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Editorial

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Value of clinical and genetic evaluation in inherited cardiomyopathy: insights and challenges

William J. McKenna[1,2,3], Luis de la Higuera Romero[1,3], Soledad Garcia Hernandez[1,3], Juan Pablo Ochoa[1,3,4]

[1]Cardiovascular Genetics, Health in Code SL, A Coruña, Galicia 15143, Spain.
[2]Institute of Cardiovascular Science, University College London, London WC1E 6DD, UK.
[3]University of A Coruña, A Coruña, Galicia 15143, Spain.
[4]CNIC (National Cardiovascular Research Center), Madrid 28001, Spain.

Correspondence to: Dr. William J. McKenna, Institute of Cardiovascular Science, University College London, Room G28 Paul O'Gorman Building, 72 Huntley Street, London WC1E 6DD, UK. E-mail: w.mckenna@ucl.ac.uk

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Pourebrahim *et al.*[1] present a comprehensive clinical and genetic evaluation of a 5 generation family with a novel splice site mutation in the gene encoding the giant sarcomere protein, titin (TTN). The splice site mutation (NM_003319.4:c.17621-1G > A) segregated with disease in individuals whose phenotypes included features of both dilated cardiomyopathy (DCM) and the most common of the arrhythmogenic cardiomyopathies (ACMs), arrhythmogenic right ventricular cardiomyopathy (ARVC)[2]. Additional desmosomal variants, particularly related to ARVC, were identified as potential disease modifiers. The report's stated aim was "to analyze the genetic basis of the phenotypic heterogeneity of cardiomyopathy" to explain the presence of DCM and ACM within the same family. The authors present a clear and detailed description of the clinical and genetic findings in their family, and they succeed in the exploration of their stated aim. The study of the family highlights many of the uncertainties of both clinical and postmortem diagnosis in ascribing specific cardiomyopathy phenotypes, particularly those associated with premature sudden death. The report also underscores the complexity of interpreting sequence data generated by whole-exome sequencing.

The exploration of the potential oligogenic basis for the phenotypic plasticity between late-onset DCM and ACM requires clarity of definition to ensure a common understanding of the terminology of these two cardiomyopathies, which have overlapping clinical features. Both are defined as heart muscle disorders that are not explained by coexistent ischemic, valvular, hypertensive, or congenital heart disease. Inherited DCM is diagnosed based on a clinical presentation with symptoms (e.g., fatigue, dyspnea), structural/functional abnormalities on imaging (e.g., ventricular dilation/impaired ventricular function), and outcomes (e.g., heart failure death/transplant, atrial fibrillation, embolic stroke, late arrhythmia) related to heart failure. ACM, by definition as a heart muscle disorder, will also have some structural and functional abnormalities of ventricular function, but the clinical presentation is dominated by arrhythmia, e.g., palpitation, syncope, conduction disease, sudden death. Thus, a correct diagnosis of DCM *vs.* ACM is important. Sudden death at a young age is an unlikely initial presentation for DCM associated with variants in TTN or myosin heavy chain, though events later in life associated with advanced disease may occur. In ACM, however, familial evaluation often reveals individuals who experienced unexpected sudden death as the initial presentation of disease in the family. This occurs most typically in ACM associated with variants in desmoplakin[3] and filamin C with young asymptomatic males at particular risk. Management in DCM focuses on heart failure treatment to attenuate disease progression and late arrhythmic/embolic complications. Management in ACM focuses on risk stratification for potentially lethal events, particularly to determine the need for an implantable cardioverter defibrillator (ICD).

PHENOTYPIC CHARACTERIZATION

What is the final diagnosis in the family? Of the seven definitely or probably affected family members, three had an arrhythmia presentation, two with preserved ejection fraction, three had heart failure symptoms and reduced ejection fraction, and one with an ejection fraction of 30% had symptoms of arrhythmia and heart failure. The definitely affected were aged 54, 56, 57, 67, and 80, while the probably affected were aged 42 and 45. Significant coronary artery disease was effectively excluded in five of the six, and an unlikely explanation for the phenotype in II-4, an 80-year-old man. Four received an ICD, while III-4 died suddenly aged 57. She was hypertensive, obese (BMI 37 kg/m^2), with minor repolarization changes, no documented or symptomatic arrhythmia, and normal imaging. 50%-80% of her RV myocardium at postmortem showed fatty replacement without mentioning significant fibrotic changes in either left or right ventricle; her death was attributed to ARVC. In a study of fat in the right ventricle, world experts in the pathology of ARVC were unable to distinguish the fatty infiltration of ARVC from the fat seen in an obese person with adipositas cordis or from the "normal" fat of aging[4]. These observations led to the revision of the pathology diagnostic criteria, which now requires the presence of fibrous tissue and or fibrofatty infiltration (but not fat alone), replacing dead or dying myocytes as the diagnostic feature[5]. Seen in isolation from her family members, her repolarization changes on EKG and postmortem findings, and perhaps even her sudden death, could reasonably be attributed to her obesity and hypertension[6,7]. Patient IV-4 also represents a diagnostic challenge. A heavy drinker, he presented with heart failure symptoms and an ejection fraction of 25%. These features would normally suggest a diagnosis of alcoholic cardiomyopathy rather than an inherited DCM. The striking feature was the normalization of his symptoms and ejection fraction after cessation of his heavy alcohol consumption. The clinical and genetic evaluation of his family with the identification of a disease-causing variant in *TTN* provided additional insights. There is a recognized interaction between mutations in *TTN* and excess alcohol consumption. Patients with *TTN* null variants and excess alcohol consumption had significantly lower left ventricular ejection fraction than patients diagnosed with "alcoholic cardiomyopathy" without *TTN* truncating variants[8]. It is of interest that his symptoms and ejection fraction improved significantly with cessation of alcohol, though it is not clear whether this was also associated with the initiation of heart failure treatment, a combination which would be the management recommendation. In both of these individuals (III-4 and IV-4), there are alternative

explanations for the observed clinical features and outcomes, and the inherited DCM associated with a disease-causing variant in *TTN* would not have been recognized without familial and genetic evaluation. In addition, the identification of the *TTN* mutation enabled cascade screening of the family and recognition that individuals V-1 and V-4 were at risk of disease development.

GENETIC INTERPRETATION

The pre-test probability of disease is important in variant interpretation and requires a correct clinical diagnosis. Variant interpretation must take into account not only computational and prediction data but also several sources of evidence on pathogenicity (population, functional, segregation data, among others). The threshold filter allele frequency in control population databases for candidate variants to determine whether a candidate variant is "too common" to be causative for a Mendelian disorder is difficult to establish. Consideration must be given to disease prevalence, the contribution of the particular gene, genetic heterogeneity, and the expected penetrance for a given genetic substrate. The population data criteria should include assessment of the enrichment of the variant in related cardiac phenotypes and healthy control populations *vs.* the disease phenotype under consideration.

The NM_003319.4:c.17621-1G > A (NM_001256850.1:c.39893-1G > A, referred to as the N2BA fetal isoform) in *TTN* is a mutation that segregated with disease in this family. It was the only disease-causing (pathogenic/likely pathogenic) variant identified in the family with certainty. It was present in all six affected/probably affected individuals. It is a splice site variant (predicted frameshift) that affects one constitutive asymmetric exon in *TTN* (including major cardiac isoforms) located in the I Band. It has been previously reported in a heterozygous carrier, a 44-year-old lady with a mild cardiac and skeletal myopathy, identified during the study of a recessive *TTN* family with centronuclear myopathy[9].

Whether the other variants are likely modifiers and contribute in a meaningful way to the phenotypic heterogeneity and are important determinants of the proposed DCM/ACM overlap is less certain.

The other *TTN* variant considered either causative or a potential modifier was NP_003310.4:p.Lys3240Arg; however, it is a VUS missense variant in the same allele as the previous splice site (*cis*), inherited as a haplotype. This fact makes its contribution to the phenotype unlikely; the splice site variant molecular effect is expected to prevail.

Several other potential modifier variants were identified in individuals harboring the *TTN* splice site variant, but none of them can be considered disease-causing, and a modifier effect is difficult to prove. For example, the other missense variants described in *TTN* have been identified in constitutive exons but are found in the general population with a frequency of common polymorphisms and are also described in homozygous carriers without disease.

Desmosomal variants are important causes of ACM, some of which cause ARVC [e.g., plakophilin (PKP2)], while others [e.g., desmoplakin (DSP)], cause an overlapping ACM/DCM phenotype. The reported p.Ile305Phe variant in *DSP* is very frequent in controls (160 homozygous carriers in the gnomAD database). Similarly, the p.GluE58Asp variant in *PKP2* and the p.Asp888Asn in *RBM20* are frequent variants in control populations. To attribute modifier status to these variants would require a large case-control study to demonstrate enrichment in a particular phenotype. None of these variants was enriched in ACM or DCM cohorts compared to internal controls and controls from gnomAD in a cohort of 23,000 correlative unrelated probands with different inherited cardiac conditions sequenced by NGS in Health In Code, questioning its effect (*Personal Communication*).

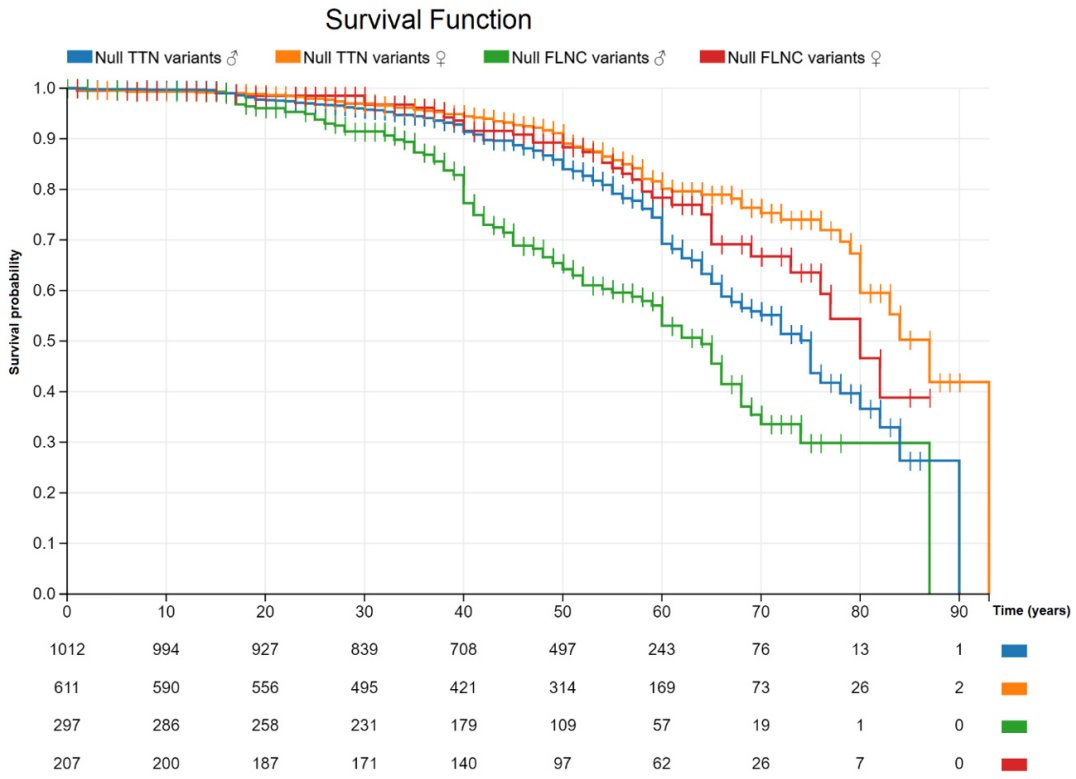

Figure 1. Survival analysis. A Kaplan-Meier curve depicting gender-related survival free from cardiac events (defined as sudden cardiac or heart failure death, transplant, appropriate implantable cardioverter defibrillator therapy, and stroke) in carriers of null variants (frameshift, nonsense, and intronic ones affecting the splicing process) in *FLNC* and *TTN*. Information was extracted from the Health in Code (HIC) proprietary database, which collects clinical and genetic data from more than 15,000 published manuscripts about inherited cardiovascular diseases, and information from > 25,000 probands and families genetically studied at HIC. In carriers of null *FLNC* variants, events start in males in their 20's, progressively increase and become more frequent after age 40. In women, events are unusual before age 30 but become more frequent after age 40. Prognosis is better in females: by age 60, 50% of men and 25%-30% of women had died of a cardiovascular cause (log-rank 0.0001). The main event in carriers of both genders is sudden death. In contrast, events are infrequent at early ages in carriers of null *TTN* variants. By the beginning of the fifth decade, only 10% of carriers suffered an adverse event, but after that, there is a trend to a higher number of events in males. By age 70, significantly more males (50%) compared with females (30%) had suffered an event (P = 0.02). The events are composed of both deaths from heart failure and transplantation and major arrhythmic events (sudden death/appropriate defibrillator therapy). More than 95% of carriers of *TTN* null variants who suffered an event had overt cardiomyopathy. In contrast, 51% of patients with an *FLNC* null variant who had events had absent or mild LV dysfunction.

Several other candidate genes which are not related to ACM or DCM were identified in the study. *FHL1* is a gene associated with skeletal myopathy and restrictive cardiomyopathy, but no variants have been reported associated with primary DCM or ACM. Although many publications and some groups have shown interest in demonstrating the association between *OBSCN* and the development of cardiomyopathies, the level of evidence is still very low, and *OBSCN* should only be considered a candidate gene in the study of cardiomyopathies. *SCN10A* and *AKAP9* are associated with ion channel disease but not ACM or DCM and, as such, are unlikely disease modifiers in this family with the *TTN* splice site variant.

The report by Pourebrahim *et al.*[1] presents a DCM titin family with heart failure in the middle decades and a 57-year-old lady with an unexplained sudden death. The exploration of the potentially overlapping phenotypes of DCM and ACM serves to highlight the presentation/survival differences which are shown in survival curves from patients with titin compared with *FLNC* mutations [Figure 1]. Mutations in *FLNC* represent a prototype of this issue (i.e., ACM and DCM within the same family) with early arrhythmic

events and later-onset heart failure complications[10]. Mutations in *TTN* are much more consistent in association with a DCM phenotype and heart failure outcomes, including arrhythmia[11] in the middle and later decades [Figure 1]. The study of this titin family highlights important issues regarding a correct clinical diagnosis and the interpretation of genetic findings/environmental factors as disease modifiers. There is a complexity in ascribing a diagnosis to a disease phenotype when other medical (e.g., hypertension) and environmental (e.g., obesity, alcohol) factors are present, even with postmortem examination of the whole heart. There is also complexity in interpreting the potential impact of the genetic findings. Both are vexing and important issues related to understanding genetic abnormalities that cause the ACM and DCM phenotypes and their differing outcomes within the same family.

DECLARATIONS

Authors' contributions

Drafted the manuscript: McKenna WJ
Provided the survival curves: de la Higuera Romero L
Provided the interpretation of the genetic variants: Garcia Hernandez S, Ochoa JP

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Not applicable.

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All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

REFERENCES

1. Pourebrahim K, Marian JG, Tan Y, Chang JT, Marian AJ. A combinatorial oligogenic basis for the phenotypic plasticity between late-onset dilated and arrhythmogenic cardiomyopathy in a single family. *J Cardiovasc Aging* 2021;1:12. DOI
2. Towbin JA, McKenna WJ, Abrams DJ, et al. 2019 HRS expert consensus statement on evaluation, risk stratification, and management of arrhythmogenic cardiomyopathy. *Heart Rhythm* 2019;16:e301-72. DOI PubMed
3. Norman M, Simpson M, Mogensen J, et al. Novel mutation in desmoplakin causes arrhythmogenic left ventricular cardiomyopathy. *Circulation* 2005;112:636-42. DOI PubMed
4. Basso C, Thiene G. Adipositas cordis, fatty infiltration of the right ventricle, and arrhythmogenic right ventricular cardiomyopathy. Just a matter of fat? *Cardiovasc Pathol* 2005;14:37-41. DOI PubMed
5. Marcus FI, McKenna WJ, Sherrill D, et al. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia: proposed modification of the task force criteria. *Circulation* 2010;121:1533-41. DOI PubMed PMC
6. Adabag S, Huxley RR, Lopez FL, et al. Obesity related risk of sudden cardiac death in the atherosclerosis risk in communities study. *Heart* 2015;101:215-21. DOI PubMed PMC
7. Finocchiaro G, Papadakis M, Dhutia H, et al. Obesity and sudden death. Pathological insights from a large pathology registry. *Heart* 2016;102:A49. DOI
8. Ware JS, Amor-Salamanca A, Tayal U, et al. Genetic etiology for alcohol-induced cardiac toxicity. *J Am Coll Cardiol* 2018;71:2293-302. DOI PubMed PMC
9. Ceyhan-Birsoy O, Agrawal PB, Hidalgo C, et al. Recessive truncating titin gene, TTN, mutations presenting as centronuclear myopathy. *Neurology* 2013;81:1205-14. DOI PubMed PMC

10. Ortiz-Genga MF, Cuenca S, Dal Ferro M, et al. Truncating FLNC mutations are associated with high-risk dilated and arrhythmogenic cardiomyopathies. *J Am Coll Cardiol* 2016;68:2440-51. DOI PubMed
11. Corden B, Jarman J, Whiffin N, et al. Association of titin-truncating genetic variants with life-threatening cardiac arrhythmias in patients with dilated cardiomyopathy and implanted defibrillators. *JAMA Netw Open* 2019;2:e196520. DOI PubMed PMC