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Relevance of Truncating Titin Mutations in Dilated Cardiomyopathy

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Conflict Of Interest

Authors disclosure: OA: None. JWK and TPA: Co-founders of Blueprint Genetics.
The funders had no role in the design, collection, analysis or interpretation of data or in the writing of this article

Acknowledgements

This work was supported by grants from the Sigrid Jusélius Foundation; Finnish Foundation for Pediatric Research; Finnish Foundation for Cardiovascular Research; Finnish Cultural Foundation, Orion Research Foundation, and Ida Montin Foundation. We would like to thank Exome Aggregation Consortium; and the groups that provided exome variant data for comparison. A full list of contributing groups can be found at http://exac.broadinstitute.org/about. Furthermore, we would like to acknowledge the authors of the four studies on DCM whose cohorts have represented the aggregated DCM cohorts in this study.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cge.12741

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Abstract

Dilated cardiomyopathy (DCM), a genetically heterogeneous cardiac disease characterized by left ventricular dilatation and systolic dysfunction, is caused majorly by truncations of titin (*TTN*), especially in A-band region. Clinical interpretation of *TTN* truncating variants (TTNtv) has been challenged by the existing inaccurate variant assessment strategies and uncertainty in the true frequency of TTNtv across the general population. We aggregated TTNtv identified in 1788 DCM patients and compared the variants with those reported in the over 60,000 Exome Aggregation Consortium (ExAC) reference population. We implemented our current variant assessment strategy that prioritizes TTNtv affecting all transcripts of the gene, and observed a decline in the prevalence of TTNtv in DCM. Despite this decline, TTNtv are more prevalent in DCM patients compared to reference population ($P = 4.1 \times 10^{-295}$). Moreover, our extended analyses confirmed the enrichment of TTNtv not only in the A-band but also in the I/A-band junction of *TTN*. We estimated the probability of pathogenicity of TTNtv affecting all transcripts of *TTN*, identified in unselected DCM patients to be 97.8% [LR = 42.2]. We emphasize that identifying a TTNtv, especially in the A-band region, have a higher risk of being disease-causing than previously anticipated, and recommend prioritizing TTNtv affecting at least five transcripts of the gene.

Key Words: Cardiomyopathy, Titin, genetic variation

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Introduction

Dilated cardiomyopathy (DCM) is the leading cause for heart transplantation, and a relatively frequent cause for heart failure and sudden cardiac death (1, 2). The estimated prevalence of DCM is at least 1:2500 and up to 30 – 50% of DCM cases are familial (3). DCM is a genetically heterogeneous disorder caused by mutations in genes encoding the components of the sarcomere, desmosome, cytoskeleton, nuclear lamina, mitochondria, and calcium-handling proteins (4, 5). Though most DCM causing mutations are unique to families, recent studies have identified same disease-causing variants in multiple DCM families (6-8). DCM causing mutations are predominantly inherited in an autosomal dominant fashion although some can be autosomal recessive, X-linked or mitochondrial.

Truncating titin (*TTN*) mutations, especially in A-band region, represent the most common cause of DCM. Recently, our research group among the others have found *TTN* truncating variants (TTNtv) - nonsense, frameshift and essential splice site, to be responsible for approximately 25% of familial cases of idiopathic DCM and 18% of sporadic cases in large patient cohorts (8-11). Collectively, TTNtv account for 14 – 20% of DCM cases. These findings have reshaped our understanding of DCM pathogenesis and improved the utility of genetic testing among these patients. Interestingly, TTNtv causing DCM in majority of the studies were not randomly distributed along the *TTN* gene, as majority of the mutations were located in the A-band region and affecting all transcripts of *TTN* (8, 10).

Furthermore, Roberts *et al*. (10) estimated a truncating variant in the A-band region to have 93% risk of being disease-causing. They also determined that C-terminal truncations affecting all transcripts were more pathogenic and mediated their effects through dominant negative mechanisms rather than haploinsufficiency. In spite of

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these findings, clinical interpretation of these variants can be challenging, as the true frequency of these variants in the general population is obscure.

Until now, it has been shown that TTNtv are prevalent in general population complicating the interpretation and classification of TTNtv in clinical diagnostics. In 2012, using the version 2 of the phase 1 integrated variant call set from the 1000 Genomes Project Cohort, Golbus *et al*. (12) reported a high prevalence, 3.2%; of *TTN* frameshift mutations (excluding nonsense mutation) in the general population. In the study by Roberts *et al*. (10), the prevalence of TTNtv was estimated 2% using a combination of 1000 Genomes call set (phase 1 version 3) and Exome Sequencing Project (ESP) call set (13, 14). Most recently, by evaluating publicly available reference populations, including, for the first time, data from Exome Aggregation Consortium (ExAC) reference population of over 60,000 individuals (15), we showed that TTNtv affecting all transcripts of the gene occur in only about 0.36% of the general population, and when evaluating the mutation types separately, the prevalence of each mutation type in A-band was lower than 0.1% (16). Moreover, we showed how quality of data analysis and larger number of reference individuals in 1000 Genomes Project has markedly reduced the prevalence of TTNtv in this database. Furthermore, we provided insights on the position, type and frequency of nucleotide change(s) leading to nonsense mutations in *TTN*, and showed that non-essential splice-site variants in *TTN* have a tendency of being frequent in the general population.

In this study, using TTNtv reported in over 1700 DCM patients from four major studies and the largest available ExAC reference population, we compared the spectrum, type and location of TTNtv identified in DCM patients and the general population, in a bid to re-evaluate the relevance of TTNtv in DCM in the light of the

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current knowledge on low prevalence of potentially disease causing TTNtv in general population.

Materials And Methods

Analysis of *TTN* truncations in DCM cohorts

We collected TTNtv identified in DCM patients from four major studies: Herman *et al*. (9); Haas *et al*. (11); Roberts *et al*. (10); and Akinrinade *et al*. (8), herewith referred to as studies/cohorts: A, B, C, and D respectively. The variants were merged by genomic position and variant type. TTNtv reported by more than one study were flagged and the frequency noted. All the variants from the four DCM cohorts were reported in hg19 coordinates. Only variants that passed all quality filters as presented by the individual studies were included.

Analysis of *TTN* Truncations in the Population

TTNtv reported in the largest available reference population, Exome Aggregation Consortium (ExAC, accessed 15 January, 2015) cohort, with over 60 000 individuals were analysed as described earlier (16). Only calls that passed all quality filters were used for downstream analysis.

Mapping of TTNtv amino acid positions to the Meta transcript

Using the genomic location of the variants, we converted the amino acid position of each variant in different transcripts as reported by the various studies to the respective positions in the inferred Meta transcript (ENST00000589042 / NM_001267550) to allow the inclusion of exons and variants not present in the canonical transcripts (ENST00000591111 / NM_001256850), otherwise referred to as

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the principal cardiac long isoform (N2BA), with the Uniprot ID Q8WZ42-1. The protein sequence of the inferred Meta transcript was downloaded from Ensembl Genome Browser (www.ensembl.org), and aligned with the Uniprot titin sequence Q8WZ42-1 using Uniprot's online sequence alignment tool (http://www.uniprot.org). Thus, protein domain annotation for the Uniprot concensus sequence Q8WZ42-1 was transferred to the inferred Meta transcript. TTNtv identified in DCM patients were compared with those detected in more than 60,000 individuals in the ExAC database.

Statistical Analyses

All statistical analyses were done using R statistical software. Comparisons between groups were performed with either Fisher's exact or Wilcoxon-Mann-Whitney tests, as appropriate. Bonferroni correction was performed on all analyses to allow for multiple testing. Odds ratios for DCM patients versus reference population cohort were calculated, and confidence intervals were determined using the conditional maximum likelihood / Fishers' method.

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Results

DCM Cohorts

DCM cohort in this study represents an aggregation of 1788 DCM patients from four major studies that have utilized either next generation sequencing (NGS) technnology and/or dideoxy sequencing to study the contribution of TTNtv to DCM (8-11). Details on the study cohort are presented in Table 1.

TTNtv in DCM Cohort

Total number, type and prevalence of TTNtv identified in each of the four DCM cohorts before and after variant assessment are summarized in Table 1. After merging all TTNtv identified in the different cohorts and excluding four missense variants classified as "other splice site" in cohorts A and C, we identified a total of 243 unique TTNtv affecting *TTN* isoforms that span the sarcomere: 104 frameshifts, 104 nonsense and 35 splice variants. Twenty-three variants: seven frameshift; nine nonsense; and 3 splice variants, were identified in at least two cohorts, with the highest overlap occuring between cohorts A and C sharing nineteen TTNtv, as some individuals in cohort A were parts of cohort C. Cohorts A and B have only one TTNtv in common while cohorts B and C shared three TTNtv. Of note, cohort D representing the Finnish DCM patients shared no variant with the other cohorts. Interestingly, 83% (202/243) of the TTNtv identified in DCM cohort were private.

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TTNtv in ExAC Reference Cohort

We identified a total of 470 TTNtv in the ExAC reference population. After excluding novex-3 isoform exon specific variants, and filtering for the number of transcripts affected, we obtained a total of 228 TTNtv. As no validation data was available, the variants were further filtered for coverage as described previously (16). Consequently, only 173 TTNtv were included in the downstream analyses. Of note, a vast majority of all TTNtv (87%) identified in the ExAC reference population cohort are private.

Variant assessment and filtering

To ensure uniformity in variant classification and interpretation, aggregated variants identified in DCM cohorts and those identified in ExAC reference population cohort were evaluated based on our current classification scheme used in Akinrinade *et al*. (8, 16) and assessed on the number of *TTN* transcripts affected. Briefly, TTNtv were filtered and assessed based on the following criteria: quality score, sequencing coverage / depth, frequency in publicly available reference population databases (including 1000 Genomes Project database, Exome Sequencing Project database), and number of transcripts the variant impacts, and all variants were classified as previously described (8). Furthermore, TTNtv identified in DCM cohorts were compared with the TTNtv in ExAC database and overlapping variants were either flagged or filtered based on ExAC frequency. We identified seven non-essential splice-site variants classified as "other splice site" by Herman *et al*. (9) and Roberts *et al*. (10) with elevated allele copies in the population for which the author did not confirm their splicing effect (Supplementary Table S1). Applying number of

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transcript-affected filter, a total of fourteen TTNtv were further filtered as they only affected ≤3 transcripts of the gene. Of note, such exons present in less than five transcripts have low expression in the left ventricle, a term coined by Roberts *et al.* (10) as Proportion Spliced In (PSI) value. Interestingly, our approach using the number of transcript affected correlates with the PSI value (*rho = 0.946*). In total, only 91% (222/243) of TTNtv identified in DCM cohorts passed our assessment criteria.

When comparing TTNtv in DCM patients with those identified in ExAC reference population, we identified twenty-two variants common to both groups. Interestingly, 64% of such variants in the ExAC cohorts were private. Moreover, 51% (242/470) of the TTNtv identified in the reference population affected less than three transcripts of the gene and were located in exons with low expression in the human left ventricle, with 19% (88/470) being unique to novex-3 isoform specific exon.

Distribution of TTNtv in DCM patients and in the general population

In this multi-cohorts study, 222 TTNtv were identified in DCM patients and 173 TTNtv in general population (ExAC cohort) that affect all transcripts of *TTN*. These variants were found in 278 DCM patients and 219 individuals in the cohorts. To validate and expand on the previous report of A-band clustering of TTNtv in DCM patients, we compared the prevalence, spectrum and distribution of TTNtv in DCM patients with those observed in ExAC reference cohort.

We observed that TTNtv affecting all transcripts of the gene were more prevalent in patients with DCM (278 of 1788 [15.6%]) compared with reference population cohort (219 of 60706 [0.4%], $P = 4.1 \times 10^{-295}$; OR = 50.8; 99% confidence interval: 39.72–65.05). When assessing the variant type, nonsense variant occurred in 7.5% of

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DCM patients compared with 0.11% of the reference population ($P = 3.8 \times 10^{-156}$; OR = 73.3; 99% confidence interval: 49.34 – 110.81) (Table 1).

We observed a non-random distribution of TTNtv within and between study groups (Figure 1). *TTN* A-band was enriched for TTNtv in DCM patients compared with controls ($P = 1.3 \times 10^{-251}$; OR = 70.4; 99% confidence interval: 52.08–96.35). Furthermore, our data showed also a significant distal I-band (I/A-band junction) enrichment of TTNtv in DCM patients as compared with reference population cohort ($P = 1.9 \times 10^{-46}$; OR = 39.1; 99% confidence interval: 21.77 – 70.79).

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Discussion

In this study, we assessed the relevance of *TTN* truncating mutations in DCM in the light of our recent report of low frequency of TTNtv affecting all transcript of the gene in the population. Our DCM cohort represents an aggregation of 1788 DCM patients from four major studies that have utilized either next generation sequencing (NGS) technology and/or dideoxy sequencing to study the contribution of TTNtv to DCM. We emphasize the need for rigorous clinically oriented variant classification and interpretation strategy in diagnostic interpretation of TTNtv. By evaluating TTNtv identified in the ExAC reference population of over 60,000 individuals and the largest available aggregated DCM cohort (1788), we showed that TTNtv affecting all transcripts are more prevalent in DCM patients and that such variants have at least 97% risk of being diseasing-causing when identified in unselected DCM patient. Moreover, our extended analyses also confirmed the enrichment of TTNtv not only in the A-band but also in the distal I-band region (I/A-band junction), affecting all transcripts of the gene. This is to our knowledge the first study to report the enrichment of I/A-band junction TTNtv in DCM patient.

The genetic spectrum of DCM is heterogeneous and multiple genes are involved. Truncations of *TTN*, especially in A-band region, represent a significant cause of DCM. Several studies have reported TTNtv to be responsible for varying proportion of DCM. Gerull *et al*. (17) reported the first observation of TTNtv in large DCM family in 2002 but due to remarkable challenge to sequence gigantic *TTN* gene thoroughly in large patient cohorts, it took until 2012 when Herman *et al*. (9) reported TTNtv to account for 21.8% of DCM. Since then, other studies have been reporting lower proportion between 14.1 – 17.9% (8, 10, 11). This variation has largely been due not only to population variations and proportion of familial cases in the individual

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cohorts, but also varying variant assessment strategies, and the uncertainties regarding the prevalence of TTNtv in the population that has compounded interpretation of TTNtv identified in health and disease. Herman *et al*. (9) and Roberts *et al*. (10) included non-essential splice-site variants in their estimation of TTNtv in DCM. Moreover, the studies included missense variants located in the splice region in the estimate. Of note, majority of the missense variants are benign polymorphisms based on their frequency in the ExAC cohort (Table S1). In our recent study (16), we described how increased quality of data analysis and larger number of reference individuals in 1000 Genomes Project has markedly reduced the prevalence of TTNtv in this database, and showed that non-essential splice-site variants in *TTN* have a tendency of being frequent in general population. This delineates the fact that the existing inaccurate variant-disease associations pose a major challenge to clinical variant interpretation and classification, and accentuate the need for a rigorous clinically oriented variant classification and interpretation strategy. By evaluating the ExAC reference population of over 60,000 individuals, we showed that TTNtv affecting all transcripts occur in 0.36% (0.32% - 0.41%, 95% CI) of the general population. In this regards, we aggregated TTNtv identified in 1788 DCM patients and obtained a total prevalence of 17.3% (15.6% - 19.1%, 95% CI) before applying our variant assessment strategy.

Using a combination of exome sequencing and genome-wide linkage analysis, Norton *et al*. (18) identified six TTNtv in regions with high logarithm of odds [LOD] score in 7 out of 17 families with DCM, two novel nonsegregating TTNtv, and illustrated the challenge in determining variant pathogenicity. However, accumulated evidence from clinical practice have shown that co-segregation of TTNtv affecting all or multiple transcripts of the gene is higher compared with those affecting few or only

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the short isoform of *TTN*. Moreover, recent studies suggest that variants affecting only a subset of gene transcripts are less likely to cause loss of function than variants affecting all isoforms (19). Consequently, our current variant assessment strategy prioritizes TTNtv affecting at least five out of seven transcripts of the gene as described in the National Center for Biotechnology Information (NCBI) database. Implementing our variant assessment strategy, only 91% (222/243) of all TTNtv identified in DCM patient fulfilled this criterion. Of note, after implementing our current variant assessment and interpretation strategy, the proportion of TTNtv positive DCM patient in cohort A reduced to 18.9%; 13.8% in cohort B; 15.0% in cohort C; and remained unchanged in cohort D (17.2%). Put together, 15.6% (13.9% - 17.3%, 95% CI) of unselected DCM patients carried a clinically significant TTNtv, though this could be higher when considering only those with family history of the disease. This however suggests that the contribution of TTNtv to DCM could have been over-estimated by Herman *et al*. (9)

Recently, Roberts *et al*. (10) estimated that observing a TTNtv produced by nonsense, frameshift, or canonical splice site mutations that affect highly expressed exons in the left ventricle (with PSI > 0.9) had a 93% probability of pathogenicity when identified in an unselected DCM patient; anticipated a higher probability of pathogenicity when such variants are identified in end-stage disease, and expected the probability to be even higher when segregation data are available. Of note, we observed that the PSI value of >0.9 correlates to exons present in at least five transcripts of the gene. Interestingly, 51% (242/470) of the TTNtv identified in the reference population affected less than three transcripts confirming/suggesting that a vast majority of TTNtv identified in the reference population have low probability of pathogenicity. In estimating this probability of pathogenicity, Roberts *et al*. (10) used

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TTNtv frequency of 0.79% in controls and 12% in unselected DCM patients respectively. Plunging our current statistics of TTNtv: 15.55% in DCM vs. 0.36% in reference population cohort; in Roberts *et al*. (10) formula, we estimated the probability of pathogenicity of TTNtv affecting all transcripts of *TTN* and identified in unselected DCM patient to be 97.8% [LR = 42.2].

Despite the observed decline in the proportion of DCM patients with a clinically meaningful TTNtv as shown in this study, current data supports and confirms the previous report that TTNtv are more prevalent in DCM patients compared to ExAC reference population cohort (P = 4.1 x 10^{-295}; OR = 50.8; 99% CI: 39.72 – 65.05). We report here that 12.3% of DCM patients carried TTNtv in the A-band region of *TTN* as against 0.19% of the ExAC reference population cohort (P = 1.3 x 10^{-251}; OR = 70.4; 99% confidence interval: 52.08–96.35). Contrary to the previous report on the paucity of I-band region TTNtv in DCM patients compared with controls (9), our data suggests an enrichment of I/A-band junction (distal I-band) TTNtv in DCM patients compared to reference population cohort (P = 1.9 x 10^{-46}; OR = 39.1; 99% CI: 21.77 – 70.79).

Assessing the distribution of TTNtv among A-band exons (exons 252 - 357), we observed an enrichment of TTNtv in exon 326 located in the C-zone of the A-band region, which apparently is the largest exon in *TTN*. It is however not clear whether this enrichment is due to the large size of the exon or that TTNtv located in this exon are highly detrimental to sarcomere function and be incompatible with life, and therefore are not seen or rare in reference population. Interestingly, using stringent bioinformatics filtering criteria, recent effort assessing the contribution of *TTN* missense mutations to DCM reported an over-represention of “likely” or “possibly” pathogenic *TTN* “severe” missense variants in the C-zone of the A-band region of the

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sarcomere (20). Furthermore, Gerull *et al*. (17) found a segregating 2bp insertion mutation (c.43628insAT), which causes a frame shift, thereby truncating A-band titin. Upon cloning the 2bp insertion mutation in human exon 326 and introducing it into the mouse genome, Gramlich *et al*. (21) reported that homozygous mice die in utero before ED9.0 and heterozygous mice recapitulate the human DCM phenotype. These probably suggest that TTNtv in exon 326 could be highly detrimental to sarcomere function and as such the exon represents a mutation hotspot in the gene.

Given that 87% of TTNtv, affecting all transcripts, identified in the population are private, and that A-band TTNtv identified in the reference population mirrors those identified in DCM patients (Figure 1), we suggest genotype positive individuals in the reference population with A-band TTNtv might represent 'genotype positive yet phenotype negative/asymptomatic patients' and are likely to develop DCM in the future. It is supported by the fact that cardiomyopathies are known to have age-dependent disease penetrance and especially high penetrance (95% by age of 40 in cohort A and 100% by age of 70 in cohort D) of DCM in families with TTNtv. If this assumption is right, TTNtv pose even higher risk to be disease causing than we observed in this study. On the other hand, it could be that such mutations need a second mutation to manifest, thus, suggesting recessive TTNtv. This highlights the need for further studies to fully understand the relevance of TTNtv and the phenotypic spectrum of cardiac disease associated with titin dysfunction.

In conclusion, our results confirm that TTNtv, especially in A-band region, represents a significant cause of DCM and that identifying a truncating variant affecting all transcripts of the gene has at least 97% risk of being disease-causing. Considering the existence of inaccurate variant assessment strategy that has compounded diagnostic interpretation of TTNtv, we recommend prioritizing TTNtv

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affecting at least five transcripts of the gene as disease-causing variants. Unless supported by RNA level evidence of splicing defect, we recommend classifying rare non-essential splice-site variants as variants of uncertain significance (VUS).

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References

1. Codd MB, Sugrue DD, Gersh BJ et al. Epidemiology of idiopathic dilated and hypertrophic cardiomyopathy. A population-based study in Olmsted County, Minnesota, 1975-1984. Circulation 1989: 80: 564-572.
2. John R, Rajasinghe HA, Chen JM et al. Long-term outcomes after cardiac transplantation: an experience based on different eras of immunosuppressive therapy. The Annals of thoracic surgery 2001: 72: 440-449.
3. Towbin JA, Bowles NE. The failing heart. Nature 2002: 415: 227-233.
4. Ahmad F, Seidman JG, Seidman CE. The genetic basis for cardiac remodeling. Annual review of genomics and human genetics 2005: 6: 185-216.
5. Dellefave L, McNally EM. The genetics of dilated cardiomyopathy. Current opinion in cardiology 2010: 25: 198-204.
6. Kamisago M, Sharma SD, DePalma SR et al. Mutations in sarcomere protein genes as a cause of dilated cardiomyopathy. The New England journal of medicine 2000: 343: 1688-1696.
7. Li D, Morales A, Gonzalez-Quintana J et al. Identification of novel mutations in RBM20 in patients with dilated cardiomyopathy. Clinical and translational science 2010: 3: 90-97.
8. Akinrinade O, Ollila L, Vattulainen S et al. Genetics and genotype-phenotype correlations in Finnish patients with dilated cardiomyopathy. European heart journal 2015: 36: 2327-2337.
9. Herman DS, Lam L, Taylor MR et al. Truncations of titin causing dilated cardiomyopathy. The New England journal of medicine 2012: 366: 619-628.
10. Roberts AM, Ware JS, Herman DS et al. Integrated allelic, transcriptional, and phenomic dissection of the cardiac effects of titin truncations in health and disease. Science translational medicine 2015: 7: 270ra276.
11. Haas J, Frese KS, Peil B et al. Atlas of the clinical genetics of human dilated cardiomyopathy. European heart journal 2014.
12. Golbus JR, Puckelwartz MJ, Fahrenbach JP et al. Population-based variation in cardiomyopathy genes. Circulation Cardiovascular genetics 2012: 5: 391-399.
13. Genomes Project C, Abecasis GR, Altshuler D et al. A map of human genome variation from population-scale sequencing. Nature 2010: 467: 1061-1073.
14. NHLBI. Exome Variant Server, NHLBI Exome Sequencing Project (ESP), Seattle, WA (URL: http://evs.gs.washington.edu/EVS/) Vol. 2015, 2015.
15. ExAC. Exome Aggregation Consortium (ExAC), Cambridge, MA (URL: http://exac.broadinstitute.org). Vol. 2015, 2015.
16. Akinrinade O, Koskenvuo JW, Alastalo TP. Prevalence of Titin Truncating Variants in General Population. PloS one 2015: 10: e0145284.
17. Gerull B, Gramlich M, Atherton J et al. Mutations of TTN, encoding the giant muscle filament titin, cause familial dilated cardiomyopathy. Nature genetics 2002: 30: 201-204.
18. Norton N, Li D, Rampersaud E et al. Exome sequencing and genome-wide linkage analysis in 17 families illustrate the complex contribution of TTN truncating variants to dilated cardiomyopathy. Circulation Cardiovascular genetics 2013: 6: 144-153.
19. MacArthur DG, Balasubramanian S, Frankish A et al. A systematic survey of loss-of-function variants in human protein-coding genes. Science 2012: 335: 823-828.
20. Begay RL, Graw S, Sinagra G et al. Role of Titin Missense Variants in Dilated Cardiomyopathy. Journal of the American Heart Association 2015: 4.

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21. Gramlich M, Michely B, Krohne C et al. Stress-induced dilated cardiomyopathy in a knock-in mouse model mimicking human titin-based disease. Journal of molecular and cellular cardiology 2009: 47: 352-358.

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Figure legends

Figure 1. Spatial distribution of titin frameshift, nonsense and splice-site mutations identified in health and disease. Titin is linearly depicted with its 152 Ig-like domains in green and 132 fibronectin type III domains in purple. TTNtv are shown as coloured bars. Depicted are nonsense (red), frameshift (turquoise) and splice-site (olivedrab) mutations identified in ExAC control cohort (bar plot above) and in DCM cohort (bar plot below). Bisque bar represents the number of transcripts of the gene affected. Variants are shown relative to the Meta transcript position. The dark grey bars indicate exons unique to the Meta transcript. The dashed lines below the number of transcripts schematic indicate the location of variants within the sarcomere.

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Table

Table 1. Number, type and distribution of TTNtv in DCM cohorts and reference population cohort.

	Cohort A (n=312)	Cohort B (n=639)	Cohort C (n=692)	Cohort D (n=145)	All DCM cohorts (n=1788)	ExAC cohort (n=60706)	P-value DCM Versus Controls
Before variant assessment							
TTNtv, n	62	76	113	21	243	470	
TTNtv +ve, n (%)	68 (21.8)	90 (14.1)	124 (17.9)	25 (17.2)	309 (17.28)	813 (1.34)	6.3×10^{-203}
Variant type							
Frameshift variants	20	40	54	10	124 (6.94)	258 (0.42)	9.1×10^{-94}
Nonsense variants	28	46	46	16	136 (7.60)	258 (0.42)	5.8×10^{-107}
Essential splice site	16	4	21	1	42 (2.35)	297 (0.49)	2.3×10^{-15}
Other splice region*	4	-	3	-	7 (0.39)	-	
After variant assessment (Including only variants affecting ≥5 transcripts), n (%)							
TTNtv, n	54	74	96	19	222	173	
TTNtv +ve, n (%)	59 (18.9)	88 (13.8)	106 (15.0)	25 (17.2)	278 (15.55)	219 (0.36)	4.1×10^{-295}
Variant type, n (%)							
Frameshift variants	19	39	52	8	118 (6.60)	95 (0.16)	2.6×10^{-123}
Nonsense variants	28	45	45	16	134 (7.49)	67 (0.11)	3.8×10^{-156}
Essential splice site	12	4	9	1	26 (1.45)	58 (0.09)	4.4×10^{-20}
Distribution by sarcomere domain, n (%)							
Z-band	-	2	-	1	3 (0.17)	19 (0.03)	2.4×10^{-02}
I-band	5	19	17	4	45 (2.52)	40 (0.07)	1.9×10^{-46}
A-band	54	61	84	20	219 (12.25)	120 (0.19)	1.3×10^{-251}
M-band	-	6	5	-	11 (0.62)	33 (0.05)	3.2×10^{-08}

Numbers (proportions) of subjects with a TTNtv are shown for each cohort before and after critical variant assessment to include only variants affecting ≥5 transcripts of TTN. Comparison between DCM cohorts and the ExAC cohort were assessed by Fisher's exact test. * Other splice region variants were excluded, as their effects on splicing were not confirmed by the authors.

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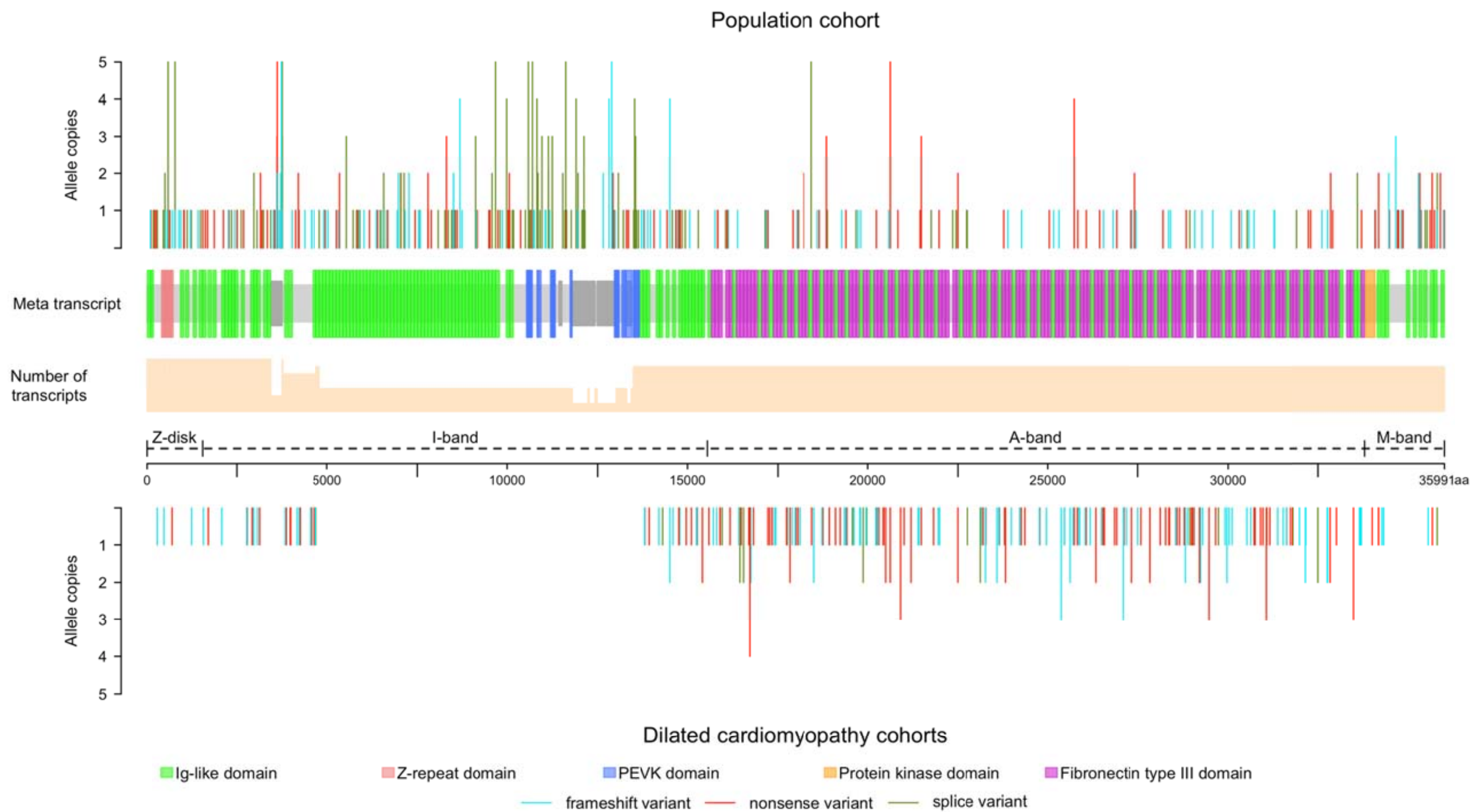

Figure 1.

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