Role of titin in dilated cardiomyopathy: from DNA variant to patient stratification

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Abstract | Dilated cardiomyopathy (DCM) affects approximately~~up to~~ 1 in 250 individuals

and is the leading indication for heart transplantation. DCM is often familial, and the most

common genetic predisposition is truncating variation in the giant sarcomeric protein titin,

which occurs in up to 15% of ambulant cases of DCM and 25% of end-stage or familial

cases. In this article, we review the evidence for the role of titin truncation in the

pathogenesis of DCM, and our understanding of the molecular mechanisms and

pathophysiological consequences of variation in the gene encoding titin (*TTN)*. Such

variation is common in the general population (up to 1% of individuals), and we consider key

features that discriminate variants with disease-causing potential from those that are benign.

We summarize strategies for clinical genetic interpretation of variants for use in diagnosis of

patients and evaluation of their relatives. Finally, we consider the contemporary and potential

future role for genetic stratification in cardiomyopathy and the general population, evaluating titin variation as a predictor of outcome and treatment response for precision medicine.

Titin is the largest human protein and a crucial component of all striated muscle, where it has structural, sensory, and signalling functions. Titin can be considered to be a molecular bidirectional spring, contributing to the contraction and relaxation of striated muscle[1], with additional roles in sarcomere organization[2], force transmission and transduction[3], and signalling responses[4,5]. A single molecule of titin spans half a sarcomere, with the N-terminal portion embedding within the Z-disc, and the C-terminus in the M-line[6–8] (FIG. 1a). The I-band of the molecule comprises mainly extensile elastic elements that uncoil during muscle relaxation and recoil during contraction, whereas the A-band is closely associated with the thick filament of the sarcomere. The full-length protein comprises 27,000–33,000 amino acids[9], with a molecular weight of approximately 3,800 kDa (the median length of a human protein is 375 amino acids)[10].

In humans, titin is encoded by a single gene[11] (*TTN*) comprising 364 exons that undergo extensive differential splicing, particularly in the I-band, across tissues and during development and disease (FIGS. 1b and 1c). In the heart, TTN isoforms can be grouped into at least four families: long and short isoforms that span the sarcomere (known as N2BA, >300 exons, and N2B, ~190 exons), ultra-long fetal isoforms that similarly span the sarcomere, and very short isoforms that do not span the sarcomere. The N2BA, N2B, and fetal sarcomere-spanning forms have the same terminal sequences, but differ in the content of the I-band spring elements, which are spliced out of the shorter form as symmetric cassettes[9], maintaining the reading frame of the protein. Considerable additional variability exists in the

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precise exon composition of individual titin molecules, which is not well characterized. For convenience, these isoforms can be succinctly represented by four distinct molecules: a short, stiff form that predominates in the adult left ventricle[12,13]; a long, compliant form that is upregulated in heart failure syndromes, including dilated cardiomyopathy (DCM)[13–16]; even longer forms expressed during development[17]; and a much shorter 46-exon isoform, often referred to as the Novex-3 isoform[11], that shares the N-terminal sequence, but utilizes an alternative C-terminal exon, and does not span the sarcomere. The physiological role of the Novex-3 isoform is unclear. In the past 2 years, several alternative N-terminal start sites have been identified[18,19], although their relevance and roles are not fully understood.

DCM is defined by left ventricular or biventricular systolic dysfunction and dilatation not explained by abnormal loading conditions or coronary disease {Pinto Eur. Heart J. 14, 1850-1858 (2016). - REF 75}, and affects approximately 1 in 250 individuals {Hershberger Nat. Rev. Cardiol. 10, 531–547 (2013) - REF 80 }. Causes of DCM can be classified as genetic or non-genetic {Elliott et al Eur Heart J. 29(2):270-6. (2008) - NEW REF}, with TTNtv the most prevalent genetic cause {Herman N. Engl. J. Med. 366, 619–628 (2012) - REF 26}, and non-genetic causes including drugs and toxins (most commonly alcohol or chemotherapy), myocarditis, and pregnancy {Pinto Eur. Heart J. 14, 1850-1858 (2016). - REF 75}, though it is increasingly recognised that a genetic predisposition may also interact with extrinsic or environmental factors e.g. {Ware N. Engl. J. Med. 374, 233–241 (2016) - reference 37}. Despite improvements in pharmacological and device-based therapy, clinical outcomes remain poor, with a 20% 5-year mortality rate {Kober et al, N Engl J Med 375:1221-1230 (2016) - NEW REF; Gulati JAMA, 309:896-908 (2013) - NEW REF}, and DCM as the leading cause of heart transplantation {Maron Circulation, 113:1807-1816 (2006) - NEW REF; Stehlik et al Journal of Heart and Lung Transplantation 30:1078-1094 (2011) - NEW REF}.

In this Review, we discuss the current understanding of cardiomyopathy associated with alterations in titin, with a broad overview of the gene, the effect of variants in *TTN* and their clinical interpretation, the molecular and physiological consequences of pathogenic *TTN*-truncating variants (*TTN*tv), and the future application of genetic stratification for precision medicine in patients with DCM.

[H1] Titin in heart disease

Titin was first implicated in human genetic disease in 1998, when linkage analysis mapped a tibial muscular dystrophy locus to chromosome 2q31[20]. The following year, an autosomal dominant DCM was also mapped to 2q31[21]. In each study the locus was noted to contain *TTN*, which was highlighted as a strong candidate gene, but in neither study was *TTN* demonstrated conclusively to be the cause of the disease. In 2002, titin variants were confirmed to cause DCM by demonstrating linkage of distinct *TTN* mutations in two unrelated pedigrees[22], while a *TTN*-mutant zebrafish displayed a DCM-like phenotype in a linked article in the same journal issue[23].

Titin was, therefore, shown to be causative in some cases of DCM in isolated families, but its overall contribution to inherited cardiomyopathy remained unknown. Comprehensive DNA sequencing of *TTN* was not possible with the technology of the time, given the size of the gene. In subsequent years, additional isolated families with *TTN* variants causing typical adult-onset DCM[24], and an early-onset recessive disease characterized by both skeletal and cardiac myopathy[25] were identified by linkage analysis. However, it was not until a decade later, in 2012, that the importance of titin as a major cardiac disease gene was fully appreciated. High-throughput DNA sequencing technology, newly available at that time, allowed comprehensive analysis of *TTN* in substantial cohorts of patients with end-stage or familial DCM ($n = 312$), and hypertrophic cardiomyopathy (HCM; $n = 231$), as well

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as healthy control individuals ($n = 249$)[26]. *TTN*tv (variants predicted to yield a truncated

protein if transcribed and translated, including nonsense variants, frameshift insertions and

deletions, and canonical splice-disrupting variants) were found in up to 27% of patients with

DCM, significantly enriched compared with controls (3%; $P = 9 \times 10^{-14}$) and patients with

HCM (1%; $P = 3 \times 10^{-16}$), and variants co-segregated with DCM in families (combined LOD

(logarithm of the odds) score 11.1), revealing *TTN*tv as the largest known genetic contributor

to DCM[26].

The association between DCM and *TTN*tv has subsequently been widely replicated[27–34] {very recent addition: TAYAL et al, JACC 18:2264-2274 (2017)}, with *TTN*tv accounting

for ~15% of DCM cases in the largest series of unselected patients[30,33] (although much more

rarely identified in paediatric cardiomyopathy[28,35]). *TTN* sequencing has become adopted

routinely in diagnostic laboratories, markedly increasing the yield and, therefore, utility of

genetic testing in DCM for confirmatory and familial screening.

As well as autosomal dominant DCM, *TTN* variants have been associated with other

phenotypes, including peripartum cardiomyopathy[36,37] and a penetrant cardiomyopathy with

predominant left ventricular noncompaction and impairment, but marked hypertrophy in

some family members[38]. *TTN* variants have also been implicated in a range of skeletal

myopathies[25,39–43], some of which (typically recessive forms) are also characterized by

cardiac abnormalities (reviewed previously[44]). Relatives of patients with recessive

titinopathies, who carry variants in the heterozygous state, can manifest cardiac pathologies[39],

and cardiac evaluation of these individuals should be considered. Although individual rare

missense variants have been reported in patients with HCM[45–47], some with evidence of

altered protein function, rare *TTN* variants are collectively prevalent in the general

population, and we are not aware of published data robustly demonstrating an increased

frequency of inherited or *de novo* variants in HCM, or familial segregation, to confirm this disease association.

Many other genes reported to be associated with DCM interact with titin, and some might act by altering *TTN* biology. For example, variants in the splice repressor RNA-binding protein 20 (encoded by *RBM20*), which regulates *TTN* splicing[48], are strongly associated with DCM, and disease-associated *RBM20* variants alter *TTN* splicing in rodent models and humans[49,50], leading to alterations in titin isoform composition and in cardiac physiology[50].

Altered *TTN* biology has also been implicated in the pathogenesis of, or molecular adaption to, several cardiac disease states. These modifications include changes in titin isoform composition, phosphorylation status, and other post-translational modifications in ischaemic cardiomyopathy, DCM, and heart failure with preserved ejection fraction. These modifications are beyond the scope of this Review, but have been discussed previously[1,51]. The remainder of this Review is focused primarily on the role of *TTN* variants, particularly *TTN*tv, in DCM.

[H1] *TTN*-truncating variants

[H3] *Molecular mechanisms*

Genetic association between a gene and a variant class can point to disease mechanism, but the experimental elucidation of mechanism is far from trivial (BOX 1). Transcripts containing a protein-truncating variant may undergo nonsense-mediated decay, so that the abnormal protein is never expressed, leading to a reduced protein dose. Alternatively, the protein may be produced, but be inactive (loss of function), or deleterious ('poison-peptide') – for example forming aggregates or inhibiting wild-type function.

Truncating variants in *MYBPC3* (the gene encoding myosin binding protein C, cardiac type) are one of the best-characterized causes of HCM[52,53] and illustrate this challenge.

Haploinsufficiency is now generally accepted to be the molecular mechanism for this variant class; however, agreement on this point was reached only after decades of debate on loss-of-function versus 'poison-peptide' effects[54,55]. The analogies with *TTN*tv are clear~~most apparent~~.

*TTN*tv associated with DCM most commonly occur in the A-band, although can be located in constitutively incorporated exons anywhere in the titin molecule from the Z-disc to the A-band[19]. In our early imaging genetic association studies, we observed a position-related effect of *TTN*tv on cardiac function with worse function linked with more distal variants[30]. This finding suggested that haploinsufficiency might not be the molecular mechanism for *TTN*tv, because all variants might be expected to have similar position-independent effect, and a poison-peptide effect was suggested. However, we were not able to demonstrate the existence of a truncated titin protein isoform in cardiac muscle extracts from patients with *TTN*tv. In subsequent studies of cardiomyocytes derived from induced pluripotent stem cells (iPSCs) from patients with *TTN*tv, or created using CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 gene editing technology, strong evidence emerged for a haploinsufficient mechanism, although in one cell line, a truncated titin protein was seen[56].

In an effort to dissect disease mechanism experimentally, our group created two mutant rat strains with *TTN*tv, either in very proximal Z-disc exons or in a distal A-band exon[19]. These were created with the goal of the Z-disc mutant being haploinsufficient because protein should not be created at all, whereas the A-band variant might produce a truncated protein product. As ever, titin biology turned out to be more complicated than expected. Although we unequivocally demonstrated the synthesis of a truncated peptide in the A-band mutant by ribosequencing (FIG. 2), the Z-disc mutant unexpectedly generated alternate 5' terminal peptides that initiated distal to the Z-disc *TTN*tv[19]. Therefore, both mutants might have generated short titin peptides that could have deleterious effects.

Nevertheless, both mutant rat strains exhibited nonsense-mediated decay of their respective *TTN*tv alleles, compensated in small part by upregulation of the wild-type allele, and overall both had ~40% less sarcomere-spanning titin[19]. On balance, we believe that these data point to loss-of-function as the dominant underlying molecular mechanism by which *TTN*tv cause DCM, although a poison-peptide effect might still have a contributory role, and has yet to be conclusively excluded.

[H3] *Molecular consequences*

DCM is characterized by cardiac dilatation and systolic dysfunction, which is manifest at the level of isolated cardiac myocytes that contract less rapidly and to a lesser degree than healthy cells[57,58]. This phenotype is unlike that produced by HCM-causing variants, which result in rapid and vigorous myocyte contraction owing to a sensitized sarcomere. The molecular consequences of *TTN*tv that lead to the hypocontractile state of the myocyte are beginning to be elucidated (FIG. 3), and we discuss some of the major advances here.

In studies of the two disparate (Z-disc and A-band) *TTN*tv in the rat, we demonstrated common metabolic and signalling perturbations showing *TTN*tv position-independent effects[19]. *TTN*tv resulted in a shift in cardiac metabolism away from the use of long-chain fatty acids, the preferred substrate of the healthy heart, and an increased reliance on glycolysis. This shift in metabolism occurred in young rats in which cardiac performance was normal[19]. A shift towards glycolytic metabolism is an established, short-term adaptive response of the heart to any stressor[59–62]. However, prolonged elevation of glycolytic intermediates and branched-chain amino acids can lead to activation of serine/threonine-protein kinase mTOR complex 1 (mTORC1) signalling[63,64], which in turn activates futile cycles of protein synthesis and inhibits autophagy, which are maladaptive[65,66]. In rats with *TTN*tv, we showed activation of mTORC1 and phosphorylation of its downstream substrates, which has been documented in cardiac biopsies from patients with end-stage DCM[66]. Future

studies will be needed to determine whether autophagy is impaired in hearts affected by

*TTN*tv, but if so, restoring normal protein processing might provide an avenue for therapeutic

intervention. This restoration might be achieved by inhibiting mTORC1 activation, either

directly or indirectly, with existing therapies such as metformin or rapamycin derivatives.

In cardiomyocytes derived from human iPSCs, *TTN*tv were associated with

diminished and disorganized sarcomeres, and cells were poorly responsive to adrenergic or

growth factor stimulation[56]. At the molecular level, major signalling pathways (involving

transforming growth factor-β, vascular endothelial growth factor, and mitogen-activated

protein kinases) that are critically important to cardiomyocyte function were inhibited[56],

although the mechanism underlying the general attenuation of signalling was not studied.

[H3] *Physiological consequences*

*TTN*tv are associated with DCM, but are also seen in the general population and can manifest

in many different ways (discussed in detail below). First we summarize the effects of *TTN*tv

on cardiac physiology.

Several studies of various *TTN* variants (including *TTN*tv) in mice have been

performed[67,68]. Some of these data remain preliminary but, overall, *TTN*tv seem to have very

limited, if any, effect on cardiac morphology or function in mice in the absence of a stressor.

However, after exposure to pharmacological or haemodynamic stress, mouse hearts with

*TTN*tv quickly dilate and develop systolic dysfunction, which is accompanied by a marked

fibrotic response[69,70]. In rats, we found a very mild phenotype of ventricular dilatation and

impaired systolic function in aged (>1.5 years) rats, but no effect in younger animals[19]. In

ex vivo studies, hearts with *TTN*tv had normal, or indeed slightly better, cardiac indices

compared with controls (perhaps reflecting a compensated state). However, hearts with

*TTN*tv did not exhibit an appropriate Frank–Starling response to volume loading, and quickly

became dysfunctional. We note that cardiac titin differs between rodents and adult humans, with distinct isoform compositions that might lead to differences in penetrance and expressivity of *TTN*tv between species. Taken together, these data show that *TTN*tv do not cause overt autosomal dominant cardiomyopathy in rodents, but that rodent hearts with *TTN*tv are primed to fail when exposed to stressors. We proposed that signalling and metabolic adaptations in *TTN*tv hearts maintain the heart in a compensated state, but at the expense of being unable to respond to a secondary insult.

The physiological effects of *TTN*tv have also been studied in cardiomyocytes derived from human iPSCs[56]. These studies showed that *TTN*tv cause impaired contractility of cardiomyocytes at baseline and in response to stress, probably related to direct effects on the sarcomere. In humans the physiological and pathophysiological effects of *TTN*tv have been studied in the general population and in patients with DCM using both echocardiography and cardiac MRI (CMR) {REFERENCES: 19-Schafer; 30-Roberts; 32-Akinrinade; 34-Franaszczyk; 79- Jansweijer; 94-Tayal; plus new reference introduced on p5 - Tayal et al, JACC 18:2264-2274 (2017).}. The association between *TTN*tv and DCM is robust and widely replicated, but not all individuals with *TTN*tv develop DCM. Moreover, the question remains as to whether the pathophysiology associated with *TTN*tv differs from other forms of DCM ~~more broadly~~. These issues will be discussed later in this Review. In echocardiographic studies, no effect of *TTN*tv on cardiac physiology was detected in the general population[30]. However, these studies were probably underpowered, because echocardiography is semiquantitative and inexact. The findings prompted careful evaluation of cardiac morphology and function in a cohort of healthy individuals ($n = 1,409$) using CMR combined with 2D studies and 3D machine learning-based analyses[19]. These investigations showed that *TTN*tv in constitutive exons leads to a slight increase in the size of the heart, with reduced

contractility, and mild eccentric remodelling[19]. Therefore, although *TTN*tv are penetrant in the general population, expressivity is limited.

[H1] Interpreting *TTN* variants

The identification in 2012 of a robust association between *TTN*tv and DCM had a major effect on the clinical investigation of patients with cardiomyopathy. The percentage of patients with DCM that could be explained genetically was previously very low (~10%[28]). Moreover, genetic testing had limited utility and was recommended only in specific circumstances, such as in the presence of concurrent conduction disease suggesting a laminopathy[71–73]. We now know that *TTN*tv account for ~15% of all DCM cases identified in the clinic, and up to ~25% of severe, end-stage, or clearly familial cases of DCM, increasing the utility of diagnostic sequencing[74,75] (BOX 2).

Nevertheless, for genetic testing to be of value, confidently ascribing causality to a specific genetic variant in an individual patient is necessary. Although *TTN*tv are more prevalent in individuals with DCM than in control populations, the burden of *TTN*tv in both unselected reference samples[30,76] and in demonstrably healthy controls[19,30] is substantial. If healthy individuals frequently carry *TTN*tv, how confident can we be that a variant found in a patient with DCM is disease-causing, and not just an innocent bystander?

[H3] *Modelling the genetic architecture of inherited diseases*

In addressing this question, the challenges and limitations that are generally applicable to many Mendelian diseases of variable penetrance and expressivity should be acknowledged. First, DCM is a categorical label, implying binary presence or absence of a disease state. However, a diagnosis of DCM is based on the assessment of continuous parameters (volumes and contractility) with superimposed diagnostic thresholds, and an underlying cardiomyopathic process can also lie on a continuum (analogous with blood pressure effects).

Second, much of our understanding of cardiomyopathies (and other inherited diseases) and associated terminology arises from a pure dominant Mendelian disease model, in which the presence or absence of a single allele determines the presence or absence of disease, and the allele can be understood as causal. This all-or-nothing eventuality is rare and, conceptually, contrasts with common diseases such as coronary artery disease and hypertension, in which many variants (each with a small effect) contribute to the phenotype, usually without any individual variant that can be meaningfully considered causal, and all variants are risk factors.

The pure (but rarely realized) dominant Mendelian model of disease can be readily extended to consider additional variants that either contribute independently to the disease process in an additive fashion, or that modify the effect of a primary deleterious allele through an interaction, and therefore contribute to the overall risk. But if the disease process is influenced by multiple contributing factors, when can an individual variant be described as causal? How much of the disease risk should be attributable to an individual variant to label it as the primary cause?

[H3] *Causative variant or risk allele?*

This issue can be addressed with well-established, mathematically-based epidemiological measures. The attributable risk percent among exposed (ARP) provides an estimate of the proportion of the risk in an exposed population that can be attributed to the exposure. In our context, this corresponds to the proportion of the risk of cardiomyopathy in mutation carriers that can be attributable to that particular mutation (derivation and interpretation reviewed previously[77]). Considering patients with DCM and any *TTN*tv, the ARP is estimated at 91% confirming, on an epidemiological basis, that the *TTN*tv is the principal determinant of DCM in this cohort.

The ARP can also be conveniently expressed as a decimal fraction, dubbed the aetiological fraction (see Supplementary information S1 (box)). When the goal is to determine whether an individual rare variant is likely to be 'causative', this can be interpreted as the proportion of variant-carrying probands in which the variant was causal; or the proportion of variants (found in affected individuals) that were penetrant; or the probability that an individual rare variant, found in a proband, was responsible for the disease. Consensus guidelines for variant interpretation in genetic testing suggest a variant be considered 'likely pathogenic' if the probability that it is causative is >0.9[78].

Conceptually, two possible explanations exist for the fact that *TTN*tv do not completely explain the risk of DCM even among carriers of *TTN*tv. First, two distinct populations of variants could be present, some with disease-causing potential and others that are benign with no role in the disease. In other words, the variants that are prevalent in the general population are qualitatively different from the disease-causing variants in patients with DCM, and a proportion of these patients will carry a benign *TTN*tv by chance. Alternatively, all *TTN*tv might have disease-causing potential, but display variable penetrance and expressivity attributable to other (genetic, environmental, or stochastic) factors that account for the residual risk. The data suggest that the truth lies in a combination of these explanations, which we and others suggest is true for many Mendelian diseases.

[H3] *Characterizing background variation in the general population*
Of the variants observed in healthy controls, 14% of *TTN*tv are found in a single exon — exon 48[30] (we use the exon numbering adopted by the Locus Reference Genomic reference transcripts, in which all possible exons are numbered 1–364 according to genomic location, irrespective of isoform inclusion). Exon 48 is an alternative 3′ (C-terminal) exon used only by the Novex-3 transcript that is expressed at low levels in the heart and does not span the

sarcomere. Variants in this exon are equally common in patients with DCM and in control individuals[19,30]. A further 12% of variants are found in other exons with minimal cardiac expression, and another 20% in exons that are expressed in the heart, but that are incorporated only into the longer isoforms[30]. In total, ~46% of the variants found in healthy controls are located in exons that are either not incorporated into the main cardiac isoforms at all, or are utilized only in the long N2BA isoform so that a fully functional N2B isoform can still be transcribed and translated from the variant allele.

A further number of the *TTN*tv observed in controls are individually fairly common, or at least insufficiently rare to be penetrant causes of DCM (population allele frequency $>1x10^{-4}$)[19]. Some of these variants are found in the nonconstitutive exons described above, and the remainder are typically variants predicted to disrupt splicing. Although variants that alter canonical splice donor/acceptor sequences are conventionally considered 'truncating', such effects can be rescued by alternate splice sites nearby, or can result in exon-skipping that might be tolerated if the resultant transcript preserves the reading frame.

[H3] *Clinical interpretation of TTNtv in DCM probands*

If we exclude categories of variants that are prevalent in healthy individuals, and consider only rare *TTN*tv in exons that are constitutively expressed in the heart, the aetiological fraction for *TTN*tv in DCM probands rises to 0.97[19]. The clear majority of disease risk can be attributed to these variants. We estimate that the probability that such a variant is causal of the observed disease is 0.97, and that a *TTN*tv in a constitutive exon found in a patient with DCM is, therefore, an actionable finding. We would, for example, consider using the variant for predictive testing in family members, reassuring and discharging from surveillance in the cardiology clinic individuals found to be genotype negative.

[H3] *Interpretation of TTNtv in healthy individuals*

Although such variants are actionable when found in a patient with known disease, this information does not indicate what proportion of family members, or unrelated individuals, who are found to have such a variant will go on to develop disease. Relatives of patients with DCM have been investigated in several studies[26,32,34,79]. Although the total number of individuals and distinct DNA variants studied remains small, the picture is of age-related penetrance: 29–65% by the age of 50 years and 83–100% by the age of 70 years (FIG. 4).

The penetrance looks rather different when *TTN*tv are examined in the general population. Again, considering only *TTN*tv in exons constitutively expressed in the heart, the prevalence of rare variants in the general population is ~1:250 (0.4%)[19]. If an upper estimate of the population prevalence of DCM of 1:250[80] is used and ~13% of cases are assumed to be associated with *TTN*tv[19], this yields an estimated penetrance of 0.12 (0.093–0.15) in the general population (calculated using a method described previously[81,82]). If the true prevalence of DCM is lower than 0.4%, then even lower penetrance for these variants is implied.

Therefore, most individuals with *TTN*tv will not develop overt DCM, but the variants are not necessarily phenotypically silent. Indeed, we have shown that individuals in the general population with *TTN*tv have slightly larger hearts, with subnormal contraction, and early features of eccentric remodelling[19]. Whether the metabolic and signalling phenotype observed in model organisms[19] is recapitulated in humans with *TTN*tv but not DCM, and whether *TTN*tv confer a substantially increased risk of heart failure as suggested by animal physiology[19] and their association with peripartum cardiomyopathy[37], remains to be determined.

Analysis of the regional distribution of *TTN*tv in cases and controls reveals further subtleties for their clinical interpretation. Missense variants that result in single amino acid substitutions can have very different effects depending on their molecular location — one

variant might disrupt a phosphorylation site resulting in disinhibition, whereas a variant at a catalytic site might ablate enzyme activity. By contrast, protein-truncating variants are generally considered equal (with some caveats)[83,84]. Cells have mechanisms to identify and remove truncating transcripts and their protein products (for example, nonsense-mediated decay and proteosomal degradation). Therefore, in most cases, the truncated peptide is not present at a detectable level in the cell, and the molecular consequence of the variant, if any, is simple deficiency[85]. Classically, the location of the truncating variant should not matter — all truncations should have the same downstream consequence, as we found in rat models with *TTN*tv at opposite ends of *TTN*[19].

We have already seen that *TTN*tv in certain regions of the protein are not associated with disease, but this observation can be explained by tissue-specific expression patterns of the various isoforms. Variants must affect cardiac isoforms to cause cardiac disease but, even so, not all *TTN*tv are created equal. In early studies of *TTN*tv in DCM, variants in the A-band were highlighted as particularly important[26]. Subsequently, we noted that A-band exons are all constitutively expressed in the heart, and that other constitutive exons are also robustly associated with disease[19,30]. For example, exon 49, encoding the N2B element that differentiates cardiac and skeletal titin isoforms, is located in the I-band, but has an odds ratio of 34 (95% CI 15–82), suggesting that perhaps all constitutive exons are equally important. In fact, a positional effect remains even among constitutively expressed exons. In one study, *TTN*tv located more distally (A-band end) were proposed to lead to a more-severe phenotype than those located more proximally (I-band)[30]. However, numbers were small, and this finding has not been replicated. In the largest meta-analysis conducted so far, the odds ratio for DCM was higher for variants in the distal I-band and the A-band, and lower for *TTN*tv at the Z-disc or M-line[19]. This finding remains poorly understood. Although transcripts containing variants in the last two exons might be expected to escape nonsense-mediated

decay[84,86], this does not account for the differences in odds ratio observed. Alternative 5′ start sites have been proposed as an explanation — perhaps isoforms that begin downstream of the *TTN*tv, such as the so-called Cronos transcript[87], might be expressed in full and rescue the situation? We consider this explanation unlikely. Although such start sites exist and are active in humans[19], the resultant transcripts would not be expected to contain the motifs to incorporate into the Z-disc, and the protein would be unlikely to span the sarcomere. Moreover, *TTN*tv immediately upstream of the Cronos start site, which might be rescued by this mechanism if active, are associated with DCM with an odds ratio similar to A-band *TTN*tv[19]. In our opinion, the mechanism underlying regionality, even within constitutive exons, remains to be determined. One explanation could be that the truncated peptide product is important, with pathogenicity at least partly attributable to a poison-peptide effect.

[H3] *Nontruncating variants*

In this Review, we focus on the role of *TTN*tv in the heart, but whether other classes of variation in *TTN* predispose to disease is also worth considering. The first report of an association between *TTN* variants and DCM included two families, one of which carried a *TTN*tv, and the other a missense variant[22]. The missense variant segregated with disease, and stem-cell-derived cardiomyocytes genetically engineered to carry the variant were later shown to develop a phenotype consistent with other DCM-causing mutations[56]. Another missense variant has been shown to segregate with a cardiomyopathy characterized by left ventricular noncompaction and impaired function, but with marked hypertrophy in some of the affected individuals[38]. Functional studies suggest partial protein unfolding, domain destabilization, and impaired binding to the titin ligand telethonin in association with the variant, providing strong evidence of causality. However, rare missense variants in *TTN* are extremely prevalent, and their prevalence in patients with DCM is indistinguishable from that in the general population. Other than a few specific variants that have been statistically

implicated in DCM through linkage studies in large families, and shown to alter protein function *in vitro* or *in vivo*, the majority of missense variants in *TTN* remain uninterpretable, whether identified in the context of genetic testing for DCM, or as an incidental finding in other sequencing.

Structural variants, including copy number variants that alter dosage of the *TTN* gene, are not well characterized. Targeted high-throughput sequencing has limited accuracy for the detection of such variants, and large-scale systematic analyses are lacking. In the seminal case series reported by Herman and colleagues, one patient with DCM carried a copy number variant leading to a partial internal duplication that would be expected to disrupt the protein structure[26]. However, larger copy number changes that might affect gene dosage — for example, the 2q31.2q32.3 deletion syndrome[88–93] — are not typically associated with cardiomyopathy (although cardiac evaluation might have been variable and not necessarily performed in late adulthood).

[H3] *Summary of clinical interpretation*

Many rare *TTN*tv found in exons that are constitutively expressed in the heart are clinically actionable when found in a patient with DCM (FIG. 5). The probability that such a variant is causative in the observed disease is >0.97, justifying a guideline-based classification[78] of 'likely pathogenic' and consideration of molecular cascade screening with suitable genetic counselling ~~is considered appropriate~~. The probability that a relative who does not carry the familial *TTN*tv will nevertheless develop DCM approaches the background in the population, and we believe that such individuals do not routinely require long-term follow-up.

By contrast, the penetrance of *TTN*tv is not fully characterized, and data are lacking to reconcile the apparently high penetrance (albeit strongly age-related) in relatives of patients with DCM with lower penetrance in the general population. The proportion of *TTN*tv-positive

individuals who are related to patients with DCM who will eventually to manifest disease cannot yet be determined. However, our practice is to maintain surveillance of these individuals, for example clinical review and imaging every 2–3 years. Variants identified incidentally in the general population, in individuals with no family history of DCM, might prove to be risk factors for heart muscle disease, but the risk is not yet well characterized. Our understanding of factors that determine penetrance is limited, but evolving, and we anticipate that both genetic and environmental modifying factors will soon be identified.

[H1] *TTN*tv and precision medicine

The identification of *TTN*tv as a major cause of DCM has had an important effect on the clinical management of patients with this condition, doubling the proportion of genetic tests that yield an informative result (i.e. identify the causative variant), which~~through a doubling of the yield of molecular genetic testing that~~, in turn, enables cascade screening. A definitive molecular genetic result in a proband with DCM will free ~50% of their relatives from the burden of long-term cardiological surveillance — electrocardiography and echocardiography, or CMR every few years, at substantial cost to both individuals and the health system. Although the study of *TTN*-related cardiomyopathy is still at an early stage, the available data suggest that molecular genetics has the potential to directly affect the management of patients with DCM. In this era of precision medicine, we can hope that the identification of a *TTN*tv will inform prognosis and treatment stratification, in addition to the benefits to the patient's family of molecular cascade testing.

To date, the power of case series to definitively characterize genetic associations with phenotype or clinical outcome has been limited. In the seminal paper that defined the importance of *TTN* in DCM, the authors reported "no significant differences between subjects with and those without *TTN* truncating mutations with respect to the age at diagnosis, left

ventricular end-diastolic dimensions, ejection fraction, or rates of cardiac transplantation, implantation of a left ventricular assist device, and death from cardiac causes", but also noted that "men with *TTN*tv had adverse events at significantly earlier ages than did women ($P = 4 \times 10^{-5}$)" [26]. Case series have reported the highest prevalence of TTNtv amongst patients with severe disease, or evaluated for cardiac transplantation{REF 26 – Herman; REF 30 – Roberts}, suggesting that the identification of a TTNtv might inform prognosis.

In our own early series of 319 patients (42 with *TTN*tv) with quantitative CMR parameters, we found that ambulatory patients with DCM and *TTN*tv had only marginal reductions in wall thickness (unadjusted indexed lateral wall thickness 2.77 ± 0.7 mm versus 3.13 ± 0.7 mm; $P = 0.003$) and contractility indices (left ventricular ejection fraction (LVEF) $33.3 \pm 13\%$ versus $37.5 \pm 12\%$, $P = 0.047$ in unadjusted analysis, $P = 0.006$ in analysis adjusted for covariates) compared with those without *TTN*tv[30]. Preliminary analyses based on a subset of the cohort suggested an increase in history of ventricular tachycardia by the time of recruitment, but the sample size was modest (111 patients with ventricular arrhythmia data, ventricular tachycardia observed in 9 of the 14 patients (64%) with *TTN*tv and 20 of the 97 individuals (21%) without *TTN*tv).

An extension of this work included comprehensive analysis of early arrhythmia history in a larger cohort of ambulatory patients with DCM ($n = 572$; 56 with *TTN*tv; mean LVEF 39±12.6%)[94]. The association between *TTN*tv and arrhythmia proved robust. Patients with *TTN*tv had a threefold increased risk of a hospital or primary care record of atrial fibrillation or ventricular tachycardia by the time of recruitment after adjusting for conventional arrhythmic risk factors (adjusted OR 2.9, 95% CI 1.5–5.8, $P = 0.002$; absolute risk increase 13%). Arrhythmias were documented in 196 patients (34%): 139 (24%) with atrial fibrillation, 69 (12%) with nonsustained ventricular tachycardia, and 11 (2%) with sustained ventricular tachycardia; 22 patients had more than one type of arrhythmia. *TTN*tv

were observed in 26 patients (13.3%) with a history of arrhythmia, and 30 patients (8%) without recorded arrhythmia. Conversely, an arrhythmia was documented in 26 patients (46%) with *TTN*tv compared with 170 patients (33%) without *TTN*tv ($P = 0.05$)[94].

In a study conducted in the Netherlands, probands with DCM and *TTN*tv ($n = 45$), or *LMNA* (the gene encoding lamin A/C) variants ($n = 28$), or variants in neither gene ($n = 60$), and their relatives were evaluated[79]. During the study period (median 2.5 years), *TTN*tv were associated with a milder clinical course than were *LMNA* variants, which are known to be associated with arrhythmia, conduction disease, and aggressive disease[95,96]. In addition, carriers of *TTN*tv were compared with patients with DCM who had neither *LMNA* nor *TTN* variants. The proportion of *TTN*tv carriers with a LVEF <35% at diagnosis was lower than among those with DCM and no identified variants, and *TTN*tv were associated with a trend towards a more favourable disease course (composite outcome of ventricular arrhythmia, transplantation, left ventricular assist device implantation, and all-cause mortality)[79]. The rate of ventricular tachycardia was nominally higher with TTNtv than in those without a variant in either gene (35% versus 11%, $P = 0.04$; HR 3.1, $P = 0.06$) in line with our own findings, although other arrhythmic end points did not differ between the groups[79].

In another study, evaluation of 72 probands with DCM (17 with *TTN*tv) revealed no difference in the incidence of adverse cardiac events between carriers of *TTN*tv and noncarriers (mean follow-up 63 months)[34], although the investigators did report a higher penetrance and poorer outcomes in male carriers of *TTN*tv than in female carriers[34].

A recent extension of our work explored clinical outcomes in a larger ambulant population (716 subjects, 83 with TTNtv, 604 with follow up data (median of 3.9 years)), alongside an expended analysis of phenotype at recruitment {NEW REF from page 5, Tayal et al JACC 18:2264-2274 (2017)}. The increased propensity to arrhythmia early in the disease course did not translate to a significant difference in prognosis over the medium-term

for DCM patients with TTNtv (hazard ratio for primary composite endpoint comprising sustained ventricular tachycardia or fibrillation, appropriate ICD shock, aborted sudden cardiac death, heart transplantation, LVAD implantation, and heart failure hospitalization: 0.92, 95% CI 0.45–1.87, $P = 0.82$), though the overall event rate (12.9%) was relatively low compared with registry data. TTNtv were also associated with thinner LV walls (mean maximum LV wall thickness 8.8 ± 1.8 mm vs. 10 ± 2.2 mm, $P < 0.001$) and lower LV mass (5.1 g/m2 reduction; $P_{adjusted} = 0.03$) in the absence of evidence of significant differences in LV dilation. While the magnitude of the difference was small and not clinically informative, such a blunted hypertrophic response could be consistent with altered mTORC1 signalling that can modulate cardiac hypertrophy, as discussed above, though a mechanistic link remains to be established.

With the exception of the reported link between *TTN*tv and early-onset arrhythmia[30,94], when taken together the published data on genotype–phenotype associations in DCM associated with *TTN* variants do not yet provide evidence of a markedly distinct phenotype or altered clinical course. It remains to be seen whether TTNtv predict outcome in the longer-term, or are informative in a subgroup of patients at higher risk. By contrast, cardiomyopathy associated with *LMNA* variants seems to be associated with poor prognosis.

Importantly, *TTN*tv are reported to be associated with a favourable response to treatment[79]. This observation is concordant with our findings in an cohort of 70 patients with end-stage DCM referred for left ventricular assist device implantation[97], and with a group of 128 patients with DCM from Canada, among whom those with *TTN*tv were no less ~~equally~~ likely to respond to medical therapy than those without such variants[98]. Together, these findings update the conventional wisdom that irreversible genetic aetiology correlates with irreversible disease[99], suggesting instead that *TTN*tv do not preclude recovery, and should not be a barrier to advanced therapies.

Evidence is accumulating that *TTN*tv are also found in association with cardiomyopathies traditionally considered as secondary, such as those related to pregnancy[37] or cardiotoxic chemotherapy[100]. The implication is that, in a proportion of cases, *TTN*tv might be acting in concert with other factors, so that treatment of the exacerbating factor might be expected to lead to substantial recovery.

The potential for *TTN* genotype to play a role in other forms of precision therapy, beyond risk stratification, remains speculative. The use of therapies based on genetic engineering is foreseeable in the long term, but the appropriate strategy — for example, gene replacement therapy for haploinsufficiency, allele-specific silencing if dominant negative, or direct gene editing to correct or ameliorate the molecular defect — will depend on an improved understanding of the molecular mechanisms involved. Antisense oligonucleotide-induced exon skipping to restore the reading frame has shown efficacy in experimental models, including mice and cardiomyocytes derived from human iPSCs[101]. An equivalent therapeutic strategy has FDA approval for human use in Duchenne muscular dystrophy[102].

The presence of a metabolic phenotype in rat models, with accompanying changes in signalling pathways including mTOR, suggests other strategies for potential evaluation, such as mTOR pathway modulation with metformin or 'rapalogues' (rapamycin analogues). Alternatively, the study of genetic or environmental modifiers of *TTN*tv expressivity and penetrance might reveal secondary pathways that are amenable to therapeutic modulation.

[H1] Conclusions

Titin is central to sarcomere structure and function, and genetic variation can lead to altered cardiac function and heart disease. However, studies to dissect the molecular mechanisms and consequences of titin variation have highlighted the subtlety and diversity of the role of

this protein. Far from being simply a large, passive structural molecule, titin is complex with varied structural, sensing, and signalling functions.

Inevitably, a gene the size of *TTN* will undergo frequent mutation. Titin function seems to be maintained in the face of much genetic variation, harbouring very many rare missense and truncating variants in the general population. Although heterozygous *TTN*tv are the most common genetic predisposition to DCM, most individuals that carry such variants do not have overt disease. However, *TTN*tv in the general population are not phenotypically silent — a picture is emerging of subclinical cardiac changes that might predispose to heart failure under conditions of physiological stress or toxic insult, or in the context of a susceptible genetic background.

In patients who present with DCM, the finding of *TTN*tv enables molecular cascade screening of family members that can free these individuals from a lifetime of surveillance in the cardiology clinic. Although the importance of *TTN*tv in DCM was not appreciated fully until 2012, in the past 5 years, *TTN* sequencing has already transitioned into clinical care with the potential to influence patient stratification. Whether a molecular diagnosis will routinely enable patient selection for targeted therapeutic intervention remains to be seen, but the signs are promising and much work is in progress.

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Author contributions

J.S.W. and S.A.C. researched data for the article, discussed the content, wrote the manuscript, and reviewed/edited the article before submission.

Competing interests statement

The authors declare no competing interests.

DATABASES

LOVD[3] Shared Database; *TTN* (titin): https://databases.lovd.nl/shared/genes/TTN. A database of genetic variants in *TTN* with phenotypic annotations.

FURTHER INFORMATION

Web resources from the Cardiovascular Genetics and Genomic Group, Imperial College London and Royal Brompton & Harefield NHS Trust: https://cardiodb.org/titin. An online resource cataloguing *TTN*tv reported in association with cardiomyopathies, with annotations to support variant interpretation.

Using high-resolution variant frequencies to empower clinical genome interpretation:

http://cardiodb.org/allelefrequencyapp

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SUPPLEMENTARY INFORMATION

See online article: S1 (box)

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Key points

- Titin truncating variants (*TTN*tv) are strongly associated with dilated cardiomyopathy (DCM), but are also prevalent in the general population

- *TTN*tv identified through genetic testing of a patient with confirmed DCM might be clinically actionable and informative for the management of the proband and their family

- The significance of TTNtv identified through sequencing for other indications is not well defined; such variants can be associated with increased risk of DCM, but in aggregate are not highly penetrant

- Haploinsufficiency caused by *TTN*tv may not explain all the associated molecular and physiological consequences, suggesting that other mechanisms might also contribute to disease pathogenesis

- Important genetic and environmental determinants of *TTN*tv penetrance and expressivity remain to be identified

Box 1 | **Mechanisms of dominance**[103]

A dominant phenotype can arise from diverse molecular processes

Loss of function

- Reduced dosage, expression, or activity, leading to haploinsufficiency

Gain of function

- Increased dosage

- Ectopic or temporally altered mRNA

- Increased or constitutive protein activity

- Dominant negative effects (mutant antagonizes wild type)

- Altered structural protein

- Other toxic peptide effects

- Novel protein function

Box 2 | **Genetic testing for cardiomyopathies**

The potential clinical benefits of a molecular diagnosis in the context of a clinically manifest inherited cardiac condition

Benefit to patients

- Diagnosis
- Precision medicine
    - o Prognostication
    - o Stratified therapy

Benefits to relatives of patients

- Diagnosis
    - o Predictive testing
    - o Molecular autopsy
- Reproductive counselling and prenatal genetic testing

All predicated on knowing the precise variant that is causative of the condition

Figure 1 | **The role of titin in the sarcomere and summary of isoforms. a** | Structure of the sarcomere. The sarcomere is the fundamental contractile unit of striated muscle, and comprises interdigitating thick and thin filaments that generate force. Four distinct regions are defined by light microscopy: Z-disc, I-band, A-band, and M-line. Individual molecules of titin span the full length of the hemisarcomere with structural, sensory, and signalling roles. The N-terminus inserts into the Z-disc, and the C-terminus into the M-line. The A-band is closely associated with the thick filament, and the I-band comprises mainly elastic elements. **b** | Structure of titin. A diversity of functions is apparent. Titin contains binding sites for other structural proteins; passive force-generating elements, such as the repetitive I-band Ig domains, PEVK region, and N2B unique sequence; and diverse binding sites for other ligands including a kinase domain. The schematic represents the titin domain structure (UniProt Q8WZ42-1) and location of known ligand-binding sites. **c** | Structure of the *TTN* gene. *TTN* is encoded in 364 exons with enormous potential for alternative splicing. An inferred-complete metatranscript comprising 363 exons can be used as a reference for convenience to describe most features. Biologically, two transcripts predominate in the heart — a long and compliant N2BA isoform, and a shorter and stiffer N2B isoform that contains fewer I-band spring elements. A third isoform, Novex-3, utilizes an alternative 3′ exon (exon 46) that cannot be represented in the metatranscript, and is expressed in the heart at lower levels. Novex-3 is not thought to span the sarcomere, and its functions are not well characterized. Other low-abundance cardiac transcripts, fetal isoforms, and skeletal muscle isoforms are transcribed from the same gene, but are not shown here. CARP, cardiac ankyrin repeat protein; DARP, diabetes-related ankyrin repeat protein; DRAL, ; FHL, four and a half LIM domains protein; HSP, heat shock protein; MARP, muscle ankyrin repeat protein; MURF, muscle-specific RING finger; myBP-C, myosin-binding protein C; PKA, protein kinase A; PKG, protein

kinase G; sAnk1, small ankyrin 1; Smyd2, N-lysine methyltransferase SMYD2. Panel b modified with permission from REF. 44.

Ribosome profiling reveals translation of truncating alleles

Figure 2 | **Ribosome profiling identifies *TTN*tv.** We studied a heterozygous A-band *TTN*-truncating variant (*TTN*tv) rat model, specifically a Fischer (F)344/ Brown Norway (BN) rat cross, in which the truncating allele is tagged by F344 strain-specific single nucleotide polymorphisms (SNPs), while the wild-type allele carries BN-specific alleles. At heterozygous SNP sites, we use the ratio of sequencing reads arising from each allele (allele balance) to infer levels of transcription and translation. **a** | RNA sequencing data (white points) contain reads from both alleles, but the truncating allele (carrying F344 SNPs) represents only 20–30% of reads, suggesting degradation of the mRNA, or upregulation of the wild-type allele, or both. In Ribo-seq data (purple points), we again see reads from the mutant allele up to the location of the truncating variant (purple line), after which the transcript is dissociated from the ribosome and no longer translated. This demonstrates that the stop codon is active, and terminates translation, but that the truncating transcript is present and translated. **b** | Titin protein synthesized after the *TTN*tvA premature stop codon is exclusively generated from the intact BN allele. In box plots, boxes show medians within the 25th to 75th percentile, and whiskers show the 10th to 90th percentile. Allele frequencies in both RNA-seq and Ribo-seq data were <50%, indicative of nonsense-mediated mRNA decay. Reprinted with permission from REF. 19.

Figure 3 | **Consequences of *TTN*tv.** A model depicting the molecular and physiological consequences of *TTN*tv, and the pathways to evolution of overt cardiomyopathy. *Might additionally be affected by poison peptides. Modified with permission from REF. 56.

Figure 4 | **Age-related penetrance of *TTN*tv.** *TTN*tv identified in probands with dilated cardiomyopathy (DCM) seem to be penetrant in family members, albeit with a strong age-dependent effect. Unbiased estimates of penetrance (the proportion of variant carriers who manifest the disease) are difficult to obtain[77], but population approaches suggest a penetrance of around 0.1. The penetrance of *TTN*tv in a familial context has been investigated in four studies, examining *TTN*tv-carrying relatives of patients with *TTN*tv-associated DCM. Herman *et al.*[26] studied 32 variant carriers aged >40 years, and reported >95% as being affected by DCM. Akinrinade *et al.*[32], Franaszczyk *et al.*[34], and Jansweijer *et al.*[79] reported penetrance by decade, yielding a picture of strongly age-related penetrance, but with most variants ultimately penetrant if followed into older age.

Figure 5 | **Clinical interpretation of *TTN*tv.** A proposed systematic approach to the interpretation of *TTN*tv. Variants should be sufficiently rare to be a plausible disease allele[78]. Truncating variants in exon 48, exons that are not expressed in the heart, or exons that are not constitutively expressed in the heart have not been shown to be associated with dilated cardiomyopathy (DCM). Variants in cardiac constitutive exons are associated with DCM, but the strength of the association differs for various protein regions. When found in a patient with DCM, variants in the A-band, distal I-band, or exon 49 (encoding the N2B unique element) have a high probability (>0.95) of being causal and are clinically actionable. Variants in other constitutive exons might require additional evidence for confident interpretation. *TTN*tv identified as secondary findings, such as during sequencing for other indications, cannot be assumed to confer the same disease risk as *TTN*tv identified in the context of disease, and are unlikely to be clinically actionable.

Supplementary information S1 (box) / **Assigning risk and causality to genetic variants.**

Attributable risk percent among exposed (ARP), provides an established statistical estimate of the proportion of the risk in an exposed population that can be attributed to the exposure. This epidemiological measure is meaningful where there is strong evidence of a biologically plausible causal relationship between exposure and disease. In our context, ARP corresponds to the proportion of the risk of cardiomyopathy in mutation carriers that can be attributable to the mutation. The measure was popularized by Cole & MacMahon[1], and defined as follows:

$$ARP(\%) = \frac{R_e - R_0}{R_e} \times 100\%$$

where

R_e = risk in the exposed (i.e. carriers of rare variant in gene of interest)

R_o = risk in the unexposed (i.e. individuals with no rare variant in gene of interest)

The equation can be conveniently rewritten as:

$$ARP(\%) = \frac{RR - 1}{RR} \times 100\%$$

where

RR = relative risk (ratio of risk among exposed to risk among unexposed)[2]

For cross-sectional (i.e. case-control) data, odds ratios (OR) provide accurate estimates of the underlying relative risk[3], leading to:

$$ARP(\%) = \frac{OR - 1}{OR} \times 100\%$$

and ARP, when expressed as a decimal fraction, has been called the etiological fraction (EF)[2] i.e.

$$EF = \frac{OR - 1}{OR}$$

In the diagnostic context, where we are treating cardiomyopathy as a Mendelian disease, and are interested in interpreting whether an individual rare variant was likely causative, the EF can be interpreted in several ways*:

- the proportion of variant-carrying probands in which the variant was causal

- the proportion of variants, found in affected individuals, that were penetrant

- the probability that an individual rare variant, found in a proband, was responsible for the disease

EF therefore represents our confidence in interpreting variation as etiologically significant when found in an individual with disease.

*Note that in each case we cannot disentangle incomplete penetrance. Without additional information we cannot determine whether an EF of 50% indicates that half of the variants found in our case cohort are fully penetrant and disease-causing (and the other half have zero penetrance), or if all of the variants are pathogenic, but with reduced (50%) penetrance.

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