Mortality Risk Associated With Truncating Founder Mutations in Titin

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ORIGINAL ARTICLE

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BACKGROUND: Truncating titin variants (TTNtv) are the most prevalent genetic cause of dilated cardiomyopathy, found in ≤25% of familial cases. Moreover, TTNtv associated with dilated cardiomyopathy are estimated to be present in 0.5% of the general population. The prognosis of asymptomatic carriers of TTNtv is poorly understood because TTNtv are associated with a highly variable phenotype. We aim to assess the natural history and clinical relevance of TTNtv by analyzing standardized mortality ratios (SMR) in multigenerational pedigrees and in close relatives of present-day patients.

METHODS: Haplotype and genealogical analyses were performed on 3 recurrent TTNtv. Subsequently, the family tree mortality ratio method was used to compare all-cause mortality of subjects at an a priori 50% risk of carrying TTNtv to the general Dutch population. SMRs were stratified for sex, age, and calendar period. Subgroups were compared with Poisson regression. Similarly, SMRs were calculated in parents of 128 present-day dilated cardiomyopathy probands with TTNtv using the reverse parent-offspring method.

RESULTS: The TTNtv were established as founder mutations and traced to 18th century ancestors. In 20 522 person-years, overall mortality was not significantly increased (SMR, 1.06; 95% CI, 0.95–1.18; *P*=0.162). However, mortality was significantly increased in subjects living after 1965 (SMR, 1.27; 95% CI, 1.04–1.53; *P*=0.009) and aged ≥60 years (SMR, 1.17; 95% CI, 1.01–1.35; *P*=0.02). The reverse parent-offspring analysis showed overall excess mortality (SMR, 1.26; 95% CI, 1.07–1.48; *P*=0.003), driven by subjects aged ≥60 years.

CONCLUSIONS: The natural history of the analyzed TTNtv shows a relatively mild disease course with significant excess mortality in elderly patients. With increasing life expectancy, TTNtv-associated morbidity and mortality will likely become more prevalent.

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Key Words: cardiomyopathy, dilated ■ mortality ■ mutation ■ natural history ■ titin

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Dilated cardiomyopathy (DCM) is characterized by dilatation of the left ventricle and systolic dysfunction that is not proportional to coronary artery disease, hypertension, or valve disease.[1] It is a major cause of end-stage heart failure and the leading indication for cardiac transplantation.[2] Estimations of the prevalence of DCM range from 1:250 to 1:400.[3] The pathogenesis of DCM is diverse, including genetic, toxic, infectious, metabolic, and endocrine causes, as well as inflammatory, infiltrative and autoimmune disease, pregnancy, and tachyarrhythmia.[1,2,4] A potential genetic cause is identified in 30% to 40% of idiopathic DCM cases.[3,5] Currently, >100 genes have been implicated in DCM, including *TTN*.[3,5–7] *TTN* encodes the largest human protein, titin, an abundant structural, sensory, and signaling filament in muscle, consisting of Z-disc, I-band, A-band, and M-band domains.[8] Truncating titin variants (TTNtv) constitute the most prevalent genetic cause of DCM, found in 19% to 25% of familial and 11% to 18% of sporadic cases.[5,7,9]

TTNtv are also present in the general population, initially estimated at 1% to 2%.[7,8,10–12] However, this high prevalence sparked controversy regarding the pathogenicity of TTNtv because it exceeded the prevalence of DCM, complicating the interpretation of genetic testing and counseling of test results.[12] Subsequent estimations of the prevalence of DCM-associated TTNtv, based on position in the A band or constitutional expression in the heart, were lower: 0.36% to 0.5%.[10,13] This still means that ≤37.5 million people globally may carry a DCM-associated TTNtv and are potentially at an increased risk of developing heart failure[7] and cardiac arrhythmia.[14,15]

Recent studies found preclinical eccentric remodeling in asymptomatic TTNtv carriers[13] and observed a relatively good response to medical treatment of TTNtv-related DCM.[16,17] These findings implicate that TTNtv carriers may benefit from early detection of cardiac involvement and treatment.

However, the prognosis of asymptomatic TTNtv carriers remains largely unknown because of reduced penetrance and highly variable disease severity. In this study, we sought to elucidate the natural history and clinical relevance of TTNtv by using the family tree mortality ratio (FTMR) method.[18–20] Multigenerational pedigrees were constructed for 3 Dutch founder TTNtv located in the A band, and standardized mortality ratios (SMR) were determined, stratified by sex, age group, and calendar period. Furthermore, as a representation of TTNtv carriers seen in current clinical practice, we analyzed SMR in the parents of present-day DCM patients carrying TTNtv, stratified by sex and age group.

METHODS

Methods are available in the Data Supplement. This study was conducted in accordance with the principles laid out in the Declaration of Helsinki and in line with guidelines provided

Table 1. Clinical Characteristics in Probands With Founder TTNtv

	Probands	
	Founder TTNtv (n=24)	
Sex male	16/24	68%
Fulfilling DCM diagnostic criteria	22/24	92%
Median age at DCM diagnosis (25%–75% percentile), y	45 (36–52)	
Median age at last follow-up (25%–75% percentile), y	54 (42–62)	
Additional variant in known DCM gene*	1/24	4%
Likely pathogenic	0/24	0%
Pathogenic	1/24	4%
Factor(s) possibly contributing to penetrance	9/24	38%
Pregnancy	2/8	25%
Alcohol abuse	1/24	4%
Drug abuse	0/24	0%
Chemotherapy	1/24	4%
Hypothyroidism or hyperthyroidism	1/24	4%
Tachyarrhythmia	3/24	13%
Mitral insufficiency	0/24	0%
Coronary artery disease	1/24	4%
(Suspected) myocarditis	1/24	4%
Rhythm disorders		
Atrial fibrillation	5/24	21%
Nonsustained ventricular tachycardia	11/24	46%
Sustained ventricular tachycardia	4/24	17%
Ventricular fibrillation	1/24	4%
Conduction disease		
Atrioventricular block grade 1	2/24	8%
Atrioventricular block grade 3	1//24	4%
(Incomplete) left bundle branch block	2/24	8%
(Incomplete) right bundle branch block	2/24	8%
Outcome		
Stroke	2/24	8%
Left ventricular assist device	3/24	13%
Heart transplant	1/24	4%
Aborted cardiac arrest or appropriate ICD therapy	2/24	8%
All-cause mortality	2/24	8%
Heart failure–related death	1/24	4%
Sudden cardiac death	0/24	0%
Composite outcome	7/24	29%
Median age composite outcome (25%–75% percentile), y	48 (44–54)	

The clinical characteristics of the 24 probands carrying 1 of the 3 different founder TTNtv. The composite outcome consisted of implantation of a left ventricular assist device, heart transplantation, aborted cardiac arrest, or appropriate ICD therapy, sudden cardiac death, and heart failure–related death. DCM indicates dilated cardiomyopathy; ICD, implantable cardioverter defibrillator; and TTNtv, truncating titin variants.

*One proband carried an additional pathogenic mutation in *MYBPC3* (c.442G>A p.[Gly148Arg, Gly148fs]), inherited from the parent not carrying the TTNtv.

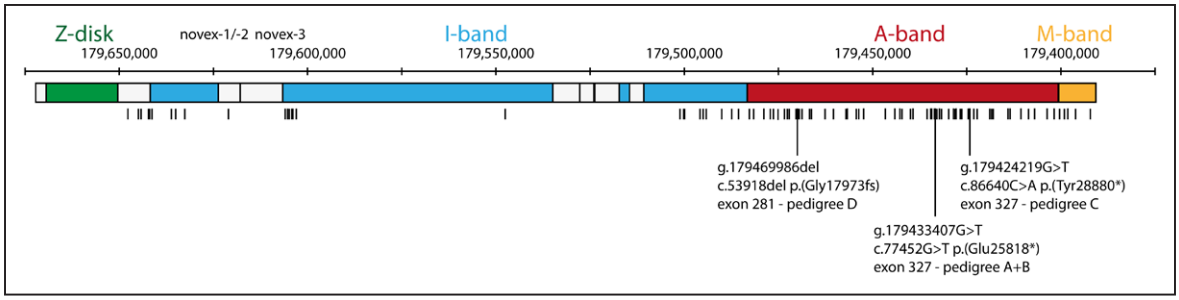

Figure 1. Schematic representation of truncating titin variants (TTNtv) within *TTN*.
Schematic representation of the *TTN* gene, based on the Hg19 genomic position data included in the overview of *TTN* transcript and exon usage data presented by Roberts et al (2015).[8] Included in the figure are the positions of the TTNtv included in the reverse parent-offspring analysis (indicated by small black bars) and the TTNtv founder mutations (large black bars with annotation of the exact mutation, exon number, and pedigree).

by ethics committees of the participating study centers. Approval was obtained from the institutional review board for retrospective data-analysis. In line with the Transparency and Openness Promotion Guidelines, the data that support the findings of this study are available from the corresponding author on reasonable request.

RESULTS

Subject Inclusion

A total of 24 DCM probands carrying the 3 recurrent TTNtv in the A band were identified. Clinical characteristics of these probands are described in Table 1; details on genetic analysis are provided in Table I in the Data Supplement. The localization of the TTNtv in the gene is illustrated in Figure 1. Extended haplotype analysis showed each of these 3 TTNtv to be on a mutation-specific chromosomal background, indicating a founder effect for all 3 mutations (Figure I in the Data Supplement). A total of 128 DCM probands carrying 92 different TTNtv were identified for the reverse parent-offspring analysis (Figure 1; Table II in the Data Supplement).

FTMR Analysis

Results of pedigree analysis are summarized in Table 2, and pedigrees are depicted in Figure 2. Pedigree analysis successfully traced 18 (of 24) probands to 4 pedigrees. Pedigrees A and B (p.[Glu25818*]) were considered to represent 1 family since haplotype analysis showed all probands to originate from a common ancestor. In total, 61 individuals on the transmission line and their 360 first-degree relatives were included in the multigenerational pedigrees, accumulating to a total of 20522 person-years, during which 317 deaths were observed, of which 279 occurred after the age of 1 year. There was no statistically significant overall excess mortality (SMR, 1.06; 95% CI, 0.95–1.18; *P*=0.162).

The SMRs according to sex, age categories, and calendar periods are depicted in Figure 3 (further detail in Table III in the Data Supplement). The overall all-cause mortality risk was not different between men and women (RR, 1.04; 95% CI, 0.83–1.30; *P*=0.734).

However, stratification by age categories revealed that among the highest age group (≥60 years), overall excess mortality was statistically significant (SMR, 1.17; 95% CI, 1.01–1.35; *P*=0.020). The point estimates for men and women aged ≥60 years were similarly directed but did not reach significance (SMR, 1.13; 95% CI, 0.92–1.37; *P*=0.118 and 1.21; 95% CI, 0.97–1.49; *P*=0.080, respectively).

Stratification by calendar periods showed significantly increased mortality in subjects living between 1965 and August 2012 (SMR, 1.27; 95% CI, 1.04–1.53; *P*=0.009). Especially men had a strongly increased mortality risk after 1965 (SMR, 1.43; 95% CI, 1.09–1.83; *P*=0.006).

Stratification by mutation did not yield evident differences. Poisson regression comparing pedigrees A+B

Table 2. Founder TTNtv and Results of Pedigree Analysis

Mutation Position in Reference Sequence, NM_001267550.2	Probands Identified	Pedigree (Probands in Pedigree)	Year of Origin	Included Subjects	Person-Years	Deaths (Deaths at Age ≥1 y)	Median Age of Death, y (interquartile Range)
c.53918del p.(Gly17973fs)	9	D (3)	1739	108	5674	70 (67)	65 (37–78)
c.77452G>T p.(Glu25818*)	11	A (9)+B (2)	1776	231	11046	176 (149)	61 (30–76)
c.86640C>A p.(Tyr28880*)	4	C (4)	1757	82	3803	71 (63)	63 (36–73)
Total	24	18		421	20522	317 (279)	62 (33–76)

The 3 founder TTNtv included in our study with a summary of the results of genealogical analysis. The year of birth of the oldest subject in the pedigree is given as year of origin. TTNtv indicates truncating titin variant.

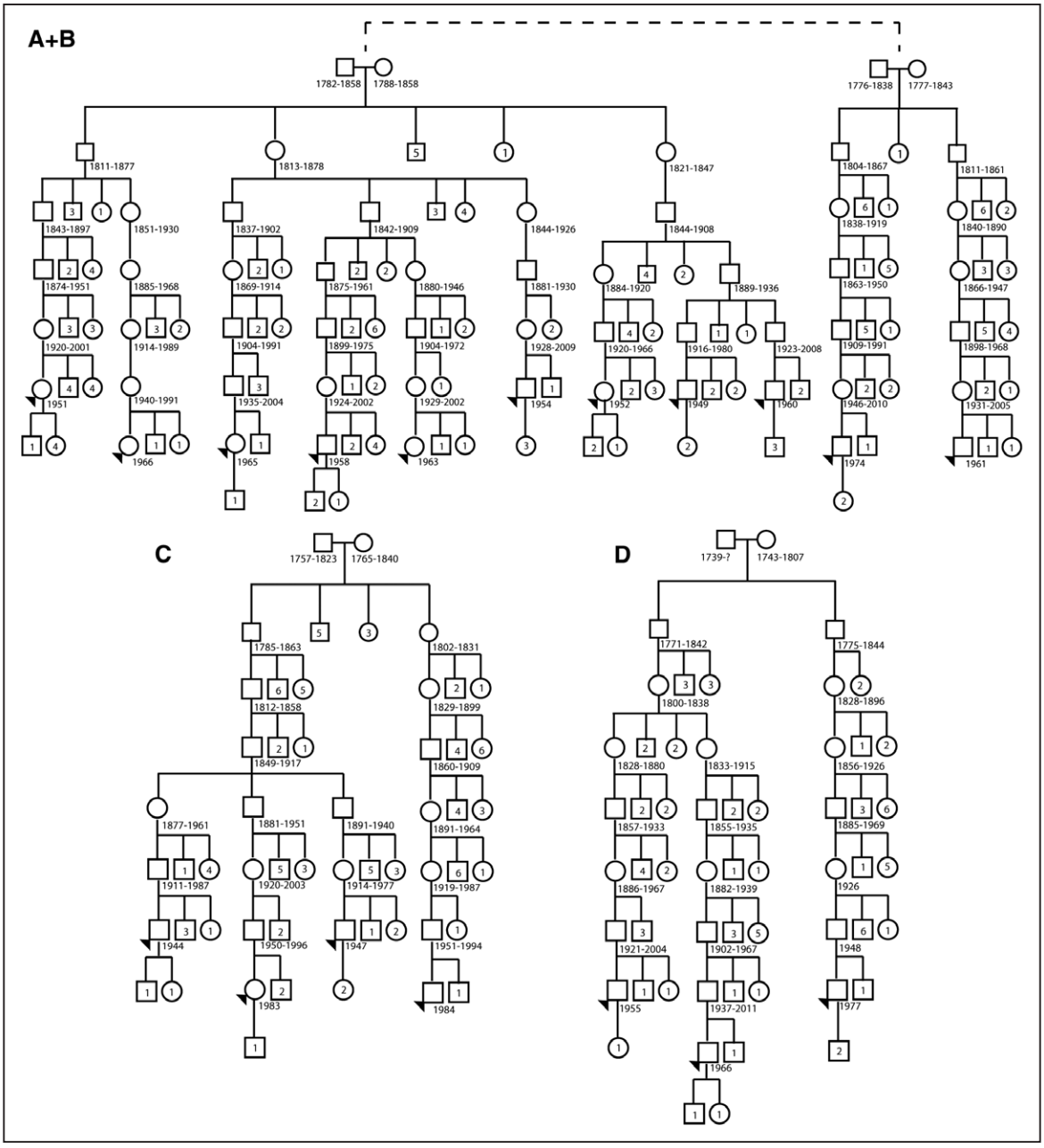

Figure 2. Pedigrees.
The 3 pedigrees included in our family tree mortality ratio analysis: A+B (c.77452G>T; p.(Glu25818*)), C (c.86640C>A; p.(Tyr28880*)), and D (c.53918del; p.(Gly17973fs)). Men are represented by squares, women by circles, and probands have been indicated with an arrow. Year of birth and death are provided for individuals on the transmission lines; siblings are shown as numbers in the symbols corresponding to their sex.

and C to D did not show differences in mortality rates (relative risk, 1.11; 95% CI, 0.8387–1.459; *P*=0.47).

Reverse Parent-Offspring Analysis

Results of the reverse parent-offspring analysis are shown in Figure 4 (further detail in Table II in the Data Supplement). In 9598 person-years, 147 deaths were observed corresponding with a significant overall excess mortality (SMR, 1.35; 95% CI, 1.14–1.59; *P*=0.0003). Stratification by age categories showed that excess mortality only reached significance among subjects aged ≥60 years (SMR, 1.47; 95% CI, 1.23–1.75; *P*=0.00001). Analyses according to sex showed that excess mortality occurred in both women and men aged ≥60 years (SMR, 1.45; 95% CI, 1.08–1.89; *P*=0.009 and SMR, 1.29; 95% CI, 1.00–1.56; *P*=0.046, respectively).

DISCUSSION

In this study, we investigated the natural history of TTNtv by analyzing all-cause mortality. In 20 522 person-years

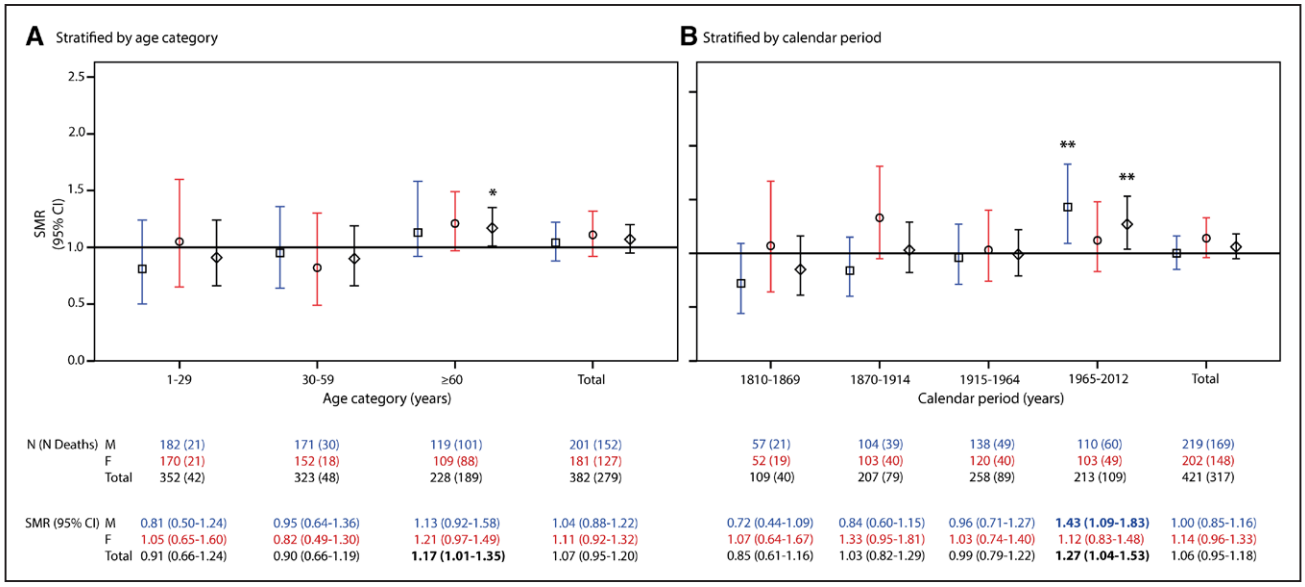

Figure 3. Standardized mortality ratio (SMR) analyses of multigenerational pedigrees.
SMR for the subjects included in the multigenerational pedigrees, stratified by age category (**A**) and calendar period (**B**) and further stratified by sex. Men are shown as squares with blue bars, women as circles with red bars, and totals of both sexes as diamonds with black bars. Provided per age category or calendar period are the number of subjects contributing person-years to the analyzed stratum, number of observed deaths, and the SMRs, stratified for male (M) and female (F) sex, as well as the totals. *SMR (vs general population) $P<0.05$, **$P<0.01$. CI indicates confidence interval.

in 3 multigenerational pedigrees, we observed that the overall SMR was not significantly increased. However, clear excess mortality occurred after 1965, predominantly in persons aged ≥60 years. The parents of present-day patients with TTNtv showed significant overall excess mortality, driven by persons aged ≥60 years.

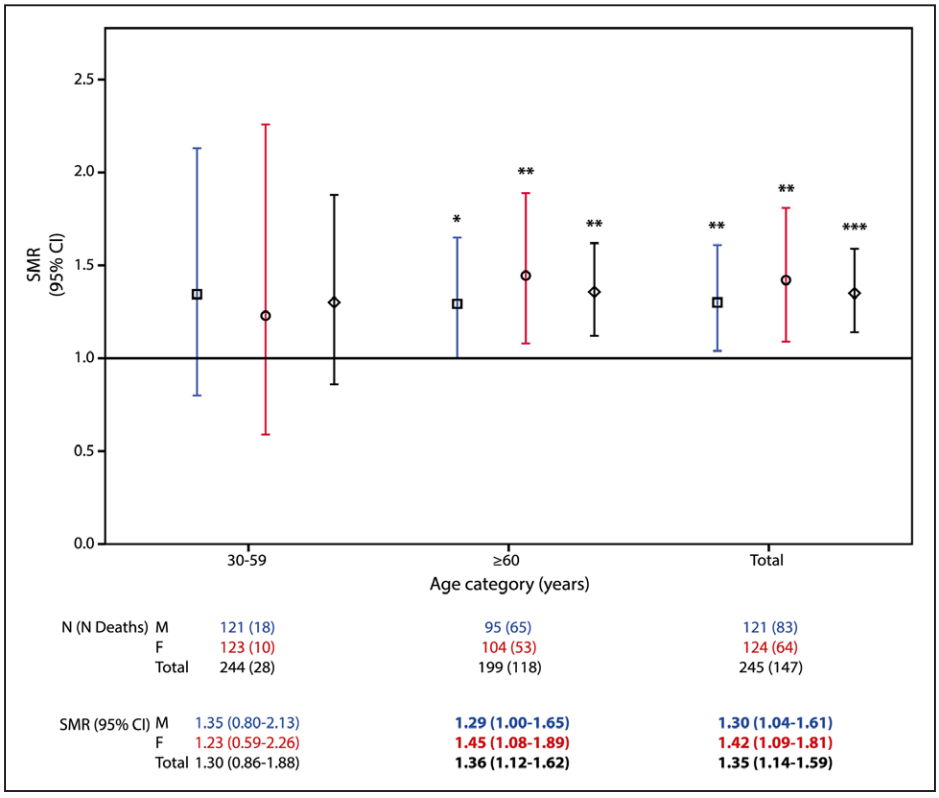

Figure 4. Standardized mortality ratio (SMR) analyses of parents of present-day probands.
SMRs for the parents of 128 present-day probands, stratified by age category and further stratified by sex. Men are shown as blue bars, women as red bars, and totals of both sexes as black bars. Men are shown as squares with blue bars, women as circles with red bars, and totals of both sexes as diamonds with black bars. Provided per age category are the number of subjects contributing person-years to the analyzed stratum, number of observed deaths, and the SMRs, stratified for male (M) and female (F) sex, as well as the totals. The 1- to 29-y age category was omitted in this figure because only 1 death occurred in it. *SMR (vs general population) $P<0.05$, **$P<0.01$, ***$P<0.001$.

At least 35% of the reported causes of deaths in our analyses were likely related to titin-associated DCM. Our results support a relatively mild disease course with excess mortality in the elderly.

The excess mortality in the elderly found in this study illustrates the clinical relevance of DCM-associated TTNtv and offers insight in the prognosis of TTNtv carriers. Previously, cohort studies have implicated TTNtv in both a relatively mild DCM phenotype with good response to medical treatment,[16,17] as well as a severe phenotype with high rates of heart transplantation, left ventricular assist device implantation, malignant arrhythmia, and death.[7,8] The FTMR method allowed us to investigate TTNtv-related mortality free from ascertainment bias, which is inherent to cohort studies and was recently observed in multiple cohorts of patients with inherited cardiac disease.[21] The SMR calculated in this study was calculated using complete sibships based on an a priori chance of carrying the TTNtv of 50%. Therefore, it should show 50% of the excess mortality. The natural history of TTNtv showed modest excess mortality compared with other FTMR studies (Table IV in the Data Supplement). To illustrate, point estimates in age categories with significant excess mortality were relatively low compared with a previous FTMR study involving mutations in the myosin-binding protein C-3 gene, associated with hypertrophic cardiomyopathy.[22] Excess mortality in studies in 2 other genes associated with DCM (lamin A/C and phospholamban) also showed higher point estimates, although it should be noted that the former only analyzed present-day patients and did not investigate natural history.[23,24] This supports a relatively mild disease course associated with TTNtv, in line with a recent report.[16]

Before 1965, no significant overall mortality was observed, whereas after this time period, there was a significant SMR of 1.27. This was confirmed by the overall excess mortality seen in our parent-offspring analysis, which acted as a validation cohort and gives additional insight in the effects of TTNtv close relatives of present-day patients. This may seem counterintuitive because treatment of heart failure in the form of medical treatment and implantation of cardioverter defibrillators has rapidly emerged since the 1970s. However, the excess mortality becoming evident after 1965 may result from a loss of competing risks. This is supported by the increase in average life expectancy in the Dutch general population.[25] The increase in life expectancy among the elderly may have been subdued by the effect of the TTNtv. As shown in Table V in the Data Supplement, the observed mean age at the time of death for individuals aged 60 years in the general population has steadily risen from 73.41 years for men and 74.06 years for women in 1861 to 1866 to 77.68 years and 82.82 years in 1961 to 1966.[25] Conversely, in the multigenerational pedigrees, this varied over time, with an age of 70.6 years for men and 77.57 years for women in 1961 to 1966. A similar effect was previously reported in an FTMR study in the cyclin-dependent kinase inhibitor 2A gene, associated with melanoma.[26] Moreover, nonadherence to guidelines, comorbidities, and polypharmacy have been shown to be important limitations in the optimal treatment of heart failure in elderly.[27,28] With life expectancy continuing to increase, TTNtv-associated morbidity and mortality will likely become more prevalent. Furthermore, in-depth phenotyping of the probands in our study revealed high degrees of response to medical treatment similar to those reported previously by Jansweijer et al (data not shown).[16] Therefore, dedicated genetic screening and cardiological follow-up of genotype-positive, phenotype-negative individuals is recommended.

Although our data support a modest disease course for TTNtv, our cohort and previous publications show that severe phenotypes do occur, even at young ages. Both genetic background and environmental factors including pregnancy, excessive alcohol consumption, and chemotherapy,[29–34] have previously been suggested to explain this heterogeneity. In our study, 1 proband was found to carry a second pathogenic mutation, and possible environmental factors were identified in a high proportion of patients. Future studies should be directed at further unraveling the disease mechanisms involved in expression and severity of TTNtv-associated DCM.

Study Limitations

The SMR analyses were performed using an undisputable outcome, that is, all-cause mortality. However, this inherently limits our analysis by omitting other aspects of disease burden. To ascertain the burden of TTNtv, causes of death were retrieved from family histories and pedigrees collected by clinical geneticists and cardiologists, shown in Table VI in the Data Supplement. A possibly DCM-related cause of death was reported in 35% of patients.

As already noted, heart failure treatment, including implantable cardioverter defibrillators, has emerged since the 1970s. Therefore, the results of the SMR after this time period may even be an underestimation of the true natural history. Concerning the FTMR analysis, as perinatal and early childhood mortality are most likely subject to under-registration, results for this age period may be subject to imprecision. Furthermore, 13 parents of probands who were included in the reverse parent-offspring analysis were excluded from the FTMR study to avoid double analysis. Because these were obligate carriers, deceased after 1965, the effect in this calendar period may be underestimated.

The reverse parent-offspring analysis is limited by the inclusion of only the parents of probands, excluding

subjects who might not have reached reproductive age. Second, a wide variety of TTNtv were included, of which pathogenicity has not been formally proven. A small number of the included TTNtv are located outside of the A band but, with the exception of 2, all are in constitutively expressed exons. Finally, there is a small chance that some of the TTNtv used in this analysis occurred de novo, resulting in neither parent carrying the mutation. However, in practice, this occurs only rarely. These limitations may have led to an underestimation of the true effects in close relatives of present-day patients.

Conclusions

In the Dutch population, we identified 3 founder TTNtv located in the A-band region. We studied the natural history of founder TTNtv across age categories and calendar periods by performing FTMR analysis. This revealed significant excess mortality in subjects aged ≥60 years and subjects living after 1965. Additionally, we studied mortality in a reverse parent-offspring analysis, observing significant overall excess mortality due to deaths occurring among subjects aged ≥60 years. Our results indicate a relatively mild disease course with significant excess mortality only seen in elderly patients. However, with life expectancy continuing to increase, TTNtv-associated morbidity and mortality will likely become more prevalent. Based on our results and previous reports that indicate that TTNtv-associated DCM has a relatively good response to medical treatment, dedicated genetic screening and cardiological follow-up of genotype-positive, phenotype-negative individuals is highly recommended.

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Disclosures

None.

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