding to cell adhesion molecules, as well as in the organization of the sarcomeres.10,11

Tucker et al used whole-exome sequencing in combination with stringent filtering and cosegregation analysis within the affected family to identify putative candidate genes associated with RCM. Based on different in silico prediction tools, a high conservation score, and on myocardial expression filtering, Tucker et al suggested FLNC-c.6889G>A as the most likely disease-causing variant in this family.

However, the elegance of this study is established by the combination of deep genetic analysis with the structural and functional analysis of explanted myocardial tissue of the mutation carrier, cell transfection studies using C2C12 myoblasts, and analysis of embryonic stem cell (ESC)-derived cardiomyocytes, which were modified by genome editing using clustered regularly interspaced short palindromic repeats-Cas9. In the myocardial tissue of the mutation carrier, the localization of filamin-C at the Z-bands was significantly reduced in comparison with control samples. Z-bands are sensitive structures with high relevance for the alignment and structural integrity of the sarcomeres, which is underlined by frequently degraded Z-bands in other genetic

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cardiomyopathies like desminopathies. However, the most convincing experiment in the study of Tucker et al supporting the pathogenicity of FLNC-c.6889G>A is the generation and characterization of an adequate cell culture model based on human ESCs in combination with genome editing using clustered regularly interspaced short palindromic repeats-Cas9. Cas9 is an endonuclease originally discovered in bacteria, which can be used in combination with a guide RNA to target and insert double DNA breaks at specific sites. Cells repair these DNA double breaks via nonhomologous end joining or by homology-directed repair. Tucker et al cotransfected, therefore, ESCs with single-stranded oligodeoxynucleotides, which serve as a repair template via homology-directed repair. Fluorescence-activated cell sorting in combination with single-cell expansion was used to generate an ESC line carrying biallelic FLNC-c.6889G>A. The expression analysis of cardiomyocytes differentiated from these ESCs excluded haploinsufficiency as the main genetic pathomechanism of FLNC-c.6889G>A because mutant filamin-C was expressed at a similar level in comparison with the wild-type form. However, the contractile function (fractional shortening) of these cardiomyocytes was significantly reduced in comparison with control cells supporting pathogenicity of FLNC-c.6889G>A. The presented data about reduced contractility caused by FLNC mutations are in good agreement with data of González-Morales et al describing filamin-C as a binding partner of the sarcomere protein titin in Drosophila melanogaster. Presumably, filamin-C is involved in the anchorage of titin and also of actin filaments to the Z-bands.

However, several interesting questions remain open, which could be investigated in further studies. For example, it would be interesting to compare the transcriptome of mutant and wild-type ESC-derived cardiomyocytes to identify underlying molecular networks involved in filaminopathies. Furthermore, the ESC-derived cardiomyocytes, carrying FLNC-c.6889G>A, could be used to investigate nanomechanical differences of the cytoskeleton. For example, it might be of interest if the cellular nanomechanics of the cardiomyocytes are disturbed by FLNC-c.6889G>A. This would explain the ventricular stiffness in RCM.

During the past 3 years, pathogenic FLNC mutations were also described by other groups in patients with hypertrophic cardiomyopathy, DCM, and also with arrhythmogenic cardiomyopathy, respectively. Furthermore, it should be mentioned that FLNC mutations were originally described in patients with skeletal myopathies.

Currently, it is unknown why different FLNC mutations cause so different clinical entities. However, usage of adequate cell culture models in combination with animal studies will hopefully reveal in future more details about the pathomechanisms of different filaminopathies. Although the article of Tucker et al is not the first report about RCM-associated FLNC mutations, it provides interesting and important functional data supporting the conclusion that FLNC mutations might be causative for familial RCM.

In summary, the report of Tucker et al in combination with other reports links mutations in FLNC to different cardiomyopathies, including RCM. Hopefully, the development of adequate cell culture and animal models as described by Tucker et al will help to decipher relevant molecular pathomechanisms leading to the development of efficient personalized therapies in future. Although novel genome-editing technologies are currently far away from entry into clinical treatment, they could be a helpful research tool to investigate the underlying pathomechanisms.

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Disclosures
None.

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