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Hoorntje, Edgar; van Spaendonck-Zwarts, Karin Y; Te Rijdt, Wouter P; Boven, Ludolf; Vink, Aryan; van der Smagt, Jasper J; Asselbergs, Folkert W; van Wijngaarden, Jan; Hennekam, Eric A; Pinto, Yigal M

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The first titin (c.59926 + 1G > A) founder mutation associated with dilated cardiomyopathy

Truncating variants in the gene encoding titin (TTNtv) are found in 13–25% of dilated cardiomyopathy (DCM) cases.^{1,2} In DCM patients, TTNtv are associated with early arrhythmic risk, composed of atrial fibrillation (AF), non-sustained and/or sustained ventricular tachycardia.³ TTNtv in the A-band region, or in constitutively expressed exons of *TTN*, are generally believed to be pathogenic, but assigning pathogenicity can be challenging because TTNtv are also found in control populations.⁴

Here we describe the *TTN* c.59926 + 1G > A splice-site variant located in the A-band (chr2:179456704C > T, build GRCh37; NM_001267550.2 reference sequence) that we have identified in multiple probands with DCM. Written informed consent was obtained from all participants following local medical ethics committee guidelines. Our study and all experiments conformed with the principles of the Declaration of Helsinki.

The Exome Aggregation Consortium dataset (ExAC) and the Genome of the Netherlands database (GoNL) were used as control populations. To study a potential founder effect, we analysed 11 microsatellite markers surrounding *TTN* and performed genealogy research. The age of the mutation was calculated as described previously.⁵

Clinical data were collected retrospectively from 11 probands, 20 mutation-positive and 17 mutation-negative family members. Subjects were diagnosed with DCM when they had a reduced left ventricular ejection fraction (<45%) and a widened ventricle (>117% of predicted). In the absence of information on end-diastolic diameter, length or weight, DCM was noted when it was declared DCM by the cardiologist. We calculated the combined LOD score using linkage programme GRONDLOD and taking into account an 80% penetrance. Frequencies are expressed as numbers and percentages and continuous variables as mean ± standard deviation. The Mann–Whitney U test was used for between-group comparisons and Fisher's exact test for comparing frequencies. Data were analysed using SPSS version 23 (IBM Corp., Armonk, NY, USA). A *P*-value <0.05 was considered statistically significant.

TTN c.59926 + 1G > A was identified in 18 family members and a further two were

obligate carriers. In total, we studied 31 carriers (11 probands, 20 family members) and 17 non-carriers. One proband had a second pathogenic *SCN5A* (c.4213G > C) variant. *TTN* c.59926 + 1G > A was found only once in 966 alleles in GoNL (0.1%)⁶ and was not present in ExAC.⁷ Haplotype analysis of nine probands and seven family members revealed a shared region of approximately 4 Mb, indicating a common ancestor. We calculated the mutation to be 300–580 years old. Most carriers, and the grandparents of the oldest generation carriers from the different families, originated from the eastern part of the Netherlands (Figure 1). Genealogy going back five to nine generations in seven families revealed three pairs of common ancestors, all born within a 10 km radius (Figure 1B). Segregation analysis yielded a combined LOD score of 3.6, strong evidence that this mutation is pathogenic.

One obligate carrier was not included in the analyses as additional clinical data were unavailable because the first symptom was sudden cardiac death. The predominant finding in mutation carriers was DCM (67%; 20/30; mean age of onset 49 ± 14 years). Atrial tachyarrhythmias were observed in 65% of DCM patients (13/20), with (paroxysmal) AF [(p)AF] or paroxysmal atrial flutter most frequently observed (60%; 12/20)

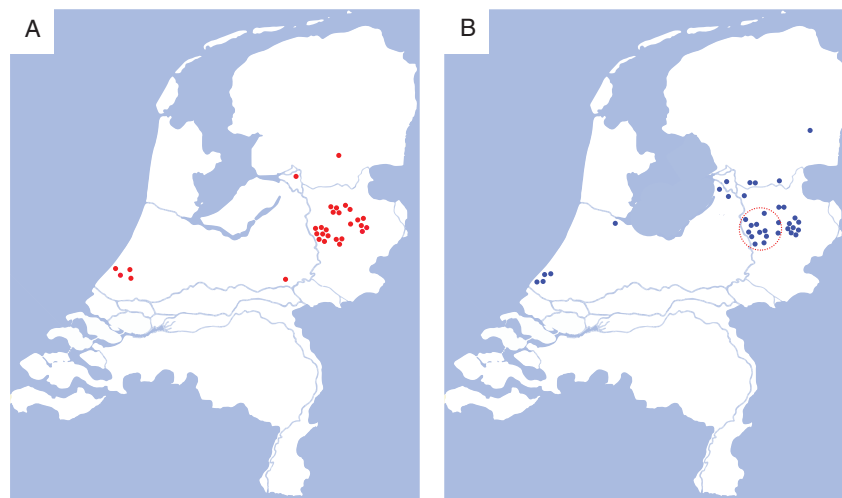


Figure 1 Geographic map of the Netherlands showing the distribution of 30 carriers and 36 grandparents. (A) Carriers' places of residence. (B) Birthplaces of the grandparents of carriers of the oldest generations from the different families. The six common ancestors linking seven of the 10 families all originated from a small area in the southern part of the province of Overijssel, indicated by the red circle (10 km radius).

Table 1 Clinical characteristics of 30 *TTN* c.59926 + 1G > A mutation carriers and 17 mutation negative family members

	<i>TTN</i> c.59926 + 1G > A +		<i>TTN</i> c.59926 + 1G > A -
	DCM (n = 20)	No DCM (n = 10)	No DCM (n = 17)
Age at diagnosis/evaluation, years	49 ± 14	47 ± 19	49 ± 10
Proband	11 (55%)	0 (0%)	0 (0%)
Male	14 (70%)	1 (10%)	13 (62%)
LVEF ^a , %	26 ± 11	53 ± 3*	57 ± 4*
LVEDD ^a , mm	63 ± 7	51 ± 6 [†]	50 ± 4 [†]
Atrial tachyarrhythmias	13 (65%)	3 (30%)	0 (0%) [‡]
(Paroxysmal) atrial fibrillation/flutter	12 (60%)	3 (30%)	NA
Ventricular arrhythmias	9 (45%)	1 (10%)	0 (0%)
Non-sustained ventricular tachycardia	5 (25%)	1 (10%)	NA
Ventricular tachycardia/fibrillation	4 (20%)	0 (0%)	NA
Out-of-hospital cardiac arrest	2 (10%)	0 (0%)	0 (0%)
ICD implantation	9 (45%)	0 (0%)	0 (0%)
Appropriate ICD therapy	4 (20%)	NA	NA
Risk factors	11 (55%)	6 (60%)	3 (18%)
Hypertension	7 (35%)	2 (20%)	2 (12%)
Diabetes mellitus	2 (10%)	0 (0%)	0 (0%)
Dyslipidaemia	3 (15%)	0 (0%)	2 (12%)
Coronary artery disease	2 (10%)	1 (10%)	0 (0%)
Obesity	1 (5%)	2 (10%)	1 (6%)
Other ^b	3 (15%)	1 (10%)	0 (0%)

DCM, dilated cardiomyopathy; ICD, implantable cardioverter-defibrillator; LVEDD, left ventricular end-diastolic dimension; LVEF, left ventricular ejection fraction; NA, not applicable; *TTN*, titin.

^aMeasurements are based on the lowest measured ejection fraction and the largest end-diastolic diameter.

^bComposition of chemotherapy, history of excess alcohol consumption, and severe mitral valve insufficiency.

*LVEF is available from five carriers and six non-carriers. In four carriers, left ventricular function is described as normal, of which one is described as >55%. In another carrier left ventricular function is described as moderate (45–55%). In the other 11 non-carriers left ventricular function is described as normal.

[†]LVEDD is missing from one carrier and available from 13 non-carriers; in the other four non-carriers, LVEDD is described as normal.

[‡]Holter monitoring only performed in three non-carriers.

(Table 1). One DCM patient had collapsed due to an atrioventricular nodal re-entry tachycardia (AVNRT), which converted to sinus rhythm after administration of adenosine. Of note, left atrial enlargement was observed on echocardiography 6 months before the AVNRT. In three patients (p)AF preceded the DCM phenotype (by 11, 12, and 14 years). Males were over-represented in the DCM group compared to carriers with no DCM ($P=0.003$).

To evaluate if the frequent occurrence of (p)AF occurred with left atrial widening, we collected information on atrial size [see Supplementary material online, Table S1, for risk factors, age at onset of (p)AF, DCM, and age at left atrial measurements]. Interestingly, in the DCM group, eight subjects had (p)AF without left atrial dilatation, including four without risk factors that could attribute to the development of (p)AF. In the carrier group without DCM, three subjects (30%) had (p)AF: two had no atrial dilatation and one had atrial dilatation measured while AF was her underlying rhythm (Figure 2).

Two carriers suffered from an aborted cardiac arrest and one obligate carrier

died suddenly (aged 58, 75, and 33 years, respectively). Autopsy in the deceased patient revealed signs of cardiomyopathy. An implantable cardioverter-defibrillator (ICD) was implanted in nine carriers: two in the context of secondary intervention and seven for primary prevention. Four ICD carriers (44%; 4/9) received appropriate treatment (three times ICD shock and one time antitachycardia pacing) within 1–4 years after implantation. Two patients underwent a heart transplantation. Hypertension, diabetes mellitus, and dyslipidaemia seemed more frequently present in the DCM group, but significant differences could not be demonstrated given our small sample. Another potential influence masking the effect of the cardiovascular risk factors could be that the carrier group with no DCM consisted almost entirely of women.

An additional stressor was reported in six carriers. The obligate carrier who died suddenly was reported to have used excessive amounts of alcohol, which was also suggested as a possible cause of DCM in another carrier. In two other carriers, a history of an infectious period preceded the development of severe left ventricular impairment. One

required intra-aortic balloon support, with myocarditis suspected but not evaluated, and his cardiac function largely recovered. In the other, myocardial biopsy was negative for cardiotropic viruses, but his left ventricular function remained poor (ejection fraction 18%). In another carrier, severe DCM (ejection fraction 15%) became apparent after anthracycline therapy. Finally, one proband with DCM carried an additional pathogenic *SCN5A* mutation. He had no spontaneous type 1 Brugada ECG but did have *SCN5A*-related features: a right bundle branch block and delayed atrioventricular conduction (PR interval 330 ms). Taken together, these data support a multiple-hit model in which a 'second genetic hit' or additional environmental stressor is required to develop overt disease.

Dilated cardiomyopathy caused by *TTN*tv has been reported to respond relatively well to treatment,⁸ which we also observed in some carriers. Cardiac function in one patient recovered after a short period after cardiogenic shock due to a suspected myocarditis. Two DCM patients responded well to treatment after a follow-up of 6 and 4 years (increases in ejection fraction of 20% to 45%

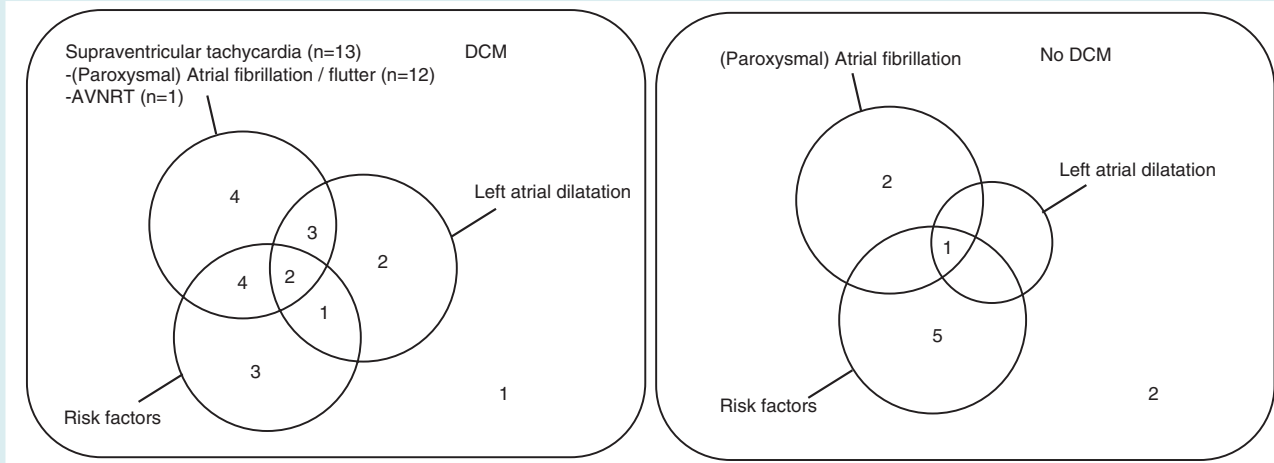


Figure 2 Venn diagrams of carriers with and without dilated cardiomyopathy (DCM) showing the presence of risk factors, atrial arrhythmias, and atrial dilatation. AVNRT, atrioventricular nodal re-entry tachycardia.

and 43% to 50%, and decreases in left ventricular end-diastolic dimensions of 65 mm to 53 mm and 60 to 47 mm, respectively).

The occurrence of (p)AF without or preceding DCM was interesting. To evaluate if this predisposition to (p)AF is inherent to this specific mutation or to TTNtv in general, we looked at age at diagnosis of DCM and (p)AF for carriers of 16 other TTNtv residing in constitutively expressed exons. We evaluated 53 carriers (16 probands and 37 family members) and found 19 asymptomatic carriers, 6 with only (p)AF (11%), 3 in whom (p)AF preceded DCM (6%) (by 1, 4, and 22 years), 11 in whom (p)AF was diagnosed the same year as DCM (21%), 6 who developed (p)AF after diagnosis of DCM (11%), and 8 with only DCM (15%). Together with the results from our cohort, this indicates that (p)AF is an important part of the clinical disease spectrum caused by TTNtv, even if gross structural abnormalities are not yet present.

Conclusion

We evaluated the clinical and genetic background of the largest cohort reported to date of carriers with an identical *TTN* (founder) variation believed to be pathogenic because of segregation with the disease (LOD score 3.6). To our knowledge, this is the first time a founder effect in *TTN* has been associated with cardiomyopathy. The phenotype is characterized by DCM and atrial tachyarrhythmias, specifically (p)AF. As (p)AF could be the first symptom, clinicians could consider performing ambulatory ECG (Holter) monitoring in asymptomatic carriers during cardiac follow-up. A role for

TTN in the aetiology of AF is further supported by a recent genome wide association study.⁹

In addition to atrial arrhythmias, TTNtv may also increase susceptibility for ventricular arrhythmias because two carriers had an aborted cardiac arrest, one obligate carrier died suddenly, and nearly half of the ICD carriers had received appropriate ICD therapy (4/9). Roberts *et al.*¹ also showed that TTNtv-positive DCM patients experienced sustained ventricular tachycardia more often than TTNtv-negative DCM patients.

We also observed male predominance in DCM (93% of males had DCM vs. 40% of females), which seems counter-intuitive given autosomal dominant inheritance. However, in a large cohort of carriers of a different TTNtv ($n=94$), males also seemed more severely affected with respect to rates of heart transplantations, implantation of left ventricular assist devices, and death from cardiac causes.² Since our cohort is relatively homogeneous (identical mutation), the significant sex difference underscores the important role of sex in the development of DCM in TTNtv. While we still do not understand which mechanisms underlie the incomplete penetrance, there are some indications from our cohort that additional stressors (cardiovascular risk factors, alcohol, myocarditis, chemotherapy, or an additional mutation, male sex) may trigger the development of DCM in a heart that is already susceptible by a TTNtv. These observations need systematic investigation in larger cohorts of different TTNtv or in a later stadium when more carriers of this mutation are identified.

Supplementary Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Shared haplotype surrounding the *TTN* mutation.

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Edgar T. Hoorntje^{1,2},
Karin Y. van Spaendonck-Zwarts³,
Wouter P. te Rijdt⁴, Ludolf Boven¹,
Aryan Vink⁵, Jasper J. van der Smagt⁶,
Folkert W. Asselbergs^{7,8,9},
Jan van Wijngaarden¹⁰,
Eric A. Hennekam⁶, Yigal M. Pinto¹¹,
Ronald H. Lekanne Deprez³,
Daniela Q.C.M. Barge-Schaapveld¹²,
Marianne Bootsma¹³, Jakub Regieli^{14,15},
Yvonne M. Hoedemaekers¹,
Jan D.H. Jongbloed^{1†},
Maarten P. van den Berg^{4†}, and
J. Peter van Tintelen^{2,3*}

¹Department of Genetics, University of Groningen, University Medical Centre Groningen, Groningen, the Netherlands; ²Netherlands Heart Institute, Utrecht, the Netherlands; ³Department of Clinical Genetics, Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands;

⁴Department of Cardiology, University of Groningen, University Medical Centre Groningen, Groningen, the Netherlands; ⁵Department of Pathology, University Medical Centre Utrecht, Utrecht, the Netherlands; ⁶Department of Genetics, University Medical Centre Utrecht, Utrecht, the Netherlands; ⁷Department of Cardiology, Division Heart & Lungs, University Medical Centre Utrecht, Utrecht, the Netherlands; ⁸Durrer Centre for Cardiovascular Research, Netherlands Heart Institute, Utrecht, the Netherlands; ⁹Institute of Cardiovascular Science, Faculty of Population Health Sciences, University College London, London, UK; ¹⁰Department of Cardiology, Deventer Hospital, Deventer, the Netherlands; ¹¹Department of Cardiology, Academic Medical Centre, Amsterdam, the Netherlands; ¹²Department of Clinical Genetics, Leiden University Medical Centre, Leiden, the Netherlands; ¹³Department of Cardiology, Leiden University Medical Centre, Leiden, the Netherlands; ¹⁴Department of Cardiology, Isala Clinics, Zwolle, the Netherlands; and ¹⁵Heart Clinic, Amsterdam, the Netherlands

*Email: p.vantintelen@uva.amc.nl

†These authors contributed equally to this work.

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