

Distinct Cardiac Transcriptomic Clustering in Titin and Lamin A/C-Associated Dilated Cardiomyopathy Patients

Citation for published version (APA):

Verdonschot, J. A. J., Derks, K. W. J., Hazebroek, M. R., Wang, P., Robinson, E. L., Adriaens, M. E., Krapels, I. P. C., van den Wijngaard, A., Brunner, H. G., & Heymans, S. R. B. (2020). Distinct Cardiac Transcriptomic Clustering in Titin and Lamin A/C-Associated Dilated Cardiomyopathy Patients. *Circulation*, 142(12), 1230-1232. <https://doi.org/10.1161/circulationaha.119.045118>

Document status and date:

Published: 22/09/2020

DOI:

[10.1161/circulationaha.119.045118](https://doi.org/10.1161/circulationaha.119.045118)

Document Version:

Publisher's PDF, also known as Version of record

Document license:

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RESEARCH LETTER

Distinct Cardiac Transcriptomic Clustering in Titin and Lamin A/C-Associated Dilated Cardiomyopathy Patients

Dilated cardiomyopathy (DCM) caused by a genetic mutation has a low rate of left ventricular reverse remodeling in response to treatment. Still, the rate of left ventricular reverse remodeling varies according to the specific gene mutated, indicating different cardiac molecular consequences.¹ For example, patients carrying an *LMNA* variant respond less favorably to pharmacological treatment. Up to 80% of patients with a truncating *TTN* variant (*TTN*tv) recovered on treatment, but the other 20% still had a more malignant clinical course, with persistent dysfunction and life-threatening arrhythmias.^{1,2} Understanding the molecular consequences of specific genetic variants on cardiac pathophysiological pathways will help us to enhance genotype-phenotype correlations. This knowledge can improve the selection of patients who will benefit the most from specific treatments. RNA sequencing makes it possible to quantify the activity of specific molecular pathways in patients with genetic DCM. Cardiac RNA profiling in *TTN*tv previously revealed a strong upregulation of the oxidative phosphorylation pathway, both in human² and rat³ hearts. This reflects a distinct transcriptomic signature in titin cardiomyopathy, which could be translated toward more specific treatment.² Here, we related cardiac transcriptomic profiles of 29 patients with genetic DCM to in-depth phenotyping.

We performed RNA sequencing on right septal biopsies gathered during diagnostic workup in 29 patients with DCM carrying a pathogenic genetic variant (13 *TTN*tv, 7 *LMNA*, 5 *RBM20*, 4 *MYH7*), as part of the Maastricht Cardiomyopathy Registry.^{1,2} None of the patients had end-stage disease. Included patients were selected on the basis of the availability of spare biopsies for research, with a prerequisite of a minimal 3 patients per gene subgroup. Only patients with a pathogenic variant in one of the earlier mentioned genes fulfilled these criteria. The study was performed according to the Declaration of Helsinki and was approved by the institutional Medical Ethics Committee. All patients gave written informed consent.

The transcriptome profile of *TTN*tv and *LMNA* (lamin A/C) carriers clustered distinctly from the other patients with genetic DCM, as revealed by a principal component analysis (Figure, left). In addition, 2 distinct subgroups were present within the patients with *TTN*tv, further denoted as *TTN*tv1 (n=6) and *TTN*tv2 (n=7). There was no clear pattern of the location of the truncating variant in the gene between these subgroups (Figure, bottom). Subsequent Ingenuity Pathway Analysis indicated an upregulation of NF- κ B (nuclear factor- κ B) signaling, cardiac necrosis, and fibrosis, and downregulation of the hypertrophy pathways in the *TTN*tv2 subgroup (Figure, right), the latter in line with a decreased cardiac mass in *TTN*tv-DCM versus other patients with DCM.² Discriminant analysis on the phenotypic data revealed a distinct malignant phenotype behind the *TTN*tv2 subgroup: a lower ejection fraction, thinner interventricular septum, increased cardiac fibrosis, more arrhythmias, a higher concentration of blood inflammation markers, and

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Key Words: cardiomyopathy, dilated
■ genotype ■ phenotype ■ principal component analysis ■ sequence analysis, RNA

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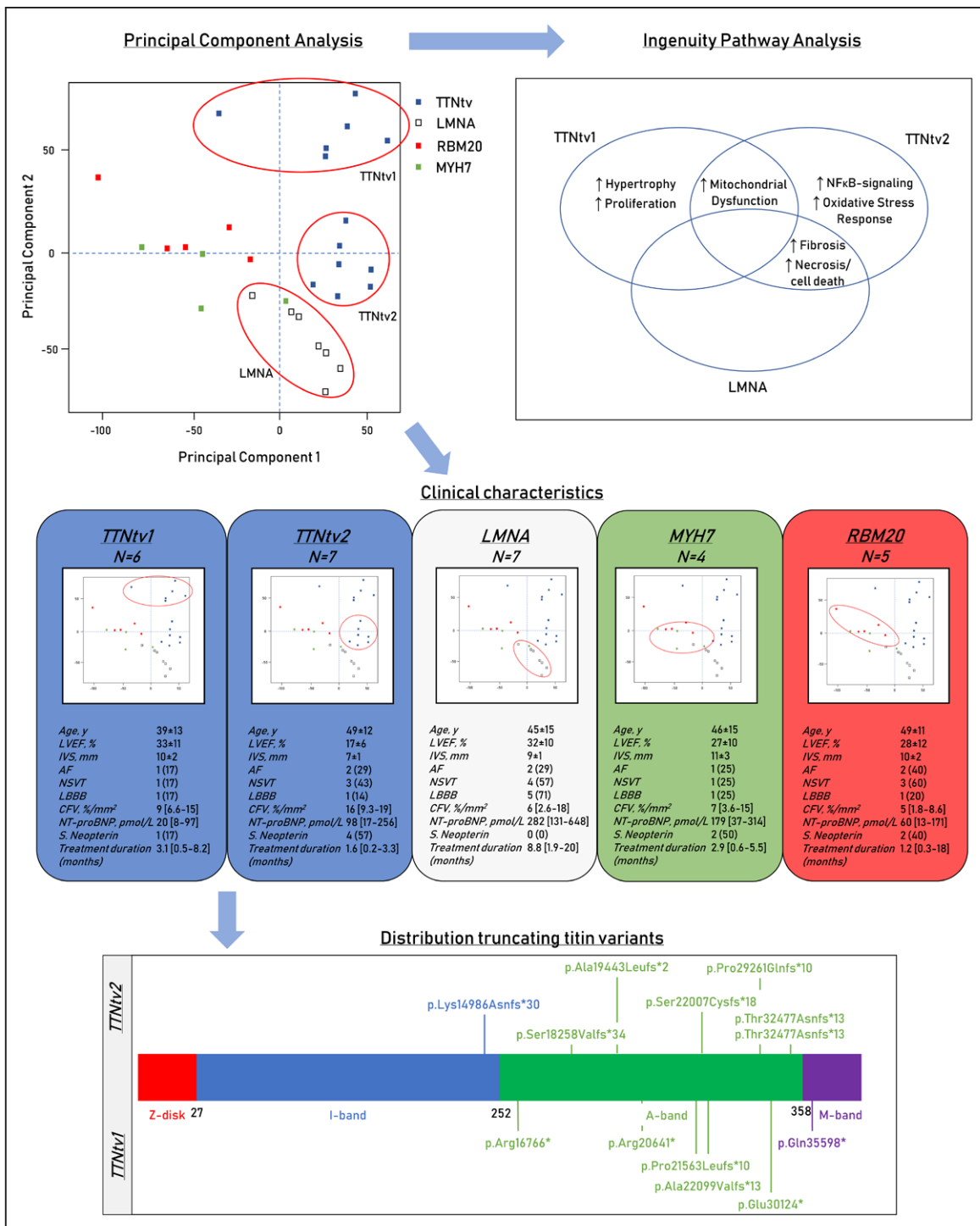


Figure. Transcriptomic results based on genotype and phenotype of the patients.

Left, Principal component analysis of the transcriptomic profile isolated from the hearts of patients with dilated cardiomyopathy with a *TTN*tv, *LMNA*, *RBM20*, or *MYH7* variant. **Right,** Venn diagram of the distinctive processes per genotype. Analysis was performed with Ingenuity Pathway Analysis. **Middle,** An overview of the clinical characteristics per genotype. Values are displayed as n (%), mean±SD, or median [interquartile range]. **Bottom,** Schematic representation of the *TTN* gene with all included truncating variants mapped across the gene. The numbers indicate the exon at the boundary of the specified gene region. AF indicates atrial fibrillation; CFV, collagen volume fraction; IVS, interventricular septum; LBBB, left bundle-branch block; LVEF, left ventricular ejection fraction; NSVT, nonsustained ventricular tachycardia; NT-proBNP, N-terminal pro-B-type natriuretic peptide; and S. neopterin, significant concentrations of serum neopterin.

N-terminal pro-B-type natriuretic peptide (Figure, middle). The transcriptomic profile of the malignant *TTN*tv2 clustered close to patients with *LMNA*, with similarities in the clinical phenotype. Ingenuity Pathway Analysis

showed increased cardiac fibrosis and necrosis and decreased hypertrophy and proliferation as most distinctive pathways between *LMNA* and *TTN*tv1 (Figure Right). NF-κB signaling and oxidative stress response

were the only differences between *LMNA* and *TTNtv2*. The transcriptomic profiles of *RBM20* and *MYH7* did not cluster together with *LMNA* and *TTNtv*, although they had clinical similarities. The separate clustering of *TTNtv* and *LMNA* from the others indicates a specific transcriptomic profile irrespective of solely cardiac function and treatment duration.

Current guidelines recommend standard heart failure medication regardless of the heterogeneous nature of the DCM phenotype and genotype. Still, 50% of patients with DCM do not functionally improve, despite improved diagnostics of the underlying causes.¹ A post hoc genetic analysis in the POSEIDON-DCM trial (Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis in Dilated Cardiomyopathy) showed that the genotype contributes to the response to treatment.⁴ This emphasized the need for a better understanding of the individual pathomechanisms and associated likelihood to respond to treatment, for which RNA profiling can provide a first insight. Here, we reveal distinct RNA profiles not only in *LMNA*-DCM, but also 2 distinct RNA profiles in *TTNtv*-DCM. These findings challenge the concept that *TTNtv*-DCM is completely benign: not only do >20% not functionally improve on standard heart failure therapy, but the malignant *TTNtv* subgroup also presents with a distinct molecular profile. Current dogma tries to classify patients in specific causes, leading to recommendations that are difficult to translate to the general DCM population. Improved DCM stratification will require unsupervised phenomapping, as recently done in heart failure with preserved ejection fraction,⁵ in combination with in-depth molecular analysis of heart tissue for effective precision medicine in DCM. Our findings show the feasibility of stratifying patients based on their pathophysiological processes, which eventually can uncover novel pharmacological targets. In conclusion, RNA profiles strongly differ in patients with *TTNtv* and *LMNA*-mutated DCM, despite clinical similarities to other pathogenic variant carriers such as *RBM20* and *MYH7*, suggesting a specific genetic effect on the cardiac transcriptome in addition to the effect of the clinical component. The understanding of these different profiles is a first step toward precision medicine.

ARTICLE INFORMATION

All RNA-sequencing data are available through gene expression omnibus (GEO) under accession number GSE146621. The clinical data that support the findings of this study are available from the corresponding author on reasonable request.

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Sources of Funding

This research was supported by the ERA-Net-CVD project MacroERA, 01KL1706, IMI2-CARDIATEAM (No. 821508), Netherlands Cardiovascular Research Initiative, Dutch Heart Foundation, CVON Arena-PRIME, 2017 to 2018.

Disclosures

None.

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