Clinical phenotypes and prognosis of dilated cardiomyopathy caused by truncating variants in the TTN Gene

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Phenotype and Prognosis of Dilated Cardiomyopathy Caused by Truncating Variants in the Titin (*TTN*) gene

Short title: Phenotypes of truncating *TTN* mutations.

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Abstract

Background:

Truncating variants in the *TTN* gene (TTNtv) are the commonest cause of heritable DCM. This study aimed to study the phenotypes and outcomes of TTNtv carriers.

Methods:

537 individuals (61% male; 317 probands) with TTNtv were recruited in 14 centers [372 (69%) with baseline left ventricular systolic dysfunction (LVSD)]. Baseline and longitudinal clinical data were obtained. The primary-endpoint was a composite of malignant ventricular arrhythmia (MVA) and end-stage heart failure (ESHF). The secondary endpoint was LV reverse remodelling (LVRR) [LVEF increase by ≥10% or normalization to ≥50%].

Results:

Median follow-up was 49 [18-105] months. Males developed LVSD more frequently and earlier than females (45±14 vs 49±16 years respectively; p=0.04). By final evaluation, 31%, 45% and 56% had AF, frequent ventricular ectopy and NSVT respectively.

76 (14.2%) individuals reached the primary-endpoint [52 (68%) ESHF-events, 24 (32%) MVA-events]. MVA-endpoints most commonly occurred in patients with severe LVSD. Male sex (HR 1.89, 95%CI: 1.04-3.44; p=0.04) and LVEF [per 10% decrement from LVEF 50%] (HR 1.63, 95%CI: 1.30-2.04; p<0.001) were independent predictors of the primary-endpoint.

207/300 (69%) patients with LVSD had evidence of LVRR. In a subgroup of 29/74 (39%) patients with initial LVRR, there was a subsequent LVEF decrement.

TTNtv location was not associated with statistically-significant differences in baseline clinical characteristics, LVRR or outcomes on multivariable analysis (p=0.07).

Conclusions:

TTNtv is characterized by frequent arrhythmia but MVA are most commonly associated with severe LVSD. Male sex and LVSD are independent predictors of outcomes. Mutation location does not impact clinical phenotype or outcomes.

Keywords: Titin, Dilated Cardiomyopathy, Sex, LV reverse remodelling, Heart Failure.

Clinical implications

The good response to optimal medical therapy emphasises the need to identify and treat patients with TTNtv as soon as left ventricular systolic dysfunction appears. Patients should remain under close surveillance to detect atrial and ventricular arrhythmia and left ventricular systolic dysfunction recurrence following initial let ventricular reverse remodelling. Malignant ventricular arrhythmia predominantly occurs in patients with severe left ventricular systolic dysfunction and supports recommendations for prophylactic implantable cardioverter-defibrillators in patients with advanced disease. Patients with TTNtv have a similar clinical phenotype and outcome irrespective of TTNtv location with respect to the A-band of the predominantly adult splice *TTN*-isoform [www.cardiodb.org/titin].

Non-Standard Abbreviations and Acronyms

ACE	Angiotensin Converting Enzyme
AF	Atrial Fibrillation
ARNI	Angiotensin Receptor-Neprilysin Inhibitor
ATP	Anti-Tachycardia Pacing
CI	Confidence Intervals
CMR	Cardiac Magnetic Resonance
CRT	Cardiac Resynchronization Therapy
DCM	Dilated Cardiomyopathy
ECG	Electrocardiogram
EF	Ejection Fraction
ESHF	End-Stage Heart Failure
FH	Family History
HR	Hazard Ratio
HTx	Heart Transplantation
ICD	Implantable Cardioverter-Defibrillator
IQR	Interquartile Range
KM	Kaplan-Meier
LGE	Late Gadolinium Enhancement
LV	Left Ventricle
LVEF	Left Ventricular Ejection Fraction
LVRR	Left Ventricular Reverse Remodelling
LVSD	Left Ventricular Systolic Dysfunction
MAF	Minor Allele Frequency
MVA	Malignant Ventricular Arrhythmia
NSVT	Non-Sustained Ventricular Tachycardia
NYHA	New York Heart Association
OMT	Optimal Medical Therapy
PLAX	Parasternal Long Axis
PSI	Percentage Spliced Index
RV	Right Ventricle
SCD	Sudden Cardiac Death
TTE	Transthoracic Echocardiogram
TTN	Titin gene
TTNtv	Truncating variants in the *TTN* gene
VAD	Ventricular Assist Device
VE	Ventricular Ectopy
VF	Ventricular Fibrillation
VT	Ventricular Tachycardia

Phenotypes and Prognosis of Dilated Cardiomyopathy (DCM) Caused by Truncating Variants in the Titin (*TTN*) gene

INTRODUCTION

Dilated cardiomyopathy (DCM) has an estimated population prevalence of 1:250 and is the commonest cause for heart transplantation (HTx) worldwide.[1, 2] More than 25% of patients with DCM have a genetic predisposition [2, 3] and emerging data suggest that genotype has an important impact on prognosis and therapy.[4-6]

Truncating variants in the *TTN* gene (TTNtv), encoding the giant protein titin, are the commonest genetic subtype of DCM, accounting for up to 25% of cases.[7, 8] Titin is an integral sarcomeric protein involved in passive force transmission and plays essential roles in, sarcomere organization, elasticity and cell signalling.[9] In some studies, TTNtv associated with DCM appear to be highly enriched in the A-band [7] but more recently, TTNtv mutations in other constitutively-expressed exonic regions have also been implicated in DCM.[10, 11] In addition, previous studies on TTNtv are inconsistent with respect to arrhythmia burden [10, 12-16], response to optimal medical therapy (OMT) [12, 13, 17, 18], impact of mutation location [7, 10, 11, 19, 20] and prognosis.[7, 10, 13, 14] In this study, we report the characteristics and outcomes of a large international cohort of consecutive probands and relatives with TTNtv.

METHODS

This study conforms to the principles of the Helsinki declaration and all authors guarantee the integrity of data from their respective institutions. Approval from a local ethics committee / internal review board was obtained at each participating center. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Cohort composition

This is a multi-center, longitudinal cohort study comprising consecutive probands and relatives evaluated in 13 European and 1 Australian cardiomyopathy unit between 1985 and 2019. Inclusion criteria for probands were patients with idiopathic left ventricular systolic dysfunction (LVSD) unexplained by abnormal loading conditions or coronary artery disease; or the presence of a familial history of cardiomyopathy with an abnormal cardiac phenotype (left ventricular dilatation, atrial or ventricular arrhythmia, abnormal T-wave inversion on ECG) (**Supplementary Methods S1)**. Only probands with a pathogenic or likely pathogenic TTNtv were included. Relatives were included if they carried a pathogenic or likely pathogenic TTNtv mutation identified in their family proband, irrespective of kinship or phenotypic expression.

Genetic testing

Genetic testing in probands was undertaken using targeted next generation, whole exome or whole genome sequencing at participating institutions or at an accredited genetics laboratory with no *a priori* selection based on clinical phenotype or adverse events. Presence of a familial history of DCM was not mandatory for genetic testing and varied according to routine institutional practice. Sanger sequencing was used for cascade screening in relatives. Likely-pathogenic TTNtv were novel or rare variants located in constitutively expressed exons with a percentage spliced index (PSI)>90% and with a minor allele frequency (MAF) <0.01% in control populations using gnomAD browser [www.gnomad.broadinstitute.org] (**Supplementary Methods S1**).[11, 20] Patients with additional pathogenic mutations in other genes implicated in DCM were excluded.

Baseline assessment

Demographics, symptoms, 12-lead electrocardiogram (ECG), transthoracic echocardiogram (TTE) and, where available, ambulatory-ECG and cardiac magnetic resonance (CMR) imaging were collated from clinical records. Data were collected independently at each participating center using uniform methodology.

LVSD was defined as left ventricular ejection fraction (LVEF) <50% on TTE or CMR. Mild, moderate and severe LVSD were defined as LVEF values of 45-49%, 36-44% and ≤35%, respectively. DCM, LV dilatation, non-sustained ventricular tachycardia (NSVT), frequent ventricular ectopy (VE≥500/24-hours) and atrial fibrillation (AF) were defined using consensus recommendations (**Supplementary Methods S1**).[21]

Study endpoints

The primary survival endpoint was a composite of malignant ventricular arrhythmia (MVA) [sudden cardiac death (SCD), aborted SCD, appropriate implantable cardioverter-defibrillator (ICD) shock] and end stage heart failure (ESHF) [destination ventricular assist device (VAD), HTx or ESHF-mortality]. Patients were censored at the time of their first endpoint event or at last evaluation. Only events occurring during follow-up at participating centers were included.

The secondary-endpoint was LV reverse remodelling (LVRR) with optimal medical therapy, defined as either LV normalization (LVEF improvement to ≥50% with a ≥5% improvement in LVEF value on TTE post LVSD onset) or an absolute increase in LVEF by ≥10%.[12, 13]

Follow-up

Follow-up duration for each patient was calculated from baseline clinical evaluation at participating centers to the date of their first study endpoint, death from another cause or the date of last evaluation.

Statistical analysis

Analyses were performed using SPSS Statistics version 25.0 (IBM, New York). A 2-sided p-value < 0.05 defined statistical significance. Variables are expressed as counts (percentages), mean ± standard deviation (SD), and median [lower quartile-upper quartile] as appropriate. Chi-squared analysis or Fisher's-exact test was used to compare categorical data between groups. The independent samples Student t-test or ANOVA test was used to compare normally-distributed continuous data between groups and the Mann-Whitney *U* test was used to compare the distribution of skewed continuous data between groups.

Univariable Cox regression was used to assess the association of baseline variables with the primary-endpoint after confirmation that the proportional hazards assumption remained valid. Predictors with p <0.05 on univariable analysis were incorporated into a multivariable Cox-regression model to calculate hazard ratios (HR) and 95% confidence intervals (CI).

Kaplan-Meier (KM) plots were used to display the cumulative probability of the occurrence of LV systolic dysfunction (penetrance) and of the occurrence of the composite primary-endpoint for the entire cohort and when stratified according to patient sex and TTNtv mutation location. The log-rank test was used to compare survival between groups.

RESULTS

565 patients with TTNtv were identified; 28 were excluded due to re-classification of a TTNtv to a 'variant of unknown significance' or due to co-inheritance of a pathogenic mutation in another DCM causing gene. The final cohort comprised 537 individuals [317 (59%) probands; 328 (61%) males] with a median family size of 1 [1-2] individual including probands. 220 families contributed only a single proband to the cohort; 97 families incorporated multiple individuals with a median family size of 3 [2-4] individuals including probands.

The overall cohort comprised 267 distinct TTNtv [125 nonsense, 129 frameshift and 13 canonical splice-site variants] with a mean of 1.2 probands per TTNtv (**Figure 1, Supplementary Tables S1 and S2**). 229 TTNtv were unique to individual probands, 28 variants were present in 2 unrelated probands, 8 variants were present in 3 unrelated probands and 2 variants were present in 4 unrelated probands.

286 (90.2%) probands were of Caucasian ethnicity, 9 (2.8%) were Asian, 5 (1.6%) were Afro-caribbean and 17 (5.4%) were of mixed or other ethnicities. 246 (78%) probands had TTNtv located in the A-band domain and 71 (22%) had TTNtv in non-A band domains [45 (14%) I-band, 18 (6%) M-band and 8 (2%) in the Z-disk region].

The baseline characteristics of the cohort are summarized in **Table 1.** 185 (58.4%) probands had a FH of DCM and 87 (27.4%) a FH of SCD. The commonest presenting symptom in the entire cohort was NYHA class ≥3 dyspnoea in 148 (28%) followed by palpitations in 109 (21%). Syncope occurred in 15 (3%) at baseline.

53 (10%) patients had AF identified on baseline ECG. The median LVEF was 30% [20-40%] in probands and 56% [45-60%] in relatives (p<0.001). Probands had a more advanced phenotype than relatives (**Supplementary Table S3**); 77/220 (35%) relatives had LVSD at baseline of whom 23 (30%) were entirely asymptomatic.

Ambulatory-ECG findings at baseline evaluation

272 (51%) patients had a baseline Holter as part of routine institutional practice, irrespective of symptoms or clinical phenotypes. AF was present on baseline Holter in 44/272 (16%).

AF was documented on baseline ECG or Holter in 86/537 (16%) individuals and preceded LVSD in 14/165 (8.5%) of patients with a normal LVEF. 53 (62%) of patients with AF on baseline ECG or Holter were asymptomatic with respect to palpitations.

The prevalence of AF at baseline evaluation was 7/42 (17%), 14/70 (20%) and 48/242 (20%) in patients with mild, moderate and severe LVSD, respectively. Patients with AF and impaired LVEF were of similar age to TTNtv patients with AF and preserved LVEF at baseline evaluation (54±14 vs 59±17 years respectively; p=0.24). Patients with AF on baseline ECG or Holter had larger left atrial dimensions than those without evidence of AF [parasternal long axis dimension on TTE 44±9 vs 39±8mm (p<0.0001) and 4-chamber left atrial area on CMR 32.2±8.1 vs 26.7±8.0 cm^2 (p=0.03), respectively].

74/187 (40%) patients had frequent VE (median 213 [11-1765] VE/24 hours) and 126/272 (46%) had NSVT (median of 2 runs of NSVT/24 hours) on baseline Holter. VEs were polymorphic in 57% and monomorphic in 43%. VE burden correlated with the severity of

LVSD (p <0.001) (**Figure 2A**). In TTNtv patients with preserved LVEF, NSVT and frequent VE on baseline Holter occurred in 5/52 (10%) and 10/43 (23%), respectively.

Cardiac Magnetic Resonance Imaging

209 patients had CMR imaging data available; 100 (48%) had late gadolinium enhancement (LGE); the presence of any degree of LGE correlated with progressive severity of LVSD; p<0.001) (**Figure 2B**). The presence of LGE on baseline CMR was associated with baseline NYHA class III-IV dyspnoea (40% vs 18%; p=0.001), AF (21% vs 8%; p=0.04), NSVT (60% vs 35%; p=0.01), and increased VE burden (median 337 [31-2457] vs 58 [3-789] /24 hour; p=0.04) on baseline Holter evaluation. Quantitative assessment of the burden of LGE were unavailable.

Sex Differences

Males were more likely to be probands (68% vs 45%; p<0.001). Male probands were also more likely to be in NYHA class III-IV (44% vs 33%; p=0.07) and had lower baseline LVEF (29±13% vs 32±11%; p=0.04). Male probands had a higher prevalence of AF on baseline Holter (20% vs 5%; p=0.009), NSVT (58% vs 42%; p=0.046) and LGE on baseline CMR (59% vs 32%; p=0.004). Male and female probands had similar VE-burden on baseline Holter (p=0.71).

Medical Therapy

332/351 (95%) patients with LVSD (at baseline or during follow-up) received ACE inhibitors or angiotensin receptor blockers, 324/350 (93%) ß-blockers and 238/346 (69%) mineralocorticoid receptor antagonists; 51/347 (15%) patients were switched to an angiotensin receptor-neprilysin inhibitor (ARNI), 50/348 (14%) were on ivabradine and

4/345 (1%) received a nitrate/hydralazine combination; 214/347 (62%) patients required oral loop diuretics. At last evaluation, 120 (22%) patients had an ICD for primary prevention and 20 (4%) for secondary prevention indications; 31 (6%) patients had cardiac resynchronization therapy (CRT).

Clinical phenotypes during follow-up

At baseline evaluation, 372 (69%) individuals had LVSD, 49 (9%) had cardiac abnormalities suggestive of phenotypic expression including abnormal T-wave inversion on ECG, isolated LV dilatation, LGE on CMR or atrial or ventricular arrhythmia, and 116 (22%) were phenotype negative. During a median follow-up of 49 [18-105] months (total of 2822 patient years), 25 (4.7%) patients with normal baseline LVEF developed LVSD. By final evaluation, 397 (74%) patients had evidence of LVSD and 39 (7%) had cardiac abnormalities suggestive of phenotypic expression (**Supplementary Methods S1**). Patients with low-normal LVEF (LVEF 50-55%) at baseline evaluation were more likely to develop LVSD during follow-up compared to those with LVEF ≥55% (21/41 (51%) vs 4/122 (3%); p<0.001).

Male TTNtv patients developed LVSD more frequently and at a younger age than females (85% vs 57%; mean age at diagnosis of LVSD (at baseline or over follow up): 45±14 vs 49±16 years, respectively; p=0.04) (**Figure 3; Supplementary Figure S1-3**).

During follow-up, there were 74 new cases of AF identified on ECG or Holter. At final evaluation, AF, frequent VE, NSVT and sustained VT were identified in 160/537 (30%), 92/204 (45%), 157/281 (56%) and 46/537 (9%) of the overall *TTN* cohort respectively.

In the 160 patients with AF at final evaluation, AF was paroxysmal in 105 (66%), persistent in 19 (12%) and permanent in 36 (22%). 6 of 140 (4%) patients with an ICD had an inappropriate ICD shock due to rapidly conducted AF. 14 patients (2.6%) had hemodynamically stable sustained VT treated with antitachycardia pacing (ATP) in the absence of a primary-endpoint event (12 patients with baseline severe LVSD and individual patients with baseline mild or moderate LVSD).

Clinical events during follow-up

Primary endpoint

There were 76 (14.2%) primary-endpoint events: ESHF in 52 (68%) and MVA in 24 (32%). The most frequent ESHF endpoint was HTx (n=40), followed by ESHF-mortality (n=11) and VAD (n=1). MVA endpoints were most commonly appropriate ICD shocks for VT / VF (n=17), followed by aborted SCD (n=4) and SCD (n=3) (**Figure 4**); 16 (3%) patients had multiple MVA and/or ESHF endpoints. All endpoint events occurred in patients with LV systolic dysfunction at baseline or over follow-up (**Supplementary Table S4**)].

Univariable baseline predictors of the primary endpoint are shown in **Table 2**. Baseline parameters with a univariable p-value of <0.05 were incorporated into a multivariable model including proband status as a first step, followed by forward conditional Cox regression multivariable modelling of other baseline variables. As only a minority of patients underwent baseline Holter monitoring, NSVT was excluded from the multivariable model to minimise case-censoring of incomplete data. Multivariable modelling included proband status as a first step, followed by male sex, A-band TTNtv mutation location, LV dilatation and LVEF [per 10% decrement from LVEF 50%]. Multivariable Cox regression incorporating 482 patients (71 events), demonstrated that male sex (HR 1.89, 95%CI: 1.04-3.44; p=0.04) and LVEF [per

10% decrement from LVEF 50%] (HR 1.63, 95%CI: 1.30-2.04; p<0.001) were independent predictors of the primary endpoint (**Table 2, Figures 5A & 5B**).

There were no statistically significant associations between TTNtv variant location with respect to *TTN*-band or cronos alternative promoter location and baseline clinical characteristics, age at LVSD penetrance or response to optimal medical therapy (**Supplementary Tables S5-6, Supplementary Figure S4**). Patients with A-band TTNtv had reduced cumulative survival from the composite primary endpoint from baseline clinical evaluation compared to patients with non-A band TTNtv on univariable analysis (HR 2.12; 95%CI: 1.11-4.05; p=0.02) only, but this was not statistically significant on multivariable modelling (HR 1.85; 95%CI: 0.96-3.56; p=0.07) from baseline evaluation or on a univariable proband-only analyses from birth (HR 1.16; 95%CI: 0.66-2.05; p=0.61) or baseline evaluation (HR 1.33; 95%CI: 0.69-2.57; p=0.39) (**Figure 5C, Supplementary Figure S5**). Similarly, when probands with TTNtv location prior to or post- cronos, the internal *TTN* promotor were assessed, there was no statistically significant difference in cumulative survival from the composite primary endpoint from birth (p=0.73) or baseline clinical evaluation (p=0.39).

Secondary endpoint

LV reverse remodelling was assessed in 300 patients with LVSD and serial echocardiograms. LVRR occurred in 207 (69%) patients [LVEF normalization in 117 and LVEF improvement ≥10% in 90]. LVRR occurred in 64%, 57% and 74% in those presenting with mild, moderate and severe LVSD, respectively. The mean absolute LVEF improvement from baseline to final evaluation was 11±14%.

Patients with LVRR had fewer primary-endpoint events compared to those without LVRR ($p<0.001$) despite similar follow-up duration from DCM-onset (77±63 vs 78±60 months respectively) (**Figure 5D**). There were no statistically significant differences in sex [134 (68%) vs 65 (75%) men; p=0.28], A-Band TTNtv [150 (77%) vs 65 (75%); p=0.74], baseline LVEF (30.0±10.9 vs 31.2±10.8%; p=0.12) or presence of LGE [49 (53%) vs 19 (56%); p=0.75] between those with and without LVRR respectively (**Supplementary Table S7**). In a sub-group of 74 patients with LVRR and subsequent serial TTE (post-LVRR), there was evidence of a reduction in LVEF by ≥10% despite OMT in 29 (39%) (**Supplementary Methods S1**).

DISCUSSION

This study demonstrates that DCM caused by penetrant TTNtv is associated with frequent atrial and ventricular arrhythmia and progression to heart failure irrespective of mutation location.

Familial screening

In our cohort, more than 30% of relatives had LVSD at baseline, of whom almost one third were asymptomatic despite the presence of impaired LV systolic function. This shows the value of cascade genetic testing and clinical screening in relatives to allow the timely commencement of prognostic medical therapy. Similarly, the fact that 4.7% of TTNtv carriers without DCM at baseline, went on to develop disease over follow-up, is an illustration of the importance of longitudinal follow-up of presymptomatic mutation carriers.

High prevalence of atrial and ventricular arrhythmia

Previous studies have reported contradictory data on the prevalence of atrial and ventricular arrhythmia in TTNtv carriers. Some have suggested that AF occurs in up to a third of patients with TTNtv [12-14] and that TTNtv are associated with a 3-fold increased odds of early atrial arrhythmia compared to non-*TTN* DCM.[15, 22, 23] In contrast, others have suggested a similar incidence of AF in *TTN*- and non-*TTN* DCM.[12, 15, 24] This study shows that the TTNtv phenotype is characterised by both atrial and ventricular arrhythmias with almost one third of patients developing atrial arrhythmias and one half NSVT. Importantly, nearly 10% of TTNtv carriers with preserved LVEF developed AF or NSVT, highlighting the need for regular Holter monitoring in all individuals with TTNtv.[22]

Prognosis and response to medical therapy

Initial studies of TTNtv suggested they are associated with fewer adverse events than idiopathic or other genetic forms of DCM.[7, 8, 12-14, 17, 24, 25] Our study demonstrates that TTNtv carriers are prone to ESHF and MVA, with a predominance of heart failure events. Importantly, the majority of malignant arrhythmic events in both sexes occurred in patients with severe LVSD supporting adherence to consensus EF <35% based thresholds for primary prevention ICD implantation.

OMT was associated with left ventricular reverse remodelling in the majority of patients. The frequency of LVRR is considerably higher than values reported previously in idiopathic DCM cohorts [26, 27] and is associated with a better prognosis. In order to determine the sustainability of structural remodelling, we examined long term changes in LVEF in a subset of patients with serial TTE after LVRR, and observed some late deterioration in LVEF. This phenomenon needs to be studied in larger cohorts, but the data suggests that continued long-term LVEF monitoring is required, even when patients respond favorably to medical therapy.

Impact of sex on outcomes

This study demonstrates that males with TTNtv have earlier disease penetrance and a more advanced phenotype including larger indexed LV dimensions, lower LVEF, more atrial arrhythmia and frequent LGE on CMR. Despite similar LVRR rates on OMT when compared to females, males also have more adverse events and reduced survival on multivariable modelling compared to females. This sex-related risk is unexplained but is not unique to *TTN* having been demonstrated in other forms of dilated or arrhythmogenic cardiomyopathy.[4, 16, 28, 29]

Mutation location and clinical phenotype

Some studies have shown that TTNtv mutation location has no effect on cardiac phenotype [15] whereas others have suggested an attenuated cardiac phenotype in patients with TTNtv located in non-A band domains or located pre-cronos due to rescue from an internal *TTN* promoter.[7, 10, 19] In this study of the largest cohort of TTNtv patients to date, we demonstrate no statistically significant difference in baseline clinical phenotypes or age of LVSD penetrance associated with TTNtv located across different *TTN*-bands or positions with respect to pre- or post-cronos alternative promoter location. There was a trend towards a poorer prognosis in TTNtv located in the A-band on univariable analysis compared to non-A band TTNtv, but this trend was statistically non-significant on multivariable modelling or on a proband-only survival analysis from birth or baseline clinical evaluation.

What is new?

- Male sex is associated with more advanced clinical phenotype and adverse outcomes compared to females.

- Both male sex and left ventricular ejection fraction are independent predictors of adverse events.
- TTNtv mutation location in the predominantly adult splice *TTN*-isoforms [www.cardiodb.org/titin], with respect to A-band or cronos does not impact on clinical phenotypes or outcomes.
- LV reverse remodelling is frequent with medical therapy, but LV systolic function can deteriorate in the long term.

CONCLUSION

Dilated cardiomyopathy caused by TTNtv is characterized by frequent atrial and ventricular arrhythmia and a high rate of LV reverse remodelling with optimal medical therapy. Malignant ventricular arrhythmias are predominantly associated with severe left ventricular systolic dysfunction. Male sex and left ventricular ejection fraction are independent predictors of adverse outcomes. When strict criteria for TTNtv pathogenicity or likely-pathogenicity is taken into account [www.cardiodb.org/titin], TTNtv location (in predominantly adult splice *TTN*-isoforms) does not influence clinical phenotype or outcomes.

LIMITATIONS

The participating centers are all tertiary referral units and so the results of the study may be prone to referral bias and may not be generalisable in all clinical scenarios. The majority of this cohort are symptomatic probands and given the incomplete penetrance of TTNtv, there may be a selection bias towards those with a more advanced phenotype. While the findings of this study were unchanged when proband-only analyses were undertaken, an impact of relatives biasing the study cannot be excluded.

Although there is a high *a priori* probability that the TTNtv variants are pathogenic due to extensive genetic panel for DCM-causing genes and strict inclusion criteria for TTNtv with PSI >90% and MAF<0.01%, cosegregation data were limited.

Given the long duration of enrolment, there is a potential for calendar year to be a confounder for clinical outcomes. The longitudinal nature of this real world data means that missing data from Holter or CMR can result in ascertainment bias. Appropriate ICD shocks were not adjudicated.

Clinical Phenotypes and Prognosis of Dilated Cardiomyopathy Caused by Truncating Variants in the Titin (*TTN*) gene

Short title: Clinical phenotypes of truncating *TTN* mutations.

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TABLE & FIGURE LEGENDS

Table 1: Baseline demographic and clinical data of 537 TTNtv patients stratified according to baseline left ventricular systolic dysfunction (ms: milliseconds; PLAX: Parasternal Long Axis; * A minority of probands had normal LVEF at baseline – this was due to some having a FH of DCM / SCD and being genetically tested due to an abnormal cardiac phenotype with LV dilatation, atrial / ventricular arrhythmia or low-normal LVEF (EF 50-55%) or due to a prior DCM diagnosis and subsequent improvement in LVEF (on OMT) prior to transfer of care to a participating institution).

Table 2: Univariable and Multivariable Predictors of the primary-endpoint using baseline clinical data obtained from clinical evaluation, ECG, TTE, CMR and Holter. *NSVT was not included in multivariable Cox regression model to minimise case-censoring. Proband status was included into the model as a first step and forward conditional Cox-regression using sex, LVEF (per 10% decrement from LVEF 50%), LV dilatation and *TTN* A-band location. *TTN* A-band location (p=0.07) and LV dilatation (p=0.23) were removed from the equation on multivariable modelling due to a lack of statistical significance. This model incorporated 71 events and 482 (90%) patients.

Figure 1: Picture demonstrating the location of TTNtv in this cohort with respect to *TTN*-band. The green triangles represent the locations of the different TTNtv. The orange bar underneath represents the corresponding exon location in the *TTN* metatranscript. The bar underneath displays the corresponding *TTN*-band. The thin grey bars represent the PSI for the different exons as described in previous studies [www.cardiodb.org/titin] [10]. All incorporated TTNtv were in exons with PSI >90% and were constitutive including in the N2B and N2BA isoforms of *TTN*.

Figure 2A: Box-Plot demonstrating the VE-burden/24 hours on baseline Holter assessment for the overall cohort (probands and relatives) - stratified according to LVEF category.

Figure 2B: Bar-chart demonstrating the percentage of TTNtv patients with LGE on CMR for the overall cohort (probands and relatives) - categorised according to LVEF on CMR.

Figure 3: Bar-chart demonstrating age-related onset of LVSD for the overall TTNtv cohort and stratified according to sex and proband status. [KM curves for LVSD penetrance demonstrated in Supplementary Figures S1-3].

Figure 4: Flow-chart demonstrating the outcomes of patients (stratified with respect to LV systolic dysfunction) with TTNtv over follow-up with respect to arrhythmic- and HF-endpoints.

Figure 5A: KM-survival analysis from baseline evaluation for the composite primary-endpoint stratified according to the presence of baseline LVSD. 71 endpoint events occurred in patients with baseline LVSD compared to 5 events in patients with baseline normal LVEF (p=0.003).

Figure 5B: KM-survival analysis from baseline evaluation for the composite primary-endpoint stratified according to male sex. 61 endpoint events occurred in male patients compared to 15 events in female patients (p=0.002).

<u>Figure 5C:</u> KM-survival analysis from baseline evaluation for the composite primary-endpoint for *TTN*-probands stratified according to *TTN* mutation location with respect to A-band. 50 endpoint events occurred in A-band TTNtv patients compared to 11 events in non A-band TTNtv patients (p=0.39).

<u>Figure 5D:</u> KM-survival analysis from baseline DCM echocardiogram for the composite primary-endpoint stratified according to occurrence of LVRR on OMT. 26 endpoint events occurred in TTNtv patients without LVRR compared to 16 events in TTNtv patients with evidence of LVRR (p<0.001).

TABLE 1:

CATEGORY	Numbers Evaluated (n)	NUMBERS IN COHORT (n=537)	Baseline LVSD (n=372)	Baseline Normal LVEF (n=165)	p-value for comparator
Proband*	537	317 (59%)	295 (79%)	22 (13%)	**<0.001**
Male Sex	537	328 (61%)	259 (70%)	69 (42%)	**<0.001**
Caucasian Ethnicity	537	496 (92%)	336 (90%)	160 (97%)	**0.04**
TTNtv PATHOGENIC / LIKELY-PATHOGENIC VARIANT CHARACTERISTICS					
A-Band	537	414 (77%)	288 (77%)	126 (76%)	0.79
Post-Cronos	537	468 (87%)	323 (87%)	145 (88%)	0.74
BASELINE CLINICAL EVALUATION					
Mean Age (years)	536	44 ± 16	47 ± 15	38 ± 17	**<0.001**
NYHA Class III-IV	523	148 (28%)	145 (40%)	3 (2%)	**<0.001**
Syncope	522	15 (3%)	13 (4%)	2 (1%)	0.17
Palpitations	522	109 (21%)	88 (24%)	21 (13%)	**0.003**
BASELINE ECG					

PR Interval (ms)	386	167 ± 30	174 ± 30	156 ± 26	**<0.001**
QRS Duration (ms)	422	96 ± 19	101 ± 20	86 ± 11	**<0.001**
Abnormal T-wave inversion	484	139 (29%)	131 (40%)	8 (5%)	**<0.001**
Low QRS Voltage Limb leads	485	60 (12%)	40 (12%)	20 (13%)	0.89
BASELINE TRANSTHORACIC ECHOCARDIOGRAPHY					
LV Maximal Wall Thickness	452	9.4 ± 1.8	9.6 ± 1.8	8.9 ± 1.7	**<0.001**
Left Atrium Size PLAX view (mm)	376	39 ± 9	42 ± 8	34 ± 7	**<0.001**
LV End-Diastolic Diameter (mm)	442	57 ± 10	62 ± 9	48 ± 5	**<0.001**
LV Ejection Fraction (%)	520	39 ± 17	30 ± 11	59 ± 6	**<0.001**
BASELINE 24-HOUR HOLTER					
AF	272	44 (16%)	37 (17%)	7 (14%)	0.55
NSVT	272	126 (46%)	121 (55%)	5 (10%)	**<0.001**
VE Burden/24hr - Median (IQR)	165	213 [11-1765]	340 [35-2974]	12 [1-256]	**<0.001**
BASELINE CARDIAC MRI					
Indexed LV End-Diastolic Volume (ml/m^2)	153	115 ± 39	123 ± 40	89 ± 18	**<0.001**
LV Ejection Fraction (%)	180	40 ± 15	35 ± 13	58 ± 7	**<0.001**

RV Ejection Fraction (%)	142	51 ± 13	49 ± 14	59 ± 7	**<0.001**
Indexed LV Mass (g/m^2)	76	69 ± 21	72 ± 22	57 ± 15	**0.004**
Late Gadolinium Enhancement	178	83 (47%)	78 (53%)	5 (16%)	**<0.001**
EXTRA-CARDIAC PHENOTYPE					
Creatine Kinase (*u*mol/l) – median (IQR)	121	100 [87-144]	100 [86-156]	100 [91-138]	0.99

TABLE 2:

PREDICTORS OF THE PRIMARY-ENDPOINT	Univariable HR	95% CI	Univariable p-value		Multivariable HR	95% CI	Multivariable p-value
Age / 10	1.08	0.92-1.26	0.33				
Proband	1.79	1.02-3.17	**0.04**		1.01	0.51-1.99	0.98
Male Sex	2.42	1.38-4.27	**0.002**		1.89	1.04-3.44	**0.04**
TTNtv Type (cf. splice):							
Nonsense	1.15	0.40-3.31	0.80				
Frameshift	1.45	0.50-4.22	0.50				
***TTN* A-Band**	2.12	1.11-4.05	**0.02**				
LV Dilatation	2.12	1.09-4.14	**0.03**				
LVEF (per 10% decrement from LVEF 50%)	1.71	1.40-2.10	**<0.001**		1.63	1.30-2.04	**<0.001**
NSVT*	3.80	1.69-8.52	**0.001**				
Frequent VE	1.25	0.35-4.46	0.74				
LGE	2.11	0.35-12.66	0.42				

FIGURE 1:

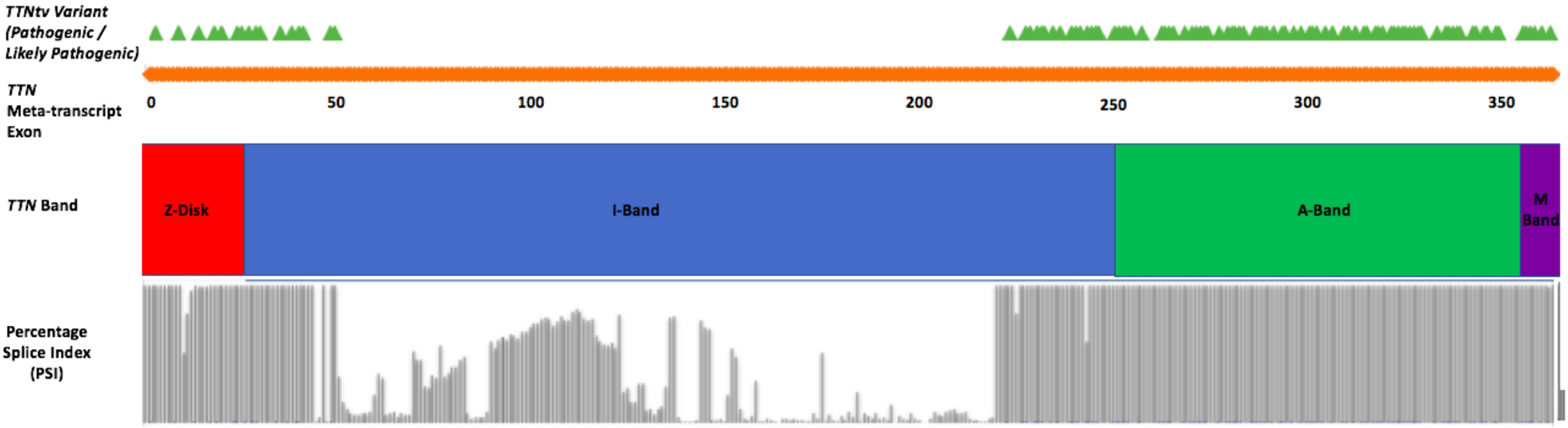

TTNtv Variant
(Pathogenic /
Likely Pathogenic)
TTN
Meta-transcript
Exon
0
50
100
150
200
250
300
350
TTN Band
Z-Disk
I-Band
A-Band
M
Band
Percentage
Splice Index
(PSI)

FIGURE 2A:

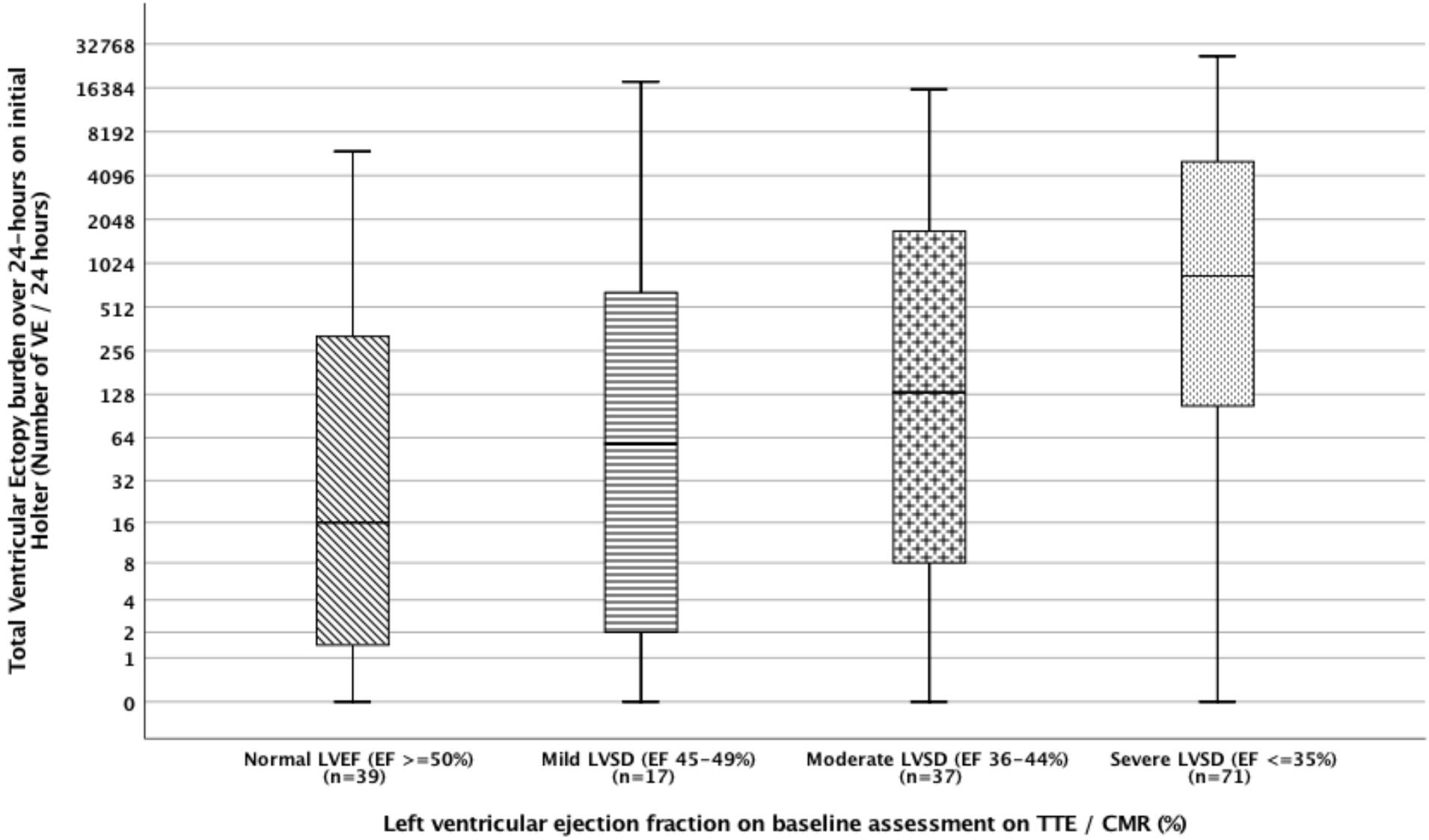

Total Ventricular Ectopy burden over 24-hours on initial Holter (Number of VE / 24 hours)
32768
16384
8192
4096
2048
1024
512
256
128
64
32
16
8
4
2
1
0
Normal LVEF (EF >=50%) (n=39)
Mild LVSD (EF 45-49%) (n=17)
Moderate LVSD (EF 36-44%) (n=37)
Severe LVSD (EF <=35%) (n=71)
Left ventricular ejection fraction on baseline assessment on TTE / CMR (%)

FIGURE 2B:

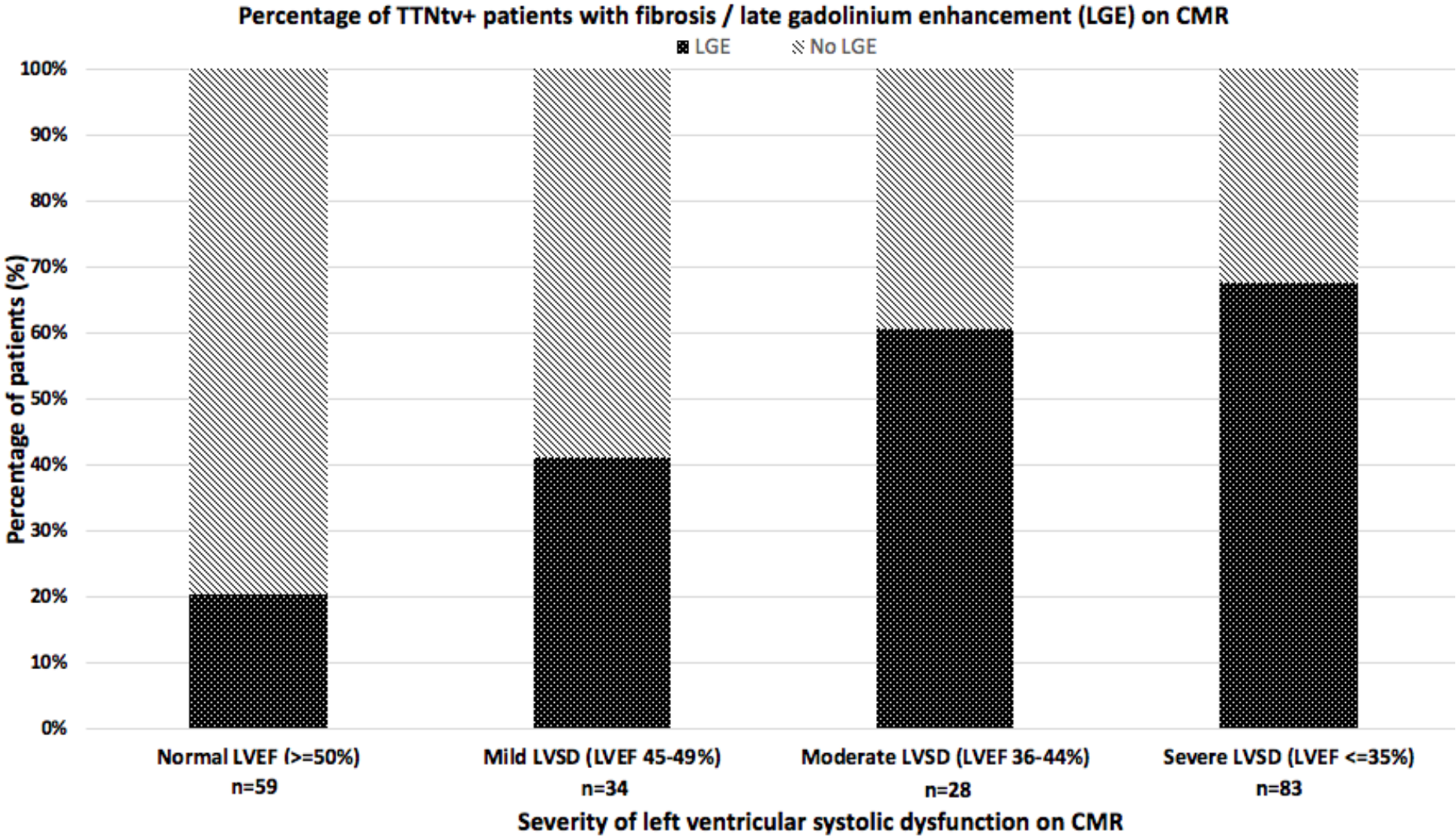

Percentage of TTNtv+ patients with fibrosis / late gadolinium enhancement (LGE) on CMR
LGE
No LGE
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percentage of patients (%)
Normal LVEF (>=50%)
n=59
Mild LVSD (LVEF 45-49%)
n=34
Moderate LVSD (LVEF 36-44%)
n=28
Severe LVSD (LVEF <=35%)
n=83
Severity of left ventricular systolic dysfunction on CMR

FIGURE 3:

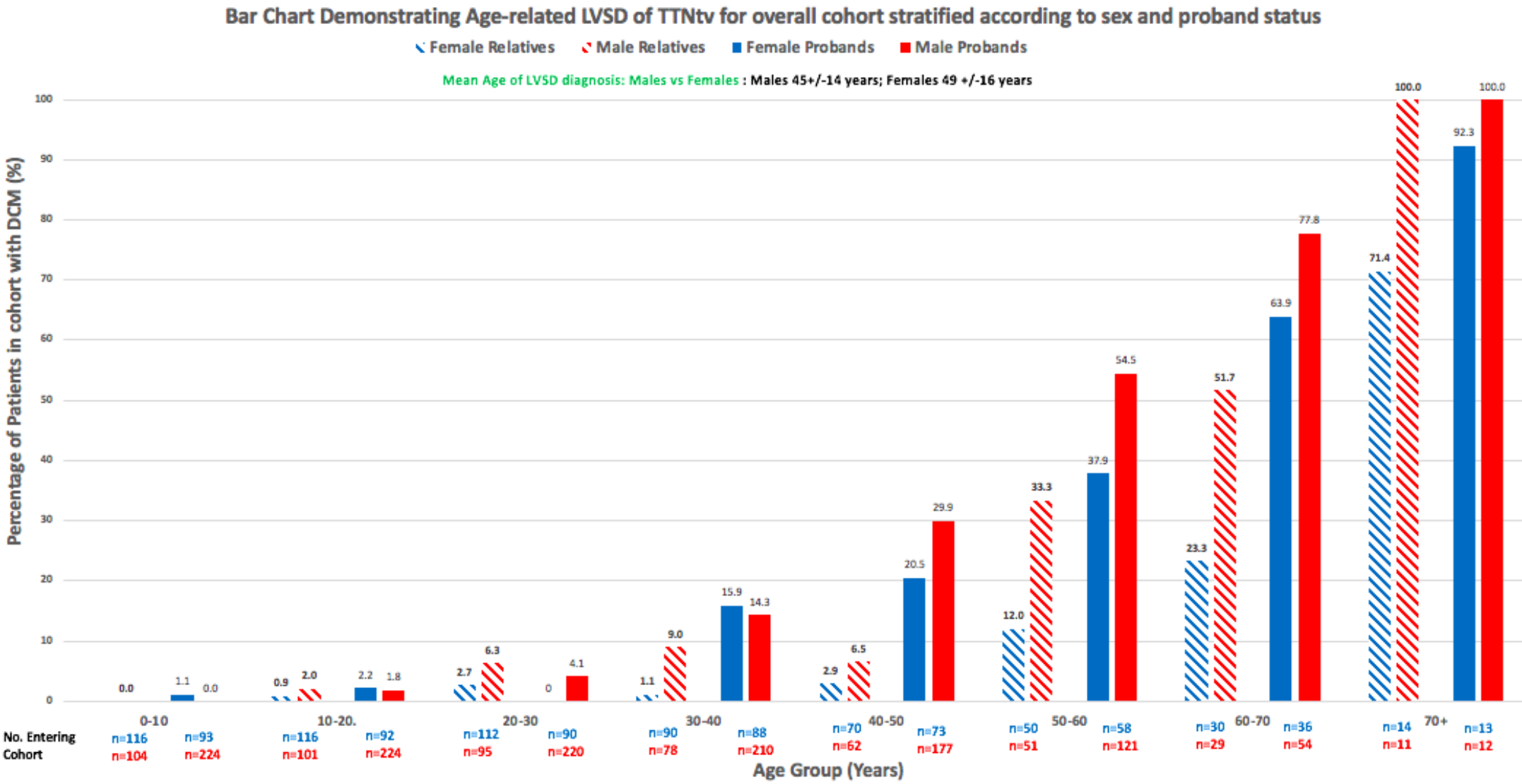
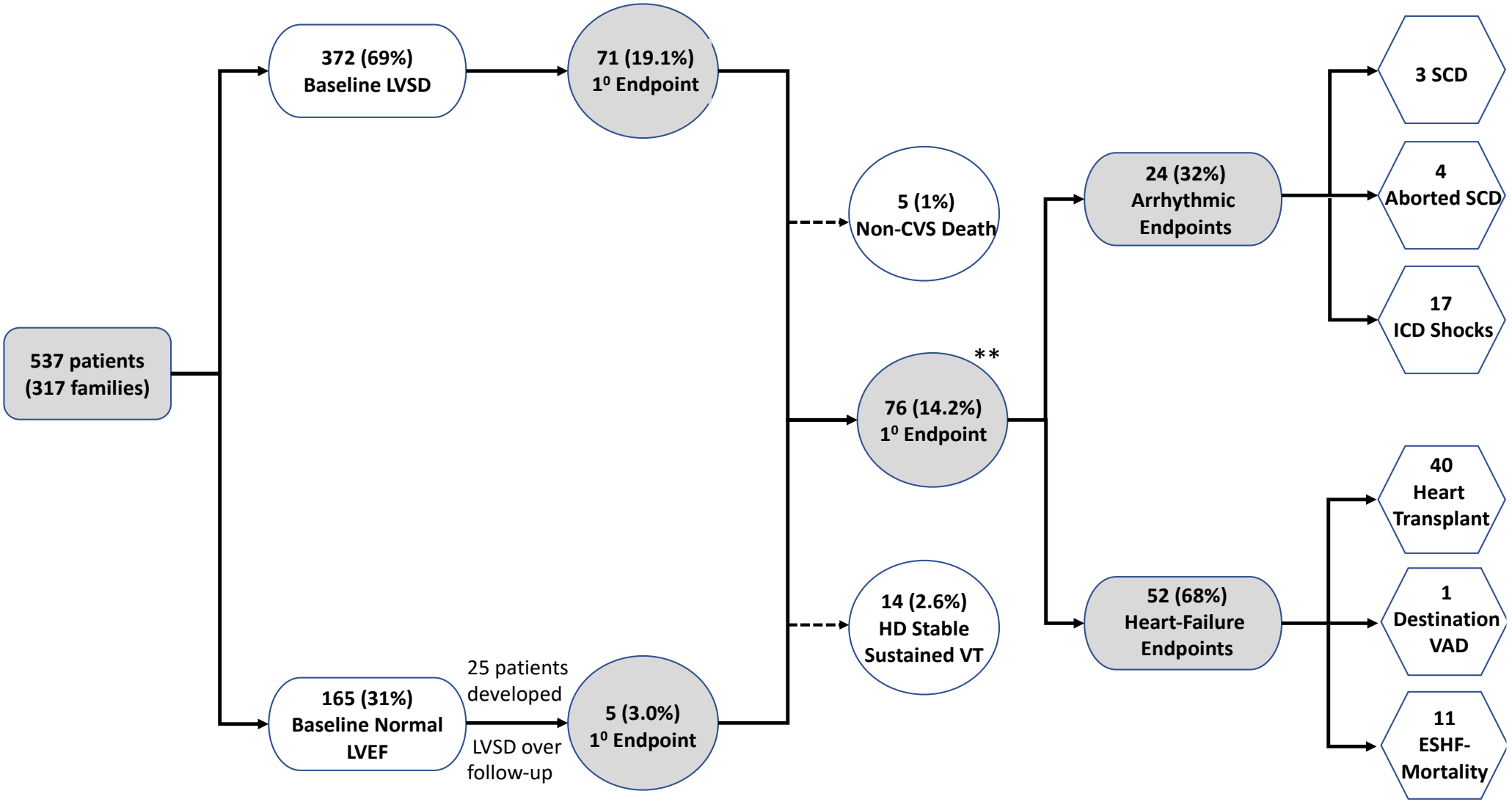

Bar Chart Demonstrating Age-related LVSD of TTNtv for overall cohort stratified according to sex and proband status
Female Relatives
Male Relatives
Female Probands
Male Probands
Mean Age of LVSD diagnosis: Males vs Females : Males 45+/-14 years; Females 49 +/-16 years
Percentage of Patients in cohort with DCM (%)
100
90
80
70
60
50
40
30
20
10
0
0.0
1.1
0.0
0.9
2.0
2.2
1.8
2.7
6.3
0
4.1
1.1
9.0
15.9
14.3
2.9
6.5
20.5
29.9
12.0
33.3
37.9
54.5
23.3
51.7
63.9
77.8
71.4
100.0
92.3
100.0
0-10
10-20.
20-30
30-40
40-50
50-60
60-70
70+
No. Entering Cohort
n=116
n=104
n=93
n=224
n=116
n=101
n=92
n=224
n=112
n=95
n=90
n=220
n=90
n=78
n=88
n=210
n=70
n=62
n=73
n=177
n=50
n=51
n=58
n=121
n=30
n=29
n=36
n=54
n=14
n=11
n=13
n=12
Age Group (Years)

FIGURE 4:



**16 (3%) patients had multiple endpoint events

FIGURE 5A:

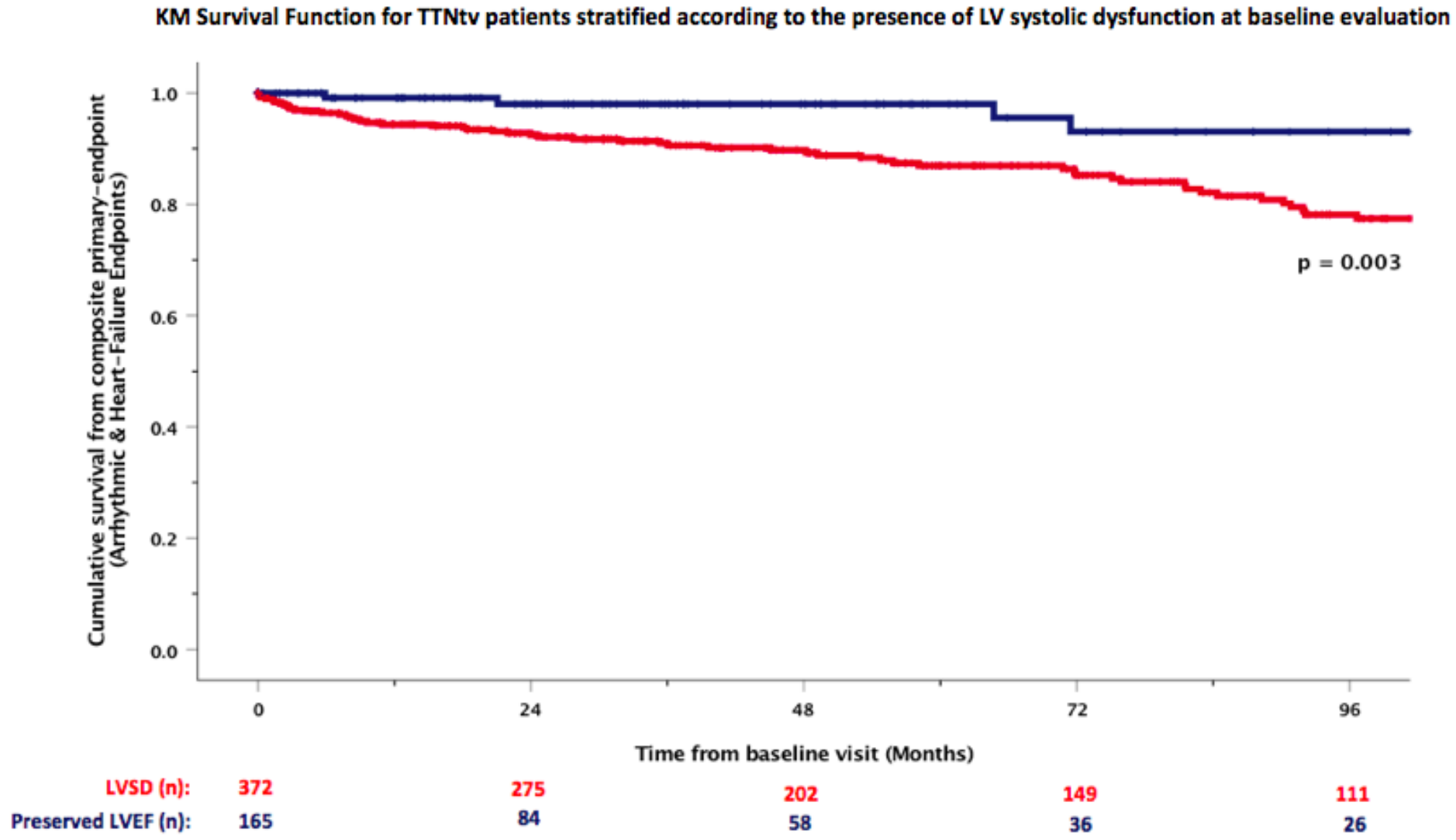

KM Survival Function for TTNtv patients stratified according to the presence of LV systolic dysfunction at baseline evaluation
Cumulative survival from composite primary-endpoint (Arrhythmic & Heart-Failure Endpoints)
1.0
0.8
0.6
0.4
0.2
0.0
p = 0.003
0
24
48
72
96
Time from baseline visit (Months)
LVSD (n): 372 275 202 149 111
Preserved LVEF (n): 165 84 58 36 26

FIGURE 5B:

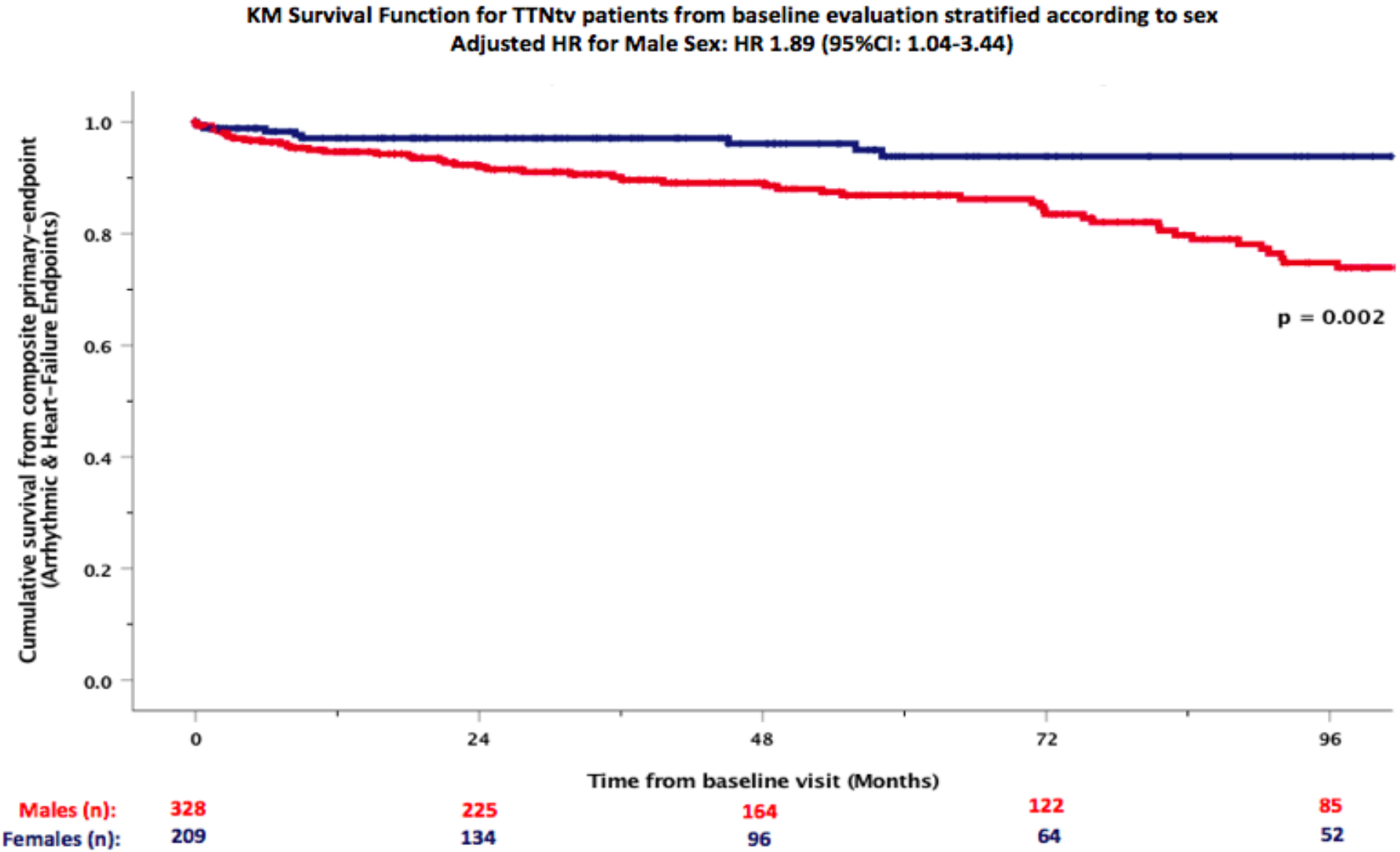

KM Survival Function for TTNtv patients from baseline evaluation stratified according to sex
Adjusted HR for Male Sex: HR 1.89 (95%CI: 1.04-3.44)
Cumulative survival from composite primary-endpoint (Arrhythmic & Heart-Failure Endpoints)
1.0
0.8
0.6
0.4
0.2
0.0
p = 0.002
0
24
48
72
96
Time from baseline visit (Months)
Males (n): 328 225 164 122 85
Females (n): 209 134 96 64 52

FIGURE 5C:

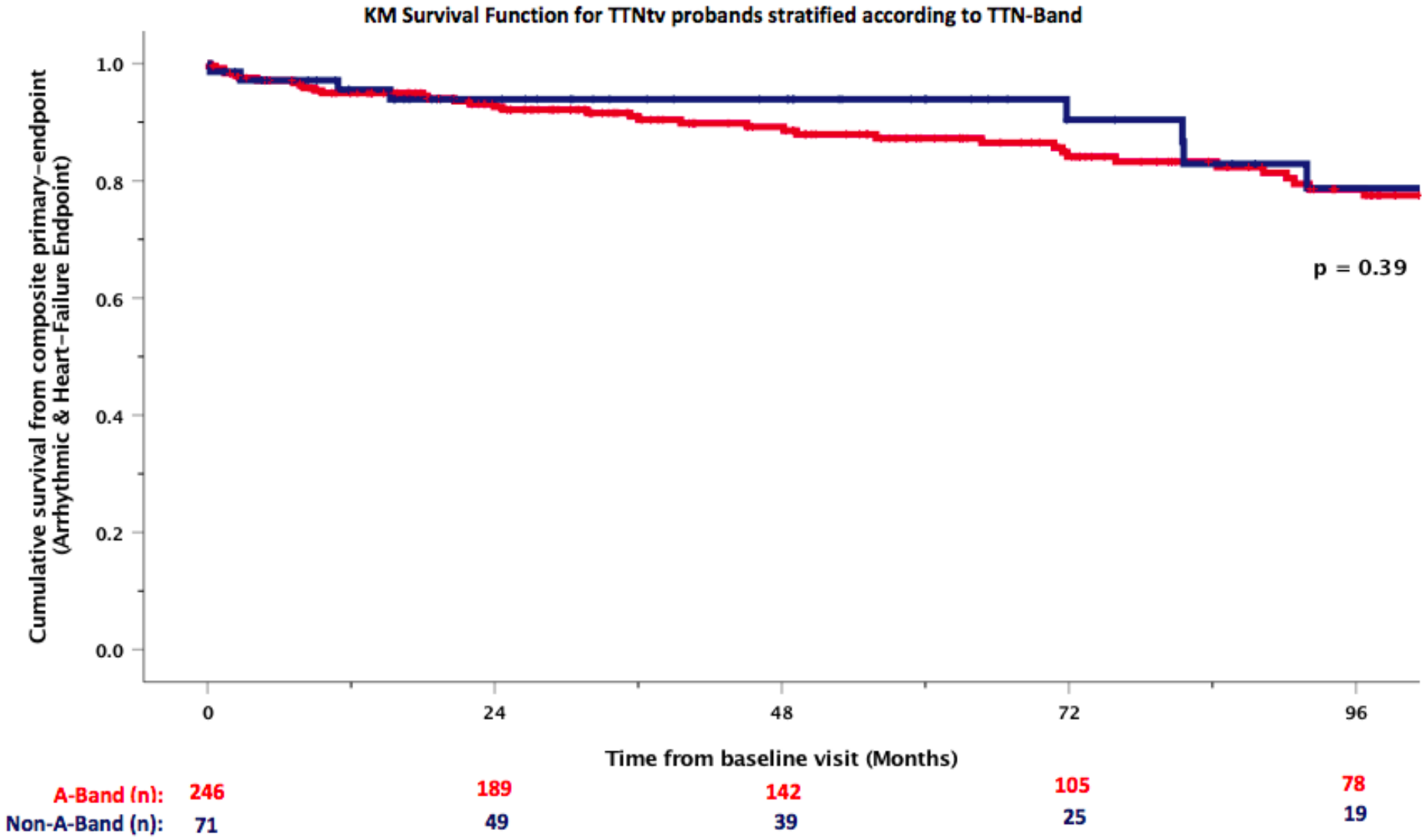
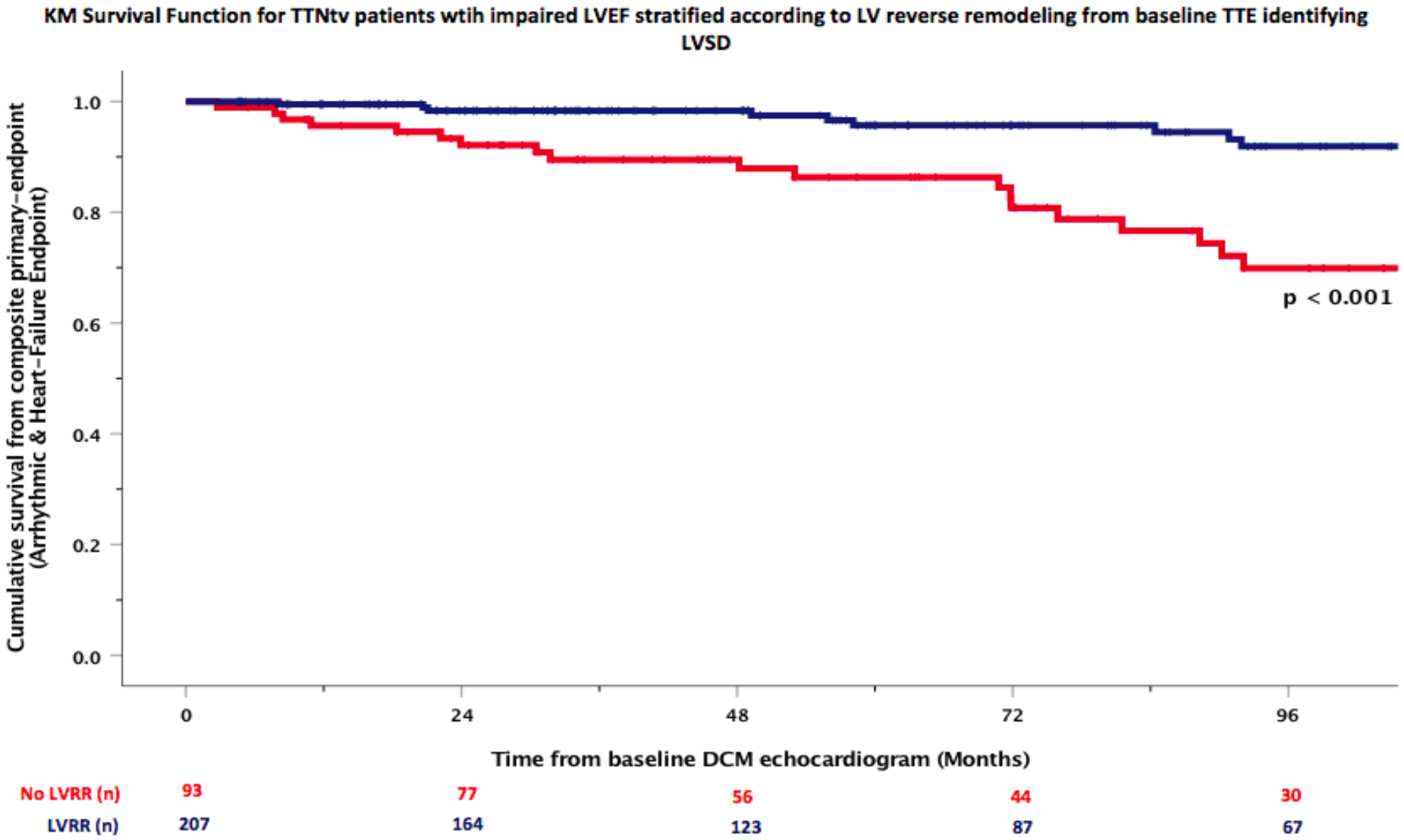

KM Survival Function for TTNtv probands stratified according to TTN-Band
Cumulative survival from composite primary-endpoint (Arrhythmic & Heart-Failure Endpoint)
1.0
0.8
0.6
0.4
0.2
0.0
p = 0.39
0
24
48
72
96
Time from baseline visit (Months)
A-Band (n): 246 189 142 105 78
Non-A-Band (n): 71 49 39 25 19

FIGURE 5D:


KM Survival Function for TTNtv patients wtih impaired LVEF stratified according to LV reverse remodeling from baseline TTE identifying LVSD
Cumulative survival from composite primary-endpoint (Arrhythmic & Heart-Failure Endpoint)
1.0
0.8
0.6
0.4
0.2
0.0
p < 0.001
0
24
48
72
96
Time from baseline DCM echocardiogram (Months)
No LVRR (n) 93 77 56 44 30
LVRR (n) 207 164 123 87 67