Recovery of cardiac function in cardiomyopathy due to titin truncation

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Introduction: Dilated cardiomyopathy (DCM) is a frequent cause of heart failure and a common indication for heart transplantation. DCM has a strong genetic basis and the most common disease-causing mutations are variants that truncate the sarcomeric protein titin (TTN truncating variants (TTNtvs); prevalence in familial or idiopathic DCM 25%[1] and 13%[2], respectively). The prognosis of DCM is poor, but functional recovery from end-stage failure has been reported following both optimal medical therapy[3] and left ventricular assist device (LVAD) support[4,5], though the determinants of successful recovery are unknown. It has been proposed that recovery from genetic cardiomyopathy may not be expected since the underlying cause is irreversible; whereas recovery may be more likely when DCM is due to reversible, non-genetic factors (e.g. myocarditis)[6]. To address this directly, we sequenced TTN in end-stage DCM patients who either recovered or did not recover following LVAD support.

Methods: We sequenced TTN in 70 cases referred to the Royal Brompton and Harefield National Health Service Trust between 1998 and 2010 for LVAD implantation due to non-ischaemic, medically refractory, end-stage DCM. Of these, 29 patients recovered cardiac function during LVAD support and had their LVAD explanted. The other 41 patients did not recover cardiac function and were transplanted or died while on LVAD support. A pharmacological regime designed to promote recovery (combination therapy[4,5]) was used in 35/70 patients and continued after explantation. The study was approved by institutional ethics committees, with written informed consent from participants. Targeted next-generation sequencing (NGS) was performed using an assay designed to assess all

known coding exons in TTN[2]. Genetic variants in NGS data were identified as previously described[2] and were confirmed independently. Statistical comparisons between groups were tested using Fisher's exact test, ANOVA and unpaired t-test as appropriate. Differences in survival rates were tested using the Mantel-Cox test. Statistical significance was defined as a P-value of <0.05.

Results: We identified TTNtvs in 10 out of 70 cases (14% of total; Table 1). All TTNtvs were either novel or very rare[α] and located in exons constitutively expressed in the heart and, as such, considered disease-causing[2]. Of the patients with a TTNtv, 6/10 recovered sufficient cardiac function to enable LVAD explanation. There was no statistical difference in TTNtv frequency between recovery cases and those who were transplanted or died on the device (6/29 [21%] vs. 4/41 [10%] respectively, P=0.30), and no evidence of clinical differences between TTNtv-positive and TTNtv-negative cases at the time of LVAD implantation (Table 1). Comparing the transplant-free survival rate in recovered patients, we found no difference between TTNtv-positive and TTNtv-negative cases; at three years post-explant, 4/6 (67%) TTNtv-positive cases were free from death and transplantation compared to 17/23 (74%) TTNtv-negative cases (Figure 1; P=0.74).

Discussion: Sustained improvement in cardiac function is observed in end-stage DCM following medical therapy and LVAD support, but it was previously unknown whether recovery could be achieved in DCM due to a genetic cause. Here, we show that recovery is possible in DCM due to a truncating mutation in the TTN gene. We also present the preliminary findings that DCM with a TTNtv is as equally recoverable

as DCM without a TTNtv and that the long-term durability of recovery is also comparable. These observations now require replication in multi-centre prospective studies. Since TTNtvs are the most common genetic cause of DCM these results have important implications for patient selection for recovery programs.

[α]Nine TTNtvs were not present in ExAC, whilst one variant had a minor allele frequency of 0.0000166 (http://exac.broadinstitute.org/).

Author contributions

Dr Felkin had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Role of the Funders/Sponsors

The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Contributions

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Table 1: TTNtv status and clinical features of LVAD-supported, end-stage DCM patients who either recovered cardiac function and were successfully explanted (recovered), or who were transplanted or died with the device *in situ* (not recovered).

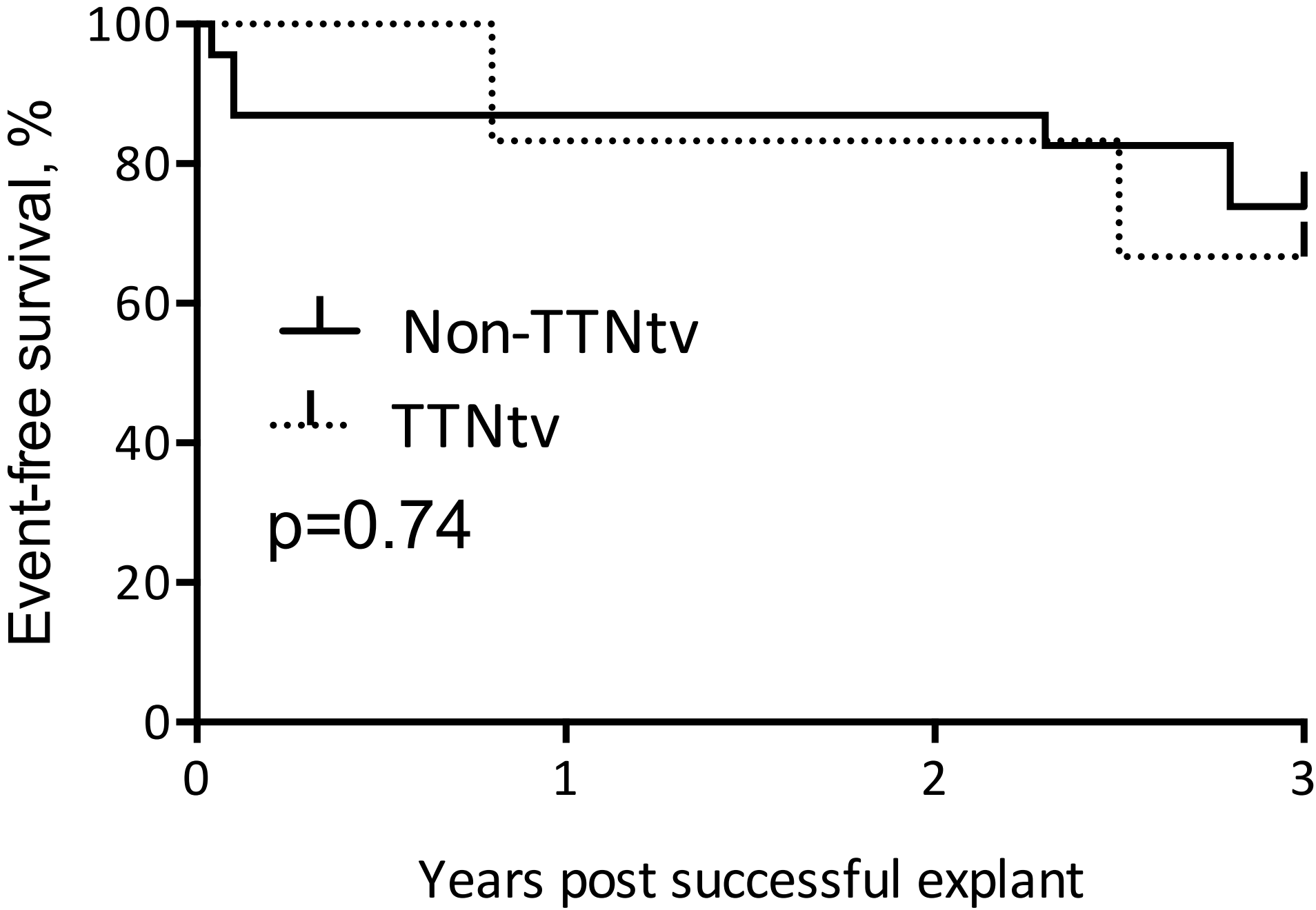
Figure 1. TTNtv and survival in recovered DCM patients. In the three years following successful LVAD explanation, the actuarial rate of survival and freedom from transplantation at 1, 2 and 3 years post-explant in TTNtv-positive cases was 83%, 83% and 67%. In TTNtv-negative cases, the rate was 88%, 88% and 75%. Differences between the survival rates were tested using the Mantel-Cox test.

Table 1.

	TTNtv, n=10		No TTNtv, n=60		
Variable	**Recovered**	**Not recovered**	**Recovered**	**Not recovered**	**P value**
Number of patients	6	4	23	37	0.30[A]
Male, n (%)	6 (100)	4 (100)	18 (78)	30 (81)	0.29[A]
Clinical comments	-	1 post-chemo	2 PPCM	3 PPCM 2 post-chemo	
Family history, n (%)	2 (33)	0 (0)	2 (8.7)	1 (2.7)	1.0[A]
Survived > 30 days post LVAD implant, n (%)	6 (100)	2 (50)	23 (100)	34 (92)	0.13[A]
Received combination therapy[3], n (%)	6 (100)	2 (50)	23 (100)	14 (38)	0.69[A]
Age at diagnosis, mean (SD), years	31.7 (10.8)	37.3 (15.6)	31.6 (12.5)	34.5 (11.9)	0.77[B]
Age at implant, mean (SD), years	33.0 (12.2)	38.6 (17.3)	35.5 (12.8)	37.5 (12.8)	0.83[B]
Implant LVEF, mean (SD), %	25.6 (13.3)	19.7 (9.5)	20.8 (10.1)	18.8 (9.6)	0.58[B]
Implant FS, mean (SD), %	10.3 (4.6)	8.5 (3.4)	9.3 (3.9)	8.6 (4.2)	0.90[B]
Implant LVEDD, mean (SD), mm	69.8 (6.6)	72.3 (10.4)	73.1 (14.3)	71.7 (9.6)	0.95[B]
Time on LVAD, mean (SD), days	214 (125)	212 (313)	317 (151)	520 (532)	0.11[B]
Explant LVEF, mean (SD), %	64.0 (4.2)	n/a	65.9 (9.5)	n/a	0.64[C]
Explant FS, mean (SD), %	29.5 (3.3)	n/a	31.9 (7.3)	n/a	0.53[C]
Explant LVEDD, mean (SD), mm	44.5 (6.4)	n/a	54.3 (8.9)	n/a	0.05[C]

TTNtvs in cohort who recovered: c.87624C>A*; c.49346-1G>A*; c.76383_76386delTAAT*; c.46782C>A*; c.81518delC*; c.71326G>T.
TTNtvs in cohort who did not recover: c.69976G>T; c.67495C>T*; c.41641C>T; c.58172delA*.
Titin variant position is given according to locus reference genomic (LRG) sequence 391_t1. A detailed overview of TTN gene structure, including the isoforms and protein domains affected by the TTNtvs described here, can be found at http://cardiodb.org/titin. *Variants reported in Roberts *et al*[2]. P-values calculated with Fisher's exact test[A], ANOVA[B] and unpaired t-test[C].
Abbreviations: TTNtv=titin truncating variant, LVAD=left ventricular assist device, DCM=dilated cardiomyopathy, PPCM=peri-partum cardiomyopathy, LVEF=left ventricular ejection fraction, FS=fractional shortening, LVEDD=left ventricular end diastolic dimension, n/a=not applicable.

Figure 1.


100
80
60
40
20
0
Event-free survival, %
Non-TTNtv
TTNtv
p=0.74
0
1
2
3
Years post successful explant