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Stressing the Giant: A New Approach to Understanding Dilated Cardiomyopathy

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Keywords

titin; mutations; dilated cardiomyopathy; knock-in

Many human cardiac diseases have a genetic cause. A large number of these occur in genes coding for the myofibrillar proteins (see [1-8] for reviews). The catalogue of mutations continues to expand. The most extensive observations have been on the beta myosins gene where over 200 different mutations have been found [9]. A wealth of structure-function information has been gleaned from studies of these altered forms.

Studies regarding mutation effects on the giant protein titin have lagged in comparison to the other myofibrillar proteins. It's 12 to 16 fold larger message size (relative to myosin) would suggest it to be a frequent mutation target. There are several reasons why so few titin mutations have been identified. First, the extreme size of titin makes conventional molecular biological approaches impossible. The protein is coded by a single gene in higher organisms [10], and there are 363 exons in the human version [11]. Sequencing a cDNA for full length titin is not possible with current techniques (no one has found ways to deal successfully with an intact 80,000 to 100,000 bp mRNA). Scanning this many exons for sequence variants has only recently been done because of time and expense. Second, there is a large diversity of titin protein forms. The gene is alternatively spliced with different isoform classes in heart and skeletal muscle [10]. The original descriptions identified unique regions near the N line in cardiac (N2B) and skeletal (N2A) that were associated with the tissue specificity. It was subsequently shown that cardiac muscle also contained isoforms with both the N2B unique and N2A unique sequences, and these have been termed N2BA. Further studies have demonstrated a plethora of sub-splicing pathways in the N2BA isoform class [11]. Splicing pathway heterogeneity is particularly diverse in the PEVK region where 10 different clones from a single PCR amplification of human heart cDNA yielded 10 different splicing patterns [12]. Third, much of the protein is believed to function as a structural chain, so unraveling a single link out of potentially 100 immunoglobulin (Ig) domains in the I-band region would change the length very little and have little effect on passive tension [13]. Even drastic changes in the number of exons expressed, as occurs in a recently described rat mutant, produce minimal changes in heart function [12,14]. Thus changes in titin length alone may not result in serious health consequences. Finally, the understanding of the diverse functions of this protein remains incomplete (see [15-17] for reviews), so its relation to cardiac disease remains even less clear.

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Several early reports linked cardiac disease to the chromosome 2q31 region of the titin gene [18,19]. The precise locations have now been determined in each case, and a total of 20 different titin mutations have been identified to date (Table1). Of these 10 are located in the 6 most 3' exons (358 to 363) that code the protein region near the M-line and 4 are found in exon 49 (N2B unique region). Four others occur in the Z-line region (see Figure 1 for a map of these sites to their approximate half sarcomere location). Undoubtedly many other mutations may have occurred with little or no functional consequences. Likewise mutations that do not cause chain termination in the PEVK region would not modify passive tension. Finally the disruption of a single domain from titin's A-band region would also likely not affect the assembly of the thick filament because of the assumed multiple binding sites.

A couple animal models with altered titin have also been found. The mdm mouse (muscular dystrophy with myositis) has an 83 amino acid deletion in the N2A region thought to be involved in calpain 3 binding [20,21]. The lack of cardiac involvement in this model is presumably related to the essential lack of calpain 3 in adult heart [22]. The pickwick mutation in Zebrafish results in a dilated cardiomyopathy like condition with poor sarcomere assembly [23]. The mutation is a T to G transversion in the N2B unique region, but the exact functional effect remains to be determined.

Because obtaining samples from living human hearts is both technically challenging and hard to justify ethically, the development of suitable cellular and animal models has driven the field. Many new mechanistic insights have been gained from transfection of cultured cells and development of transgenic animals, primarily rodents [24-29].

In the current issue Gramlich and coworkers [30] report for the first time the properties of a knock-in mouse containing a titin mutation similar to one reported previously in humans [31]. This autosomal dominant mutation (an AT insertion in the large exon 326 in the myosin binding protein C region of the middle half A band) results in a termination codon and a truncated expressed protein. Heterozygote individuals developed dilated cardiomyopathy with sudden death, but the penetrance was incomplete. Curiously, although this exon is constitutively expressed in skeletal muscle as well, there has been no skeletal muscle mutant phenotype detected to date.

Homozygocity of the current knock-in mouse was found to be lethal by embryonic day 9.5. This was consistent with previous cell culture work showing that near full length titin was essential for myofibrillogenesis [24,25]. Heterozygotes, however, were essentially normal in regard to health and fertility. Hearts contained normal ratios of titin to myosin, and had similar passive tension properties to wild type. Systolic and diastolic function of heterozygotes was not significant different when compared to normal animals. Echocardiography revealed no dilation or altered cardiac structural dimensions. This lack of the effect of the mutation on the mouse model was probably not too surprising in light of the mild effects in humans. Why would we expect to see changes in a mouse when it takes 20 to 40 years for the phenotype to develop in humans and only in a certain proportion of mutation carriers?

The novelty of the current work was that the investigators found ways to induce the DCM phenotype in the knock-in heterozygotes. Two different approaches were used. In the first angiotensin II was continuously infused into wild type and heterozygote mice to cause arterial hypertension. Both groups responded with increased ejection fraction and fractional shortening after one week, but by two weeks the performance of the heterozygotes had significantly declined for both these parameters. Echocardiography also indicated increased dilation between one and two weeks in the heterozygotes. Microscopic examination showed essentially normal myofibril structure by electron microscopy. However, large increases in interstitial fibrosis (~12% of area in heterozygotes versus ~4% in wild type) were observed. The second

approach was the continuous infusion of the beta agonist isoproterenol. After one week, the heterozygotes had significantly lower ejection fraction (~39% vs ~53%), significantly lower fractional shortening (~24% vs ~34%) and significantly enlarged left ventricle chamber size.

Western blotting using antibodies from the expressed titin region and those from the carboxyl end deleted part verified that a truncated titin was expressed in the knock-in heterozygotes. The amount of this shortened titin was much lower than expected, perhaps due to elevated proteolysis. Real time PCR indicated that the message levels for the wild type allele were elevated in heterozygotes, but this compensation was incomplete.

The use of stressors to induce a dilated cardiomyopathy phenotype in rodent models of human disease may prove to be a valuable approach for study of mechanisms related to other mutations. Many unanswered questions remain: what controls the level of up-regulation of wild type alleles in heterozygotes and could we fully express an adequate amount of wild type protein from a single allele? Was the truncated protein fully incorporated into the myofibril structure? Would it be possible to completely shut down expression of the mutant allele, and would this provide a rescue of the wild type cardiac phenotype? Could we further increase the rate and extent of breakdown of mutant protein and thus achieve the normal phenotype? What are the signals that lead a heart to develop dilated cardiomyopathy? These questions must await further studies, but our knowledge appears to have taken a step forward from stressing the giant.

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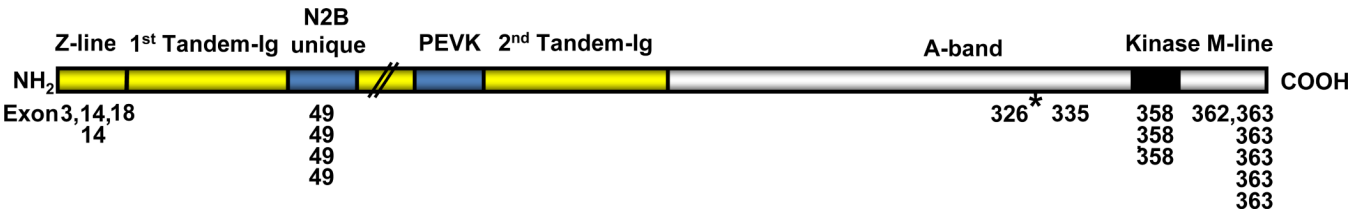

Figure 1.

Diagram of human titin regions and locations of mutations in the half sarcomere. The single titin molecules extend from the Z-line to the M-line in the middle of the sarcomere. The I-band region background is shown in yellow and the A-band region in white. Each number listed refers to a single titin mutation in the corresponding exon annotated in GenBank AJ277892. The mutation for the knockin-mouse report in the current issue [30] is denoted with an asterisk.

Table 1

Human titin mutations and their effects. Abbreviations: [1]DCM – dilated cardiomyopathy; [2]HCM- hypertrophic cardiomyopathy; [3]TMD – Tibial Muscular Dystrophy; [4]LGMD – Limb Girdle Muscular Dystrophy; [5] – base pair position of mutation in genomic sequence of human titin (GenBank Accession no. AJ277892).

Disease	Country	Heart	Skeletal muscle	Titin exon	AJ277892 Position[5]	Sarcomere position	Mutation type	Cell level effects	Base change	Ref
DCM[1]	Japanese	yes	no	3	17750	Z-line (Z repeat 1)	1 base change	decreased T cap binding affinity	G to A	32
HCM[2]	Japanese	yes	no	14	34029	Z-line (Z repeat 7)	2 base transversion	increased α-actinin binding affinity	G to T	33
DCM	Japanese	yes	no	14	34038	Z-line (Z repeat 7)	1 base change	decreased α-actinin binding affinity	C to T	32
DCM	Native American	yes	no	18	37048	Z-line to I-band	1 base change		T to C	19,31
DCM	Japanese	yes	no	49	79690	N2B unique	1 base change	increased FHL2 binding affinity	C to A	34
DCM	Japanese	yes	no	49	80313	N2B unique	1 base change, stop codon	decreased FHL2 binding affinity		32
DCM	Chinese	yes	no	49	82126	N2B unique	1 base change		G to A	35
DCM	Japanese	yes	no	49	82324	N2B unique	1 base change		G to A	32
DCM	Australian	yes	no	326	245037	A-band	2 base insertion, stop codon		AT insert	31
DCM	Australian	yes	no	335	267662	A-band	1 base change, stop codon		del G	36
Edstrom myopathy	Swedish	no	yes	358	286133	A-band, kinase	1 base change	reduced nbr1 binding affinity	C to T	37
Fatal cardiomyopathy	Sudanese	yes	yes	358	289385	M-line (Mex 1)	8 base deletion, stop codon	disrupted M line complex	del 8 bp	38
DCM	Japanese	yes	no	358	287772	M-line (Mex 1)	1 base change	FHL2 binding region	G to A	34
Fatal cardiomyopathy	Moroccan	yes	yes	360	291297	M-line (Mex 3)	1 base deletion, stop codon	disrupted M line complex	del A	38
TMD[3]	French C	no	yes	362	292298	M-line (Mex 5)	1 base deletion, stop codon		del T	39
TMD/LGMD[4]	Finnish maj	no	yes	363	293269–293279	M-line (Mex 6)	11 bp deletion/insertion		del/ins	40
TMD	Belgian	no	yes	363	293329	M-line (Mex 6)	1 base change		T to A	41
TMD	French A	no	yes	363	293356	M-line (Mex 6)	1 base change	reduced calpain 3 binding	T to C	39,40
TMD	Spanish	no	yes	363	293378	M-line (Mex 6)	I base deletion, stop codon		del A	39
TMD	French B	no	yes	363	293379	M-line (Mex 6)	1 base change		C to T	39