Cardiomyopathy Paper Review

Cardiomyopathy is a severe disease which affects the muscles of the heart. Even though its occurrence is rare, it is a common reason for heart failure in children and is the main cause of heart transplantations in children over the age of one year. The most prevalent forms of cardiomyopathy in children are dilated and hypertrophic myopathy, which result in the abnormal structure and functioning of the cardiac muscles and lead to neonatal heart failure in extreme cases. Dilated cardiomyopathy is a disease which causes the stretching out and thinning of the muscles of the left ventricle(LV), while hypertrophic functional form causes the thickening of themuscles of the heart. Much about the incidence, prevalence, risk factors and causes of these types of pediatric cardiomyopathy were unknown for a long time. In this review, we will focus on what was previously known and what was newly discovered about pediatric dilated cardiomyopathy(DCM) in particular.

The annual incidence of pediatric DCM was 1.13 cases per 100,000 (based off of a census performed in the US, between 1996 and 1999). However, these cases may be underrepresented, because many children who died suddenly of heart failure may not have been diagnosed with this disease. For a long time, the causes of pediatric DCM were unknown, or the disease was thought to arise spontaneously. Only in a few cases, the cause was identified as either myocarditis, a neuromuscular disorder, familial cardiomyopathy or inborn metabolic errors[1]. Recently, it was found through genetic testing of the cardiomyopathy genes of pediatric DCM patients that a certain pathogenic genetic mutation may be responsible for DCM.Mutations in the genes which produce proteins such as troponin and tropomyosin, i.e., thin filament proteins which regulate the interaction between actin and myosin, were known to be one of the causes of DCM. However, not much was known about how mutations in proteins which regulate the

assembly of thin filaments may cause DCM. Leiomodin(Lmods) are a family of actin binding proteins, which help in the regulation of the assembly of these thin filaments by binding to and initiating the elongation of f-actin. There are multiple isoforms of Lmods.
Mutations in Lmod1, the form prevalent in skeletal muscles, results in Nemaline myopathy, while mutations in Lmod3, found in smooth muscles, leads to megacystis microcolon intestinal hypoperistalsis. However, mutations in Lmod2, the isoform found mostly in cardiac muscles, was not known to cause any diseases in humans, though its absence in mice was observed to result in cardiac failure and death.

In this paper, the first known case of pediatric DCM caused by a homozygous mutation in LMOD2, the gene that produces Lmod2 protein was presented. It was hypothesized that this

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[1] J. D. Wilkinson, D. C. Landy, S. D. Colan, J. A. Towbin, L. A. Sleeper, E. J. Orav, G. F. Cox, C. E. Canter, D. T. Hsu, S. A. Webber, S. E. Lipshultz, The pediatric cardiomyopathy registry and heart failure: Key results from the first 15 years. Heart Fail. Clin. 6, 401–413 (2010).

loss of function mutation led to abnormal length of actin filaments, resulting in human Dilated Cardiomyopathy.

The hypothesis was tested using various genetic and molecular experiments performed on the explanted heart tissue of an infant with DCM, in parallel with studies of mice without the Lmod2 protein being expressed in them(Lmod2 knockout mice). The experiments were conducted to understand how a mutation in LMOD2 affects the cells of the heart and causes cardiomyopathy. Through Western Blot Analysis, it was found that no full-length or even shortened Lmod2 proteins were found in the LV of the patient's heart. To find the effect of the mutation of actin length regulation, they measured the levels of the other integral proteins(cardiac troponin, tropomyosin, tropomodulin-Tmod1), which work in association with actin. They found that the quantities of most of them were reduced, showing that Lmod2 mutation does cause abnormalities in the length and number of actin filaments.
Reverse transcriptase quantitative PCR was performed to find out if the lack of Lmod2 in the patient was because there was a decrease in the transcript being produced or if the protein was being degraded after its production. It was found that compared to a normal person, there was a large decrease in the amount of mature Lmod2 produced in the LV, while there was a relatively normal amount of the pre-mRNA. This meant that there was Nonsense mediated decay(NMD) occurring, where mRNA having premature stop codons are eliminated, leading to lack of protein expression.
Through deconvolution immunofluorescence microscopy of sections of the patients LV, (using phalloidin to stain actin, antibodies to stain Tmod, α-actinin to stain Z-discs), it was found that the myofibrils of the LV were disordered, and that the the Z-discs were much wider. The actin filaments barely extended out from the Z-discs and Tmod1(the protein which caps actin filaments) was localized near the Z-discs.

By studying cardiomyocytes taken from the patient, it was also found that these cells were less sensitive to Calcium ions, and that the maximum force they could produce was very less. These are all common hallmarks of DCM.

The mutated LMOD2 gene was expressed in Lmod2 knockout mice and it was found that this nonsense mutation prematurely encodes the stop codon, thus producing proteins with a shorter C-terminal. These truncated proteins lack the site that helps it bind to actin, and thus function inefficiently. However, it was also found that, when compared to mice which produce no Lmod2at all, mice producing mutated Lmod2 in small quantities show a later onset of cardiac dilation and dysfunction.

Thus, from the above methods, it was found that a LMOD2 mutation leads to highly reduced Lmod2 protein expression in patients and that this reduced expression leads to shorter thin filament formation, as well as reduced sensitivity of cardiomyocytes to calcium and reduced force production. The decrease in LMOD2 mature transcripts, but normal levels of pre-mRNA suggest the NMD occurs on mutant LMOD2. It was also found that the mutant Lmod2 does have some limited positive function, but is produced in too small quantities in humans to show any phenotypic effect.

From these conclusions, a potential therapeutic intervention arises, where specific components of NMD are inhibited, such that these mutant LMOD2 transcripts are preserved and can

produce sufficient quantities of functional Lmod2 to improve the cardiac function of patients with this nonsense mutation.

The method in which the researchers found the nonsense LMOD2 mutant mRNA was eliminated through Nonsense mediated decay(NMD was interesting. Two different primers were used in the process of RT-qPCR to find the difference in the quantities of mature mRNA and pre-mRNA produced. To find the amount of mature mRNA, intron spanning primers present inside exons were used, which prevented any DNA containing introns from being amplified. Only the DNA which did not possess introns(cDNA) was amplified. To find the quantity of pre-mRNA, primers located in the introns were used. This ensured that only the DNA which was reverse-transcribed from RNA containing non-coding introns(pre-mRNA) was amplified and thenquantified. Using this technique, they found that pre-mRNA levels were normal, but mature mRNA was less, leading to the conclusion that mutant mRNA were being degraded by NMD.

The biallelic loss of function mutation in LMOD genes causes the aberrant formation of actin filaments, inducing cardiac dysfunction and leading to Dilated Cardiomyopathy. While this was only observed in one patient, the pathogen causing ability of this mutation is supported by the fact that it is a nonsense mutation and is absent in its homozygous form in most of the general population. Thus, it can be assumed that this mutation is deadly. However, there should be efforts to identify additional cases by adding LMOD2 sequencing to cardiomyopathy screening panels.