Titin truncating mutations: a rare cause of dilated cardiomyopathy in the young

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SUMMARY

Truncating mutations in the *TTN* gene (*TTN*tv) are the most common genetic cause of dilated cardiomyopathy (DCM) in adults but their role in young patients is unknown. We studied 82 young DCM subjects and found that the prevalence of *TTN*tv in adolescents was similar to adults but surprisingly few *TTN*tv were identified in children. The *TTN*tv identified in children with DCM included a confirmed *de novo* variant and in several cases there was clear evidence of additional clinical or genetic risk factors. These findings have implications for genetic testing and suggest that single *TTN*tv are insufficient alone to cause early-onset disease.

DCM is the most common cardiomyopathy in the young with an annual incidence of 0.57 to 0.87 cases per 100,000.[1-3] It is associated with high rates of cardiac transplantation and sudden death, particularly within the first year after diagnosis.[2,3] DCM may result from diverse inflammatory, metabolic, mitochondrial, neuromuscular or syndromic conditions, but in a majority of cases no specific cause can be identified. Up to 15% of young DCM patients have a positive family history[1-4] suggesting a genetic etiology. However, relatively few genetic analyses of primary early-onset DCM have been performed.[5,6]

Truncating mutations in the gene encoding the giant sarcomeric protein titin (*TTN*tv) have recently been identified in 13-27% cases of adult-onset non-ischemic DCM.[7,8] These remarkable findings indicate that *TTN*tv are the most common genetic cause of DCM in adults[9] and have profound implications for genetic testing of patients and their families. The contribution of *TTN*tv in young DCM patients is unknown. Here we report the results of genetic testing of cardiomyopathy-related genes, including the *TTN* gene, in 82 patients aged 21 years or less at the time of DCM diagnosis.

METHODS

Study subjects

Study subjects diagnosed with DCM from birth to 21 years of age were recruited from Boston Children's Hospital, the Royal Brompton and Harefield NHS Trust, the Kanuni Sultan Suleyman Training and Research Hospital, and a familial DCM research program at the Victor Chang Cardiac Research Institute. All subjects were of European descent. Routine clinical assessment was performed including medical history, family history, ECG, and transthoracic echocardiography. Protocols were approved by local ethics committees and written informed consent was obtained.

Genetics studies

Genomic DNA libraries that were enriched for genes implicated in cardiovascular disease, including *TTN,* were constructed using custom hybridization capture probes and sequenced as described.[7,8] Sequencing data were aligned to the human reference genome hg19 and analyzed using a custom pipeline.[7,8] *TTN* variants were annotated using a Locus Reference Genomic sequence (LRG) and an inferred complete *TTN* meta-transcript (LRG_391_t1).[8] Variant frequency was assessed using the ExAC database (http://exac.broadinstitute.org, accessed November 2014).

Myocardial tissue studies

Left ventricular tissue samples obtained from pediatric hearts (n=6) and from control adult hearts (n=50) were subjected to RNA sequencing and the relative exon usage across *TTN* isoforms was compared (See the Methods section in the Supplementary Appendix).

RESULTS

Eighty-two subjects with early-onset DCM were identified. There were 54 males and 28 females aged from birth to 21 (median 10) years at the time of DCM diagnosis. All subjects had severe symptomatic heart failure, with 63 subjects (77%) requiring heart transplantation (n=52) or a left ventricular assist device (n=11) and 4 subjects experiencing premature death. Twenty-seven subjects had a family history of DCM and 61 subjects (74%) had no identifiable clinical risk factors (Table 1, and see Table 1 in the Supplementary Appendix).

TTN screening

Genetic testing with a cardiomyopathy gene panel was performed in all subjects. Nonsense, frameshift or splice-site disrupting variants in cardiomyopathy genes were identified in 18 of 82 (22%) cases (Table 1, and see Table 1 in the Supplementary Appendix). Ten patients had *TTN*tv (12%). The characteristics of these variants are shown in Table 2. Notably, none of these variants were present in the ExAC database that contains >60,000 exomes from the general population. Truncating variants in genes other than *TTN* were present in 9 in patients (11%), similar to findings in adult DCM populations REF.

Effects of age and family history

The frequency of *TTN*tv differed according to the age of clinical presentation. Seven of 31 adolescents (23%) aged between 15 to 21 years at the time of DCM diagnosis were found to carry a *TTN*tv (see Table 1 in the Supplementary Appendix). This high prevalence mirrors recently-reported data for

adult patients with end-stage heart failure (22%, P = 1.0).[8] Three of the *TTN*tv identified in adolescents have previously been seen in adult patients with DCM[7,8,10] and four of the seven adolescent patients had a positive family history with adult-onset DCM in other affected relatives.

In contrast, only three variants were identified in the 51 children diagnosed with DCM aged <15 years (6%, Table 1). The prevalence of *TTN*tv in children was lower than in adolescents (P=0.037) and in adults with end-stage heart failure[8] (P=0.010). When 13 children with other possible causes of DCM were excluded, there were only two of 38 children who carried a *TTN*tv (5%), with significant differences when compared to adolescents (P=0.044) and adults[8] (P=0.019). Of the three variants found in childhood-onset cases, one was a heterozygous nonsense *TTN* variant, p.Q4656X, that was identified was in a male child who presented with DCM at 18 months of age. The nonsense change was deemed to occur *de novo* as it was absent from both parental DNA samples. This child also carried a heterozygous splice site variant in the *TBX20* gene, p.297_-2A>G, that was present in his unaffected mother. A second heterozygous nonsense *TTN* variant, p.4749X, was found in a female child with DCM diagnosed at 12 months of age, but no further clinical history or family DNA samples were available. The third *TTN* variant was a frameshift, p.T19345SfsX2, that was found in a male child diagnosed with DCM at 10 years of age. This child had no family history, but developed rapidly-progressive severe DCM that required heart transplantation subsequent to receiving conventional doses of chemotherapeutic agents (daunorubicin, etoposide, cytosine, amsacrine) for treatment of hematological malignancy.

Effects of variant location

We have demonstrated previously that truncating *TTN* variants associated with adult-onset DCM cluster in the titin A-band while *TTN* variants seen in healthy control subjects are more commonly found outside this region.[7,8] Eight of 10 truncating *TTN* variants identified in this study were located in the

A-band or at the I-band/A-band junction. The remaining two variants were both found in childhood-onset cases and were located in the proximal I-band, in exons 49 and 50, respectively.

Exons that are constitutively present in cardiac isoforms of titin have a higher proportion spliced-in (PSI) score than exons that are variably spliced, and truncating *TTN* variants seen in adult DCM patients are enriched in exons with high PSI scores.[8] To determine whether these PSI patterns are replicated in pediatric hearts, we compared relative exon usage in pediatric and adult left ventricular tissue (Figure 1). Overall patterns of exon usage were similar in pediatric and adult ventricles. There were no differences in the A-band and M-band regions but mean PSI was lower in pediatric samples for some exons in the I-band. For each of the seven exons harboring pediatric DCM variants, including the I-band exons 49 and 50, the mean PSI was 100% (Table 2) with no statistically significant differences when compared to adult controls (see Table 2 and Figure 1 in the Supplementary Appendix).

DISCUSSION

Here we report the first comprehensive evaluation of truncating *TTN* variants in young patients with DCM. We found that patients diagnosed with DCM during adolescence had a high prevalence of *TTN* variants (23%), similar to adult DCM populations.[7,8] Adolescent patients frequently had a history of DCM in adult family members and the *TTN* variants in these patients were all located within the A-band cluster that is characteristic of adult-onset DCM.[7,8] Three of the seven variants in adolescent patients do not appear in population databases but have been seen previously in adult DCM cases.[7,8,10]

These findings contrast with the very low prevalence of truncating *TTN* variants found in patients diagnosed with DCM during childhood. Two of the three variants in childhood-onset cases occurred in children with no known family history of DCM, including one variant that was confirmed to occur *de novo.* The position of these variants differed to adult DCM cases, with two variants occurring in the proximal I-band region. Several factors suggest that these I-band variants could be pathogenic, including their location in exons with high PSI values in both pediatric and adult hearts. The p.Q4656X variant in exon 49 is situated within the cardiac-specific N2B unique sequence. Zebrafish that are homozygous for a N2B nonsense variant (*pik*^{m171}) show severe early-onset DCM and a nonsense variant in this exon has been reported in an adult patient with DCM.[11,12]

Titin truncations due to nonsense, frameshift and splice site variants may disrupt sarcomere function by dominant negative effects or haploinsufficiency. Our recent observations of normal titin protein levels in heart tissues from patients with A-band *TTN* variants implicates a dominant negative model.[8] Whether I-band truncations might be more susceptible to nonsense-mediated decay and haploinsufficiency remains to be determined. We have previously demonstrated a strong positive correlation between the severity of DCM and the distance of variant loci from the N-terminus.[8] This positional effect seen in adult patients is at odds however with the early onset of DCM in children with I-band variants.

The earlier presentation and increased severity of disease in children with *TTN*tv compared to adolescents and adults could be due to gene "dose" or combination with a "second hit" genetic or acquired factor. Although the patients in our series were all heterozygous carriers of *TTN*tv, homozygous deletions of the titin M-band region have been associated with a severe recessive congenital myopathy that includes childhood-onset DCM.[13] We found that one of our childhood cases had double truncating variants in the *TTN* and *TBX20* genes, while another had a *TTN* variant and a history of chemotherapy. TBX20 deficiency has been associated with DCM in humans and in mice,[14] and cardiac toxicity is a frequent complication of chemotherapeutic drugs.[15]

Our data suggest that genetic testing for *TTN* variants is not only highly indicated in adult DCM patients but also in those with DCM onset during adolescence. We propose that genetic testing is worthwhile in children with familial and idiopathic DCM although the yield of truncating *TTN* variants is likely to be lower than in adolescents and adults. The discovery of a truncating *TTN* variant in a child with DCM is clinically-significant for that patient and should prompt a search for concurrent cardiomyopathy gene variants and/or acquired DCM risk factors.

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Figure Legends

Figure 1. Distribution of truncating *TTN* variants.

TTN variant loci in DCM patients aged <15 years (red) and 15-21 years (blue), and in healthy volunteers, a population cohort derived from the Framingham Heart Study and the Jackson Heart Study[8] and unselected adult DCM patients.[8] The *TTN* meta transcript is shown with sarcomere regions differentiated by color: Z-disk (red), I-band (blue), A-band (green), and M-band (purple). "Proportion spliced-in" (PSI) for each *TTN* meta-transcript exon in pediatric and adult left ventricle (LV) is shown with exon usage represented by the height of gray bar (0%-100%).

Table 1. Clinical and Genetic Characteristics of Subjects with Childhood-Onset DCM (0 to 14 years)

Subject	Sex	Age at diagnosis (or surgery)	Family history	DCM severity	ECG	Other risk factors	Truncating genetic variants*
DCM-2	M	Birth	No	Heart Tx			
DCM-25	M	Birth	No	Heart Tx		Ventricular septal defect	
DCM-26	F	Birth	No	Heart Tx		Ventricular septal defect	
DCM-33	M	Birth	No	Died 6 yr			
DCM-28	M	2 wks	No	LVEF 37%		Ventricular septal defect	*VCL* p.Q247X
DCM-21	F	6 wks	No	Heart Tx			
CW-III-1	F	6 wks	Yes	Heart Tx	1st degree heart block		
DCM-6	M	2 mth	No	Heart Tx		Atrial septal defect	*ACTN2* p.W259X
UK-1	M	(2 mth)	NA	Heart Tx			
UK-2	F	2 mth	No	Heart Tx			
DCM-1	F	3 mth	No	Heart Tx			
DCM-7	F	3 mth	No	Heart Tx			
DCM-20	F	3 mth	Yes	LVEF 32%			

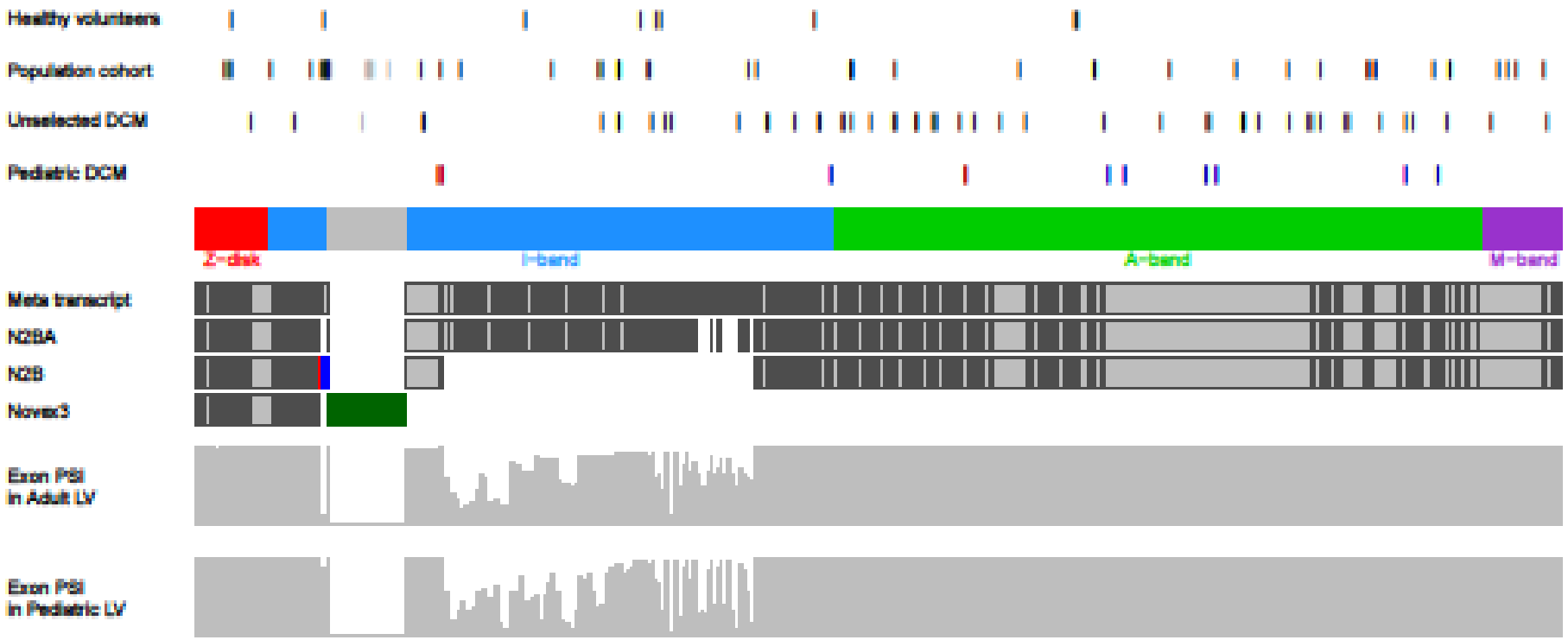
Table 2. Characteristics of truncating *TTN* variants identified in young DCM subjects

Patient ID	Genomic start position (Hg19)	Transcript effect*	Protein effect*	Variant type	*TTN* domain	Exon no.*	Mean exon PSI	Reported disease association	Minor allele frequency (ExAC)
DCM-22	179603994	c.13966C>T	p.Q4656X	Nonsense	I band	49	1.00	Novel	Absent
UK-4	179602935	c.14245C>T	p.R4749X	Nonsense	I band	50	1.00	Novel	Absent
UK-19	179483495	c.46782C>A	p.Y15594X	Nonsense	I/A junction	252	1.00	Adult DCM[8†,10]	Absent
UK-10	179459187	c.58034_58035delCT	p.T19345SfsX2	Frameshift	A band	297	1.00	Novel	Absent
UK-16	179440883	c.69976G>T	p.E23326X	Nonsense	A band	327	1.00	Novel	Absent
UK-27	179439533	c.71326G>T	p.E23776X	Nonsense	A band	327	1.00	Novel	Absent
AV-IV-2	179432761	c.78095_78098delAAAG	p.R26032TfsX41	Frameshift	A band	327	1.00	Novel[8†]	Absent
BR-IV-1	179431868	c.78991C>T	p.R26331X	Nonsense	A band	327	1.00	Adult DCM[8†]	Absent
AG-III-1	179411203	c.94855C>T	p.R31619X	Nonsense	A band	343	1.00	Novel[8†]	Absent
DCM-30	179406990	c.97492+1G>C		Splice site	A band	350	1.00	Adult DCM[7]	Absent

ExAC, Exome Aggregation Consortium database; PSI, proportion spliced-in.

* Variants are annotated according to position in meta-transcript (LRG_391_t1); exon numbering is in accordance with HAVANA recommendations.

† This patient was reported in Roberts et al[8]; p.R26331X was present in one additional adult-onset familial DCM proband in that series.


Healthy volunteers
Population cohort
Unselected DCM
Pediatric DCM
Z-disk
I-band
A-band
M-band
Meta transcript
N2BA
N2B
Novex3
Exon PSI
in Adult LV
Exon PSI
in Pediatric LV