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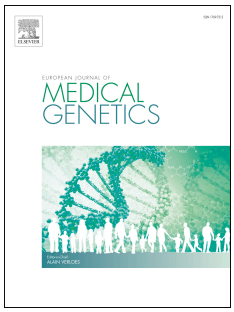
Accepted Manuscript



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PII: S1769-7212(17)30449-4

DOI: 10.1016/j.ejmg.2017.10.003

Reference: EJMG 3350

To appear in: *European Journal of Medical Genetics*

Received Date: 19 July 2017

Revised Date: 28 September 2017

Accepted Date: 7 October 2017

Please cite this article as: A. Nijak, M. Alaerts, C. Kuiperi, A. Corveleyn, B. Suys, B. Paelinck, J. Saenen, E. Van Craenenbroeck, L. Van Laer, B. Loeys, A. Verstraeten, Left ventricular non-compaction with Ebstein anomaly attributed to a *TPM1* mutation, *European Journal of Medical Genetics* (2017), doi: 10.1016/j.ejmg.2017.10.003.

Left Ventricular Non-compaction with Ebstein Anomaly attributed to a *TPM1* Mutation

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Running title: Identification of *TPM1* mutation in LVN(C)-EA

Abstract

Left ventricular non-compaction (cardiomyopathy) (LVN(C)) is a rare hereditary cardiac condition, resulting from abnormal embryonic myocardial development. While it mostly occurs as an isolated condition, association with other cardiovascular manifestations such as Ebstein anomaly (EA) has been reported. This congenital heart defect is characterized by downward displacement of the tricuspid valve and leads to diminished ventricular size and function. In an autosomal dominant LVN(C) family consisting of five affected individuals, of which two also presented with EA and two others with mitral valve insufficiency, we pursued the genetic disease cause using whole exome sequencing (WES). WES revealed a missense **variant** (p.Leu113Val) in *TPM1* segregating with the LVN(C) phenotype. *TPM1* encodes α-tropomyosin, which is involved in myocardial contraction, as well as in stabilization of non-muscle cytoskeletal actin filaments. So far, LVN(C)-EA has predominantly been linked to **pathogenic variants** in *MYH7.* However, one sporadic LVN(C)-EA case with a *de novo TPM1* **variant** has recently been described. We here report the first LVN(C)-EA family segregating a **pathogenic** *TPM1* **variant**, further establishing the association between EA predisposition and *TPM1*-related LVN(C). Consequently, we recommend genetic testing for both *MYH7* and *TPM1* in patients or families in which LVN(C)/non-compaction and EA coincide.

Key words: TPM1, Ebstein anomaly, left-ventricular non-compaction, exome sequencing

Introduction

Non-compaction is a rare hereditary cardiac condition, resulting from abnormal embryonic myocardial development. Anatomically, patients present with extensive myocardial trabeculations and deep intertrabecular recesses. These most frequently occur in the left ventricle, hence the term left ventricular non-compaction (cardiomyopathy) (LVN(C)) is also commonly used. LVN(C) prevalence has not been thoroughly investigated at the population level. However, in patients referred for echocardiographic examination, it is estimated at 0.14% (Aras D, 2006). LVN(C) is predominantly inherited in an autosomal dominant manner and causal **variants** mostly occur in genes encoding sarcomeric proteins (Table 1). The clinical presentation of mutation carriers ranges from asymptomatic to ventricular arrhythmias, thromboembolic events and heart failure with risk for sudden cardiac death in severe cases.

LVN(C) can occur as an isolated condition or in association with other congenital heart disorders (CHDs), suggesting that genetic factors predisposing to multiple cardiovascular manifestations exist. Ebstein anomaly (EA) is the most prevalent congenital heart disease in LVN(C) patients (Stähli BE, 2013). EA has a prevalence of 1 in 200 000 live births and can be either sporadic or inherited in an X-linked or autosomal dominant manner (Attenhofer Jost CH, 2007). It involves enlargement of the anterior leaflet of the tricuspid valve, while the posterior and septal leaflets are adherent to the underlying myocardium. This leads to partial atrialization of the right ventricle and, consequently, diminished ventricular size and function. Disease presentation ranges from asymptomatic valve disease in adult life to partial heart failure or sudden cardiac death at fetal and neonatal stage.

Most of the yet identified EA genes encode cardiac transcription factors and sarcomeric proteins (Table 1), but little is known about the genetic etiology of EA associated with LVN(C) (LVN(C)-EA). Although some genes have been linked to either isolated LVN(C) or isolated EA (i.e. *NKX2.5* & *ACTC1*) (Klaassen S, 2008; Schott JJ, 1998), the only recurrent LVN(C)-EA gene is *MYH7,* encoding the cardiac β-myosin heavy chain (Vermeer AMC, 2013). The recent identification of a *de novo* p.Asp159Asn substitution in the α-tropomyosin 1 (*TPM1*) gene in a sporadic LVN(C)-EA patient also implicates *TPM1* in LVN(C)-EA development (Kelle AM, 2016).

Clinical report

Here, we report on an autosomal dominant LVN(C)-EA family, consisting of three patients with LVN(C) and two patients with the combined LVN(C)-EA phenotype, in which a pathogenic *TPM1* variant (c.377C>G; p.Leu113Val, NM_001018007) was identified using whole exome sequencing in individuals III:1 and III:4 (Figure 1). p.Leu113Val is absent from the Exome and Genome Aggregation Consortium, Exome Variant Server, and 1000 Genomes databases. *In silico* pathogenicity analyses, including MutationTaster, SIFT and PolyPhen-2, predict a damaging effect on protein structure and/or function. Moreover, p.Leu113 is highly conserved throughout evolution (up to Zebrafish). Segregation analysis confirmed presence of the variant in all available affected individuals (III:1; III:4; IV:1; IV:2), and absence in an unaffected individual (III:3). **No variants of note were found in any other candidate gene (Supplementary Table 1).**

Patient III:1 was diagnosed with LVN(C) and mitral valve insufficiency as a neonate. Delivery of his son (IV:1) was induced at the 30th week of pregnancy, because of premature closure of the ductus arteriosus and hydrops fetalis. Neonatal complications comprised

ascites, pericardial effusion, bronchopulmonary dysplasia after high frequency ventilation, and grade 2 left-sided subependymal hemorrhage with leukomalacia. Subsequently, he was diagnosed with LVN(C), progressive mitral valve insufficiency and pulmonary hypertension. He underwent a mitral valve replacement at age 3.5 years. Patient III:4 was diagnosed with LVN(C) after both of his children were found to suffer from non-compaction. MRI at age 33 showed also a mildly dilated left atrium. His left ventricular function was normal. His oldest daughter (IV:2) was diagnosed with EA at 22 weeks of gestation using fetal echocardiography. She was born at 39 weeks and neonatal echocardiography revealed also the presence of LVN(C). At 2 years of age, echocardiography demonstrated an abnormal left ventricle with pronounced apical and posterior-lateral trabeculation as well as reduced contractile function, dilatation of the left atrium, venae pulmonales and progressive pulmonary hypertension. The tricuspid valve demonstrated an abnormal septal leaflet with grade 2/4 insufficiency. Owing to progressive heart failure, she was placed on a mechanical biventricular assist. Awaiting her heart transplant she died at age 3.5, due to acute respiratory distress and right ventricular failure. Her younger sister (IV:3) was diagnosed postnatally with a spongious appearance of the left ventricle and non-compaction, predominantly in the apical region, **which** confirms LVN(C). She also showed mild EA with an anteriorly implanted insufficient tricuspid valve (grade 1-2). Mild mitral valve insufficiency was also observed. Under ACE-inhibitor treatment, her left ventricular function currently remains normal.

Discussion

TPM1 encodes α-tropomyosin 1, a sarcomere myofilament that mediates both stabilization of thin filaments and the interaction between actin and myosin. **Pathogenic** missense or

splice site *TPM1* **variants** have been found in patients with **LVN(C) without EA** (Chang B, 2011; Hoedemaekers YM, 2010; Probst S, 2011; Tian T, 2015), hypertrophic cardiomyopathy (HCM) (Selvi Rani D, 2015) and dilated cardiomyopathy (DCM) (Li YD, 2015). Recently, England et al. detected three novel **variants** in *TPM1* in a cohort of 380 patients with different CHDs (Tetralogy of Fallot – **c.114+2T>C**; atrial septal defect, pulmonary atresia with persistent *ductus arteriosus*, and hypoplastic right ventricle – p.Ile130Val; atrial septal defect – p.Ser229Phe) (England J, 2017). Subsequent functional analyses indicated that *TPM1* plays a major role in cardiogenesis and mutations in this gene can cause a broad spectrum of cardiac malformations. We here describe the second LVN(C)-EA-associated *TPM1* variant. Both our patient (IV:2) and the one sporadic patient from literature (Kelle AM, 2016) were diagnosed with LVN(C)-EA and died prematurely while awaiting heart transplantation. In **the reported** family, we also identified one less severely affected LVN(C)-EA sibling, an adult with LVN(C) only and two LVN(C) patients with mitral valve insufficiency. The latter condition was previously not described in the p.Asp159Asn mutation carrier from Kelle et al. Further genetic studies are warranted to confidently link mitral valve disease to the clinical spectrum of *TPM1* genetic variability. **In brief, pathogenic *TPM1* variants have been linked to a multitude of phenotypes. Considerable genotype-phenotype correlation is currently lacking though (http://www.lovd.nl/3.0/).**

In conclusion, we confirmed a role for *TPM1* mutations in the genetic etiology of familial LVN(C)-EA. We therefore recommend genetic testing for both *MYH7* and *TPM1* in sporadic LVN(C)-EA patients and/or families in which EA and LVN(C) coincide or co-segregate.

Acknowledgements

We would like to thank all family members for their collaboration. A.N. holds a PhD fellowship of the Fund for Scientific Research Flanders (FWO). B.L. and E.M.V.C. are senior clinical FWO investigators. A.V. is supported by a FWO postdoctoral grant. B.L. holds a starting grant from the European Research Council (ERC- StG-2012-30972-BRAVE).

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Table 1 Genes involved in the genetic etiology of EA and LVN(C)

Disease phenotype	Genes	Functional classes	References
LVN(C)	*ACTC1*	Sarcomere protein	(Klaassen S, 2008)
	DTNA	Cytoskeletal protein	(Ichida F, 2001)
	MYH7	Sarcomere protein	(Budde BS, 2007)
	MYBPC3	Sarcomere protein	(Arbustini E, 2014)
	TNNI3	Sarcomere protein	(Ehlermann P, 2008)
	TNNT2	Sarcomere protein	(Klaassen S, 2008)
	TPM1	Sarcomere protein	(Chang B, 2011)
	LDB3	Cytoskeletal protein	(Vatta M, 2003)
	MIB1	Ubiquitin ligase	(Luxán G, 2013)
	NKX2.5	Transcription factor	(Schott JJ, 1998)
	PRDM16	Transcription factor	(Faivre L, 1999)
	CASQ2	Sarcoplasmic reticulum protein	(Hoedemaekers YM, 2010)
	DMD	Cytoskeletal protein	(Statile CJ, 2013)
	LMNA	Nuclear lamina protein	(Hermida-Prieto M, 2004)
	TAZ	Mitochondrial protein	(Chang B, 2011)
	SCN5A	Ionic channel protein	(Shan L, 2008)
EA	*ACTC1*	Sarcomere protein	(Augière C., 2015)
	MYH6	Sarcomere protein	(Posch MG, 2011)
	MYH7	Sarcomere protein	(Budde BS, 2007)
	NKX2.5	Transcription factor	(Gioli-Pereira L, 2010)
	FLNA	Cytoskeletal protein	(LaHaye S, 2015)
LVN(C)-EA	*MYH7*	Sarcomere protein	(Vermeer AMC, 2013)
	TPM1	Sarcomere protein	(Kelle AM, 2016)

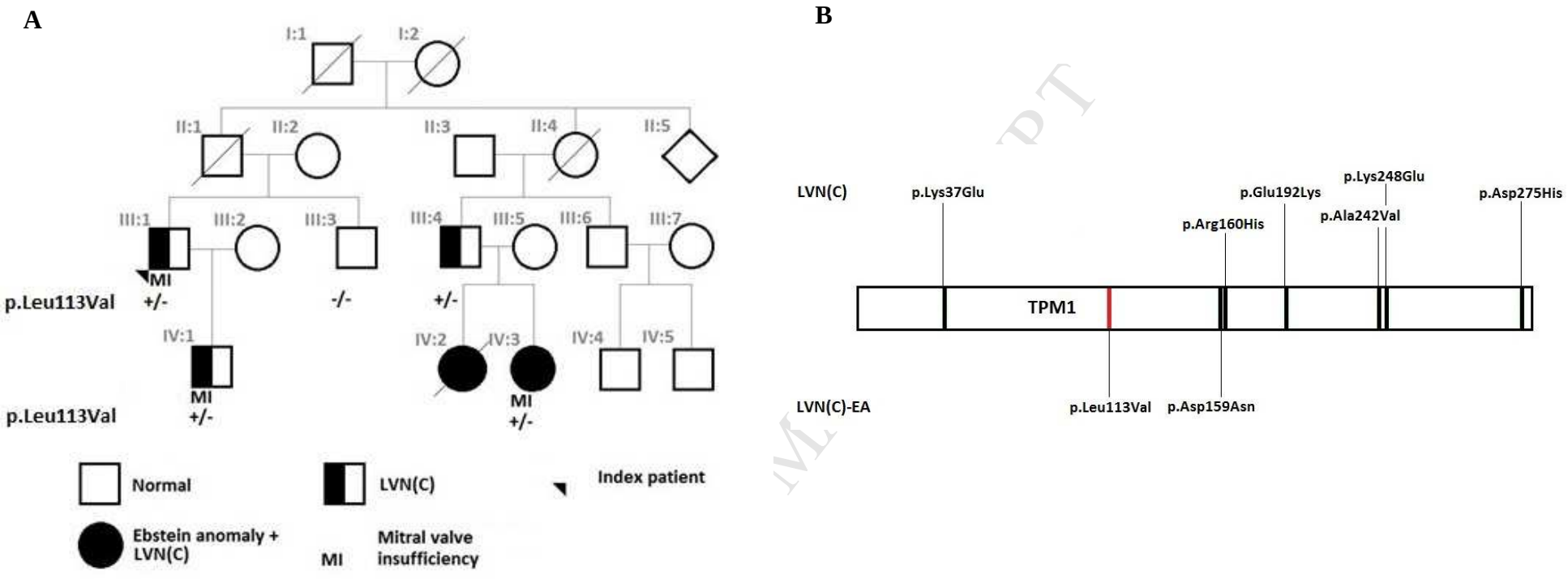

Figure 1. A Pedigree of the presented LVN(C)-EA family. **B** TPM1 gene mutations reported in LVN(C) (on top) and LVN(C)-EA (below) cases (Bainbridge MN, 2015; Chang B, 2011; Hoedemaekers YM, 2010; Kelle AM, 2016; Probst S, 2011 ; Tian T, 2015). Variant reported in this paper is indicated in red.