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Titin Missense Variants as a Cause of Familial Dilated Cardiomyopathy

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High-throughput sequencing technologies have revolutionized the identification of genetic variants responsible for genetic diseases such as dilated cardiomyopathy (DCM). However, a causal genetic variant is still not identified in ≈60% of DCM.[1] Although truncating variants in *TTN* (TTNtv) are the main genetic cause of DCM, the role of rare missense variants in *TTN* as a cause of DCM remains unknown. We describe 2 families with DCM in which clinical and in vitro investigations support that missense variants affecting a conserved cysteine of *TTN* are causative of DCM. The study was approved by the local ethics committee, and the data that support the findings of this study are available from the corresponding author on reasonable request.

A Spanish family with 12 individuals with DCM was studied. Three phenotype-positive distant relatives underwent exome sequencing and shared a variant (Chr2(GRCh38):g.178741559A>T) in *TTN* predicted to cause the substitution of a cysteine by a serine: p.Cys3892Ser (NM_001267550.2:c.11674T>A). The affected amino acid is highly conserved (PhastCons100way score of 1.000, PhyloP100way score of 9.293). Several in silico predictors suggest that the change is deleterious, including the meta predictor (score, 0.708).[2] Exome sequencing did not reveal additional shared rare variants in other cardiomyopathy-associated genes.

The proband was a man who underwent cardiac transplantation at 57 years of. Among 36 family members, 14 were carriers and 12 had DCM (86%; Figure A). Median age at DCM diagnosis was 33 years (interquartile range, 18–45). Mean left ventricular (LV) ejection fraction was 43±6% and mean LV end-diastolic diameter was 57±4 mm. The 2 carriers without DCM exhibited LV ejection fraction in the lower limit of normal range. Twenty-two relatives were noncarriers and showed normal phenotype (median age, 51 years; interquartile range, 24–60; LV ejection fraction, 62±5%; LV end-diastolic diameter, 46±2 mm). Family-specific 2-point logarithm of the odds score was 3.96 (dominant model, 80% penetrance).

A Danish family with the same mutated residue but with Cys replaced by Arg (NM_001267550.2:c.11674T>C) was identified. In silico evaluation also predicted the variant to be pathogenic, with a REVEL score of 0.688. The proband was a female (LV ejection fraction of 20%, LV end-diastolic diameter 75 mm) who underwent heart transplantation at 17 years of age. Her father (obligate carrier) was diagnosed with DCM at 54 and died at 73 years of age. A 52-year-old half-sister exhibited the variant without DCM (Figure B).

To further characterize the p.Cys3892Ser variant, induced pluripotent stem cell (iPSC) lines were generated using CRISPR/Cas9 to introduce a point mutation T>A c:11,674 in the titin (TTN) allele of a wild-type human iPSC line (HDF-iPS-SV10, Spanish National Stem Cell Bank), wild-type human iPSC, hetero- and

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CORRESPONDENCE

Nonstandard Abbreviations and Acronyms

DCM	dilated cardiomyopathy
iPSC	induced pluripotent stem cell
LV	left ventricular
TTN	titin

homozygous human iPSC-TTN^{Cys3892Ser} lines were differentiated to cardiac lineage.

Video for single-cell contraction amplitude showed deficient contraction in homozygous p.Cys3892Ser cardiomyocytes (Figure C). We did not detect decreased contraction in heterozygous p.Cys3892Ser cells, consistent with other DCM iPSC lines.[3]

Circular dichroism spectroscopy was used to study thermal denaturation of recombinant purified TTN I21 domains with and without the p.Cys3892Ser variant. Circular dichroism signal at 215 nm was monitored as temperature increased from 25 to 85 °C at a rate of 30 °C/h. The recombinant TTN I21 domain containing p.Cys3892Ser preserved the global fold of the wild-type protein (Figure D) but was not stable at physiological temperatures (Figure E). We also attempted to study variant p.Cys3892Arg by circular dichroism. However, this was not possible due to the insolubility of the mutant domain, which is probably caused by the higher

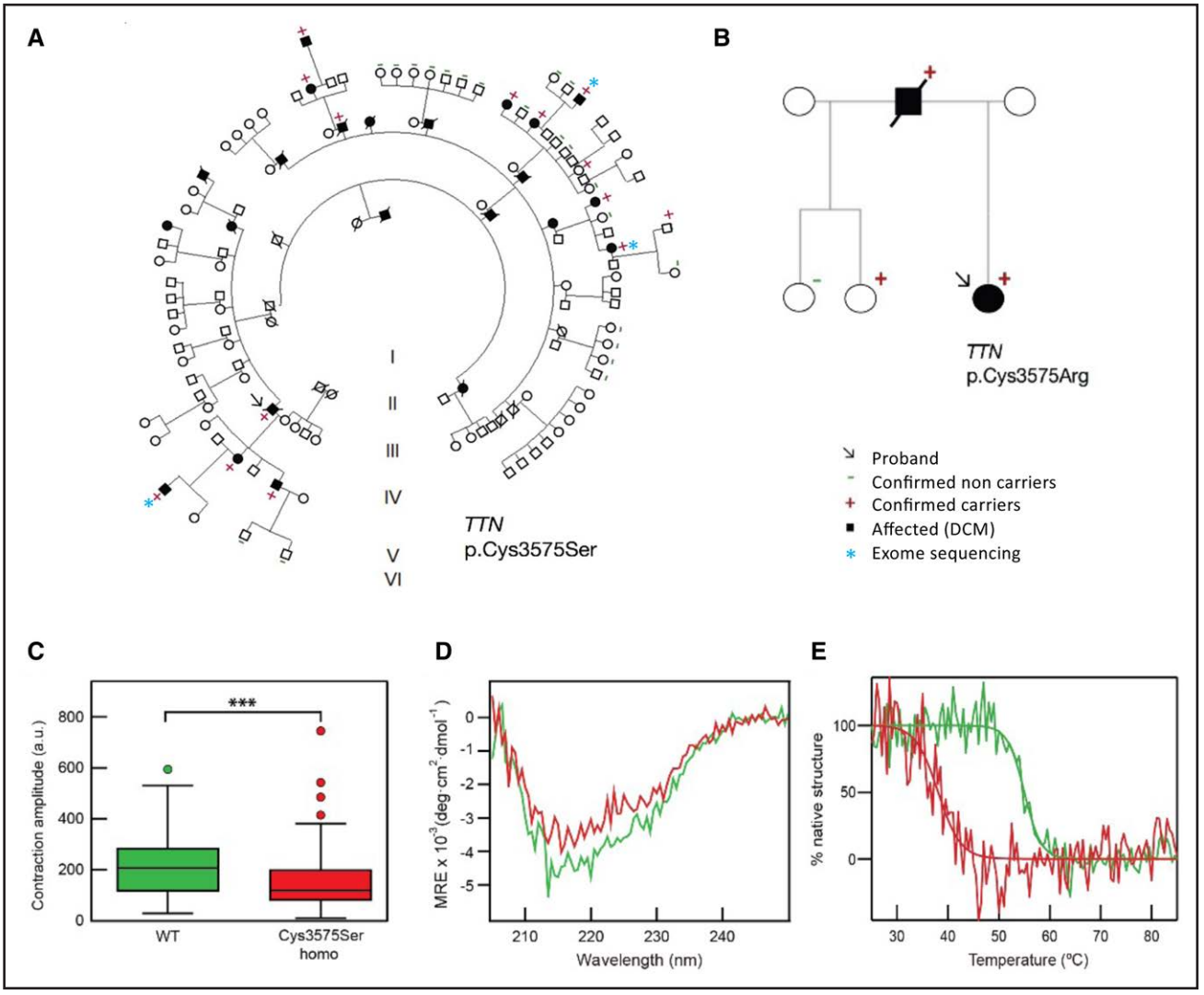

Figure. Evidence supporting the TTN missense variants p.Cys3575Ser and p.Cys3575Arg as a cause of dilated cardiomyopathy. **A**, Pedigree of a large Spanish kindred with 14 TTN p.Cys3575Ser carriers (12 with DCM) and 22 noncarriers. Blue asterisks show individuals who underwent Exome sequencing. **B**, Pedigree of the Danish family with the TTN p.Cys3575Arg variant, with 3 carriers (2 with DCM) and 1 noncarrier. **C**, Amplitude of contraction of WT (green) and homozygous Cys3575Ser (red) induced pluripotent stem cell–induced cardiomyocytes (***P=0.0001, Mann-Whitney). **D**, Far-ultraviolet circular dichroism spectra at 25 °C of WT (green) and Cys3575Ser (red) recombinant I21 protein domains. **E**, Thermal unfolding curves of recombinant I21 WT (green) and Cys3575Ser (red) domains. DCM indicates dilated cardiomyopathy; MRE, molar residue ellipticity; TTN, titin; and WT, wild type.

destabilizing nature of the Cys to Arg substitution (data are not shown).

We provide strong evidence supporting that *TTN* missense variants involving the conserved cysteine p.Cys3892 can cause DCM. Cysteine is one of the least abundant and most conserved amino acids. Cys3892 corresponds to a conserved cysteine of the cardiac specific I21 domain, present in 27 species ranging from zebrafish to humans. None of the 2 variants have been described in the literature or in ClinVar, and they are not reported in gnomAD. Regarding molecular mechanisms of pathogenicity, it is tempting to speculate that TTN domain destabilization could lead to reduced titin levels (haploinsufficiency) or saturation of protein quality control systems, both of which have been linked to pathogenicity in DCM *TTN* truncating variants. The Spanish family exhibited a logarithm of the odds score of 3.96. Logarithm of the odds scores of 3 support odds 20:1 in favor of linkage, and a variant strongly related to the phenotype.

Our study is the first to unequivocally confirm that *TTN* missense variants can cause DCM. Gerull et al[4] reported that a missense *TTN* mutation could cause DCM in a moderately large family with a logarithm of the odds score of 2.73, which is less than the value of 3 usually considered to prove association.

A recent study showing that, in 530 patients with DCM, almost 7% had rare TTN missense variants predicted to be deleterious by bioinformatics filtering. However, they were not over-represented in DCM compared with ExAC, and authors concluded that TTN missense variants should be classified as likely benign.[5] Our results confront with this conclusion, because we proved that missense variants involving Cys3892 are causative of DCM, supporting the pathogenic role of certain missense TTN variants.

ARTICLE INFORMATION

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Disclosures

None.

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