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BRIEF REPORT

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KBTBD13 is a novel cardiomyopathy gene

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Abstract

KBTBD13 variants cause nemaline myopathy type 6 (NEM6). The majority of NEM6 patients harbors the Dutch founder variant, c.1222C>T, p.Arg408Cys (*KBTBD13 p.R408C*). Although *KBTBD13* is expressed in cardiac muscle, cardiac involvement in NEM6 is unknown. Here, we constructed pedigrees of three families with the *KBTBD13 p.R408C* variant. In 65 evaluated patients, 12% presented with left ventricle dilatation, 29% with left ventricular ejection fraction< 50%, 8% with atrial fibrillation, 9% with ventricular tachycardia, and 20% with repolarization abnormalities. Five patients received an implantable cardioverter defibrillator, three cases of sudden cardiac death were reported. Linkage analysis confirmed cosegregation of the *KBTBD13 p.R408C* variant with the cardiac phenotype. Mouse studies revealed that (1) mice harboring the *Kbtbd13 p.R408C* variant display mild diastolic

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dysfunction; (2) *Kbtbd13*-deficient mice have systolic dysfunction. Hence, (1) *KBTBD13* is associated with cardiac dysfunction and cardiomyopathy; (2) *KBTBD13* should be added to the cardiomyopathy gene panel; (3) NEM6 patients should be referred to the cardiologist.

KEYWORDS
cardiomyopathy, congenital myopathy, *KBTBD13*, NEM6

1 | INTRODUCTION

Kelch repeat and BTB (POZ) domain containing 13 (*KBTBD13*) is predominantly expressed in striated muscle(Sambuughin et al., 2010), but its function is largely unknown. KBTBD13 is a substrate adaptor for Cullin-3, a muscle-specific ubiquitin ligase that mediates protein turnover (Sambuughin et al., 2012). Recently, we showed that KBTBD13 serves an additional function, namely that of an actin-binding protein that modulates the relaxation kinetics of skeletal muscle (de Winter et al., 2020). Indeed, variants in *KBTBD13* cause nemaline myopathy type 6 (NEM6; MIM# 609273), a nondystrophic congenital myopathy characterized by peculiar slowness of movement due to slow relaxation kinetics of skeletal muscles (Gommans et al., 2002; Gommans et al., 2003). The majority of NEM6 patients harbors the Dutch founder variant, c.1222C>T, p.Arg408Cys (*KBTBD13 p.R408C*), yet patients are globally dispersed with reported cases in Europe, Asia, Australia, and Northern America (Garibaldi et al., 2018; Kang et al., 2020; Olivé et al., 2010; Sambuughin et al., 2010).

Although KBTBD13 is expressed in cardiac muscle at a level comparable with that in skeletal muscle (Sambuughin et al., 2010), cardiac involvement in NEM6 is unknown (Finsterer & Stöllberger, 2015). Prompted by a NEM6 patient with the *KBTBD13 p.R408C* variant, who visited our cardiogenetic outpatient clinic with heart failure, a family history of sudden cardiac death (SCD), and no variants in established cardiomyopathy genes, we constructed pedigrees and studied medical reports in three families with the *KBTBD13 p.R408C* variant. Furthermore, we took advantage of two mouse models, developed for unraveling NEM6 pathophysiology (de Winter et al., 2020), to study the role of KBTBD13 in the myocardium: one harboring the R408C variant in *Kbtbd13* and one in which *Kbtbd13* is deleted. Importantly, the homozygous $Kbtbd13^{R408C}$-knockin (KI) mice closely phenocopy NEM6 pathology, including nemaline bodies in skeletal myofibers and slow kinetics of skeletal muscle relaxation, whereas mice in which the *Kbtbd13* gene was deleted developed no NEM6 phenotype (de Winter et al., 2020). Based on these and other findings, we proposed that *KBTBD13 p.R408C* is a gain-of-function variant (de Winter et al., 2020). The cardiac phenotype of these mouse models is unknown and is studied here. Details regarding the applied methods are in the supplement. All data collection was in accordance with the principles of the Declaration of Helsinki and written informed consent was obtained from the subjects.

Figure 1 shows the pedigree of Family 1. Cardiac evaluation of Family 1 revealed that 24 out of 28 NEM6 patients display cardiac abnormalities, including left ventricle (LV) dilation ($n = 5$), reduced left ventricular ejection fraction (LVEF) ($n = 14$; including several patients with LVEF < 45%, indicating systolic heart failure), repolarization abnormalities ($n = 12$) and atrial fibrillation ($n = 5$). Two patients received an implantable cardioverter defibrillator (ICD) because of nonsustained ventricular tachycardia, and three patients died because of SCD ($n = 3$; Figure 1; Table S1). In Family 1, two consanguineous parents (II.1 and II.2, Figure 1) had multiple children who died prenatal or in early childhood of whom five were reported having signs of severe congenital myopathy including reduced fetal movements. Although we cannot exclude the role of other conditions in these children, for instance, an autosomal recessive disorder, taken together, the above may suggest a more severe cardiac phenotype in patients homozygous for the *KBTBD13 p.R408C* variant (note that their genotype is unknown). In Family 2, 16 of the 17 evaluated patients had a cardiac phenotype (Figure S1; Table S2). Structural abnormalities such as a dilated LV were observed ($n = 2$) as well as functional abnormalities such as reduced LVEF ($n = 10$; including several patients with systolic heart failure). Six patients displayed ventricular arrhythmias, of whom two received an ICD because of nonsustained tachycardia and one because of severely reduced LVEF (25%). In Family 3, three out of 19 patients were evaluated. One family member had peripartum cardiomyopathy (age 22) of which she recovered, and one patient displayed nonsustained arrhythmias and mildly impaired LV systolic function (Figure S2; Table S2). Thus, in total, 65 NEM6 patients were evaluated of whom 12% presented with LV dilatation, 29% with LVEF < 50%, 8% with atrial fibrillation, 9% with ventricular tachycardia, and 20% with repolarization abnormalities. Linkage analysis revealed a logarithm of the odds (LOD) score of 6.02, at zero recombination, confirming significant cosegregation of the *KBTBD13 p.R408C* variant with the cardiac phenotype.

Homozygous $Kbtbd13^{R408C}$-KI mice were studied at 9 months after birth. This age was chosen as the NEM6 skeletal muscle pathology in the mice is slowly progressive, similar to disease progression in the patients (de Winter et al., 2020; Gommans et al., 2002; Gommans et al., 2003). The level of mutant *Kbtbd13* transcript in the LV of homozygous KI mice was comparable with the level of WT transcript in the LV of WT mice (Figure 2a). Heart mass (Figure 2B; Table S3) and LV inner diameter and wall thickness at

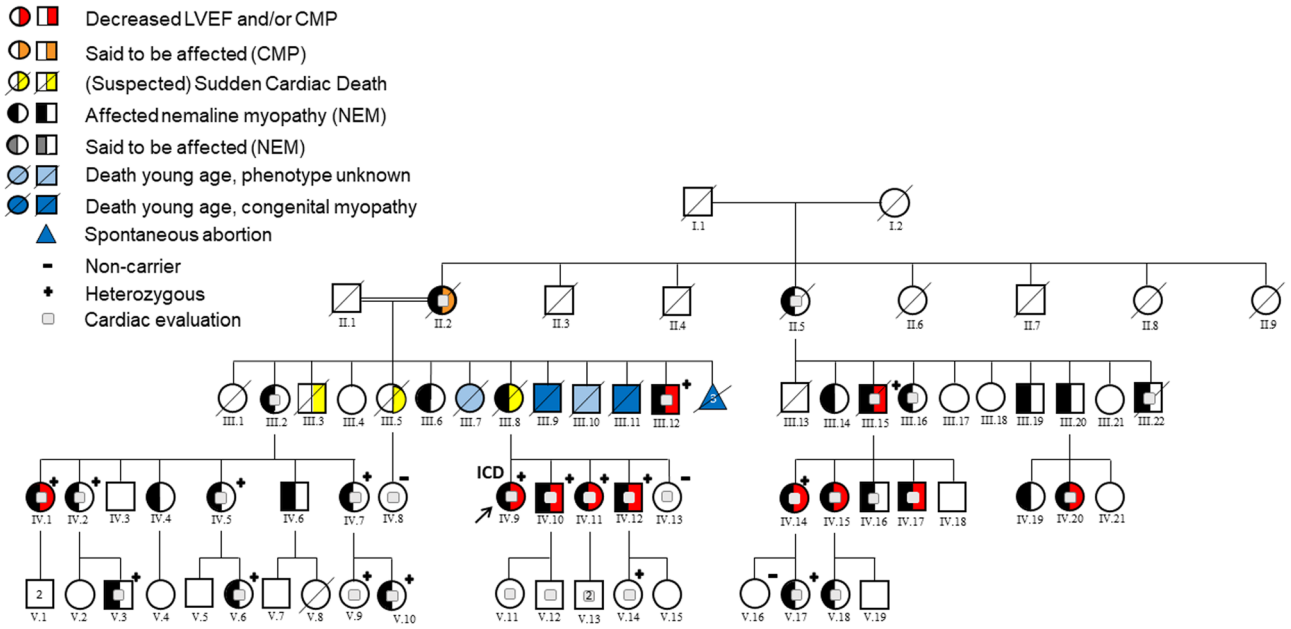

FIGURE 1 Pedigree Family 1. Partly or fully filled symbols represent affected individuals (see box for explanation); open symbols: unaffected individuals; diagonal line: deceased; arrow: proband; circle: female; square: male; the number in the symbols indicate the number of individuals (if >1). CMP, cardiomyopathy; LVEF, left ventricular ejection fraction; NEM, nemaline myopathy.

diastole were unaffected in KI mice (Figure 2B; Table S3). Histological evaluation of LV myocytes revealed no abnormalities (Figure 2c). A complete overview of the parameters obtained with echocardiography and pressure-volume analyses are shown in Table S3. In brief, echocardiography showed unaffected LVEF (Figure 2d), heart rate, and strain rate (Table S3); the response upon dobutamine administration was not affected. Pressure-volume relations showed unaffected end-systolic pressure-volume relation (ESPVR), whereas the end-diastolic pressure-volume relation (EDPVR) was significantly steeper in KI mice (Figure 2e). Thus, mice with the *Kbtbd13*^{R408C} variant have mild diastolic dysfunction.

To study whether the diastolic dysfunction in KI mice is an effect of the *Kbtbd13*^{R408C} variant, next we studied *Kbtbd13*-knockout (KO) mice. Similar to the KI mice, the KO mice were studied at 9 months after birth. As expected, KO mice expressed no *Kbtbd13* transcript (Figure 2f). Heart mass was lower in KO mice (Figure 2G; Table S3), in line with the lower end-diastolic LV posterior wall thickness (Figure 2g). Histological evaluation revealed no abnormalities (Figure 2h). A complete overview of the parameters obtained with echocardiography and pressure-volume analyses are shown in Table S3. In brief, echocardiography showed unaffected LVEF; upon dobutamine administration, KO mice had a blunted increase in LVEF (Figure 2i) and heart rate (Table S3). Pressure-volume loop analysis revealed a less steep ESPVR in KO mice, indicating impaired systolic LV function (Figure 2j). EDPVR was unaffected in KO mice (Figure 2j).

Thus, the *Kbtbd13*^{R408} variant results in mild diastolic dysfunction at nine months of age.

This is the first study to demonstrate cardiac dysfunction in individuals with the myopathy-causing *KBTBD13 p.R408C* variant. The cardiac phenotype, which includes LV dilatation, reduced LVEF, and arrhythmia, is heterogeneous, presumably due to disease progression over time (Olivotto et al., 2015; Pinto et al., 2016). Although some patients meet criteria for dilated cardiomyopathy (DCM), others have normal LV dimensions and meet criteria for arrhythmogenic cardiomyopathy (ACM), or display arrhythmia in the absence of cardiomyopathy. Thus, caution is warranted when classifying the patients, as a spectrum of cardiomyopathic features from ACM to mild LV dilatation and/or dysfunction, to overt DCM might be present. Thirty-seven variants have been identified in *KBTBD13*, dispersed throughout Europe, Australia, Asia, and Northern America (Garibaldi et al., 2018; Kang et al., 2020; Olivé et al., 2010; Sambuughin et al., 2010) (Leiden Open Variant Database). Of these 37, the *KBTBD13 p.R408C* variant is the most prevalent one, and the only variant of which the cardiac consequences have now been studied. The pathomechanism underlying the cardiac dysfunction in NEM6 patients is unclear. To date, only very little is known regarding the functions of KBTBD13 in striated muscle, but insights are increasing. KBTBD13 is a member of the KBTBD subfamily of Kelch proteins that contains a BTB domain and Kelch repeats but lacks a BACK domain (Sambuughin et al., 2012). KBTBD13 forms a complex with nbr1 and Cul3 ubiquitin ligase through its N-terminal BTB domain and this interaction is required for the formation of a functional Cul3 ubiquitin ligase complex, suggesting that the pathogenic mechanism in NEM6 may involve dysregulation of cellular protein turnover (Sambuughin et al., 2012). As NEM is considered a myopathy of the actin-based thin filaments, it has been postulated that KBTBD13 is involved in the turnover of thin filament proteins, but evidence in support of this postulation has been lacking. In fact, recently we showed that KBTBD13^{R408C} slows skeletal muscle relaxation kinetics through a direct effect of the binding of KBTBD13^{R408C} to actin monomers in the thin filament (de Winter

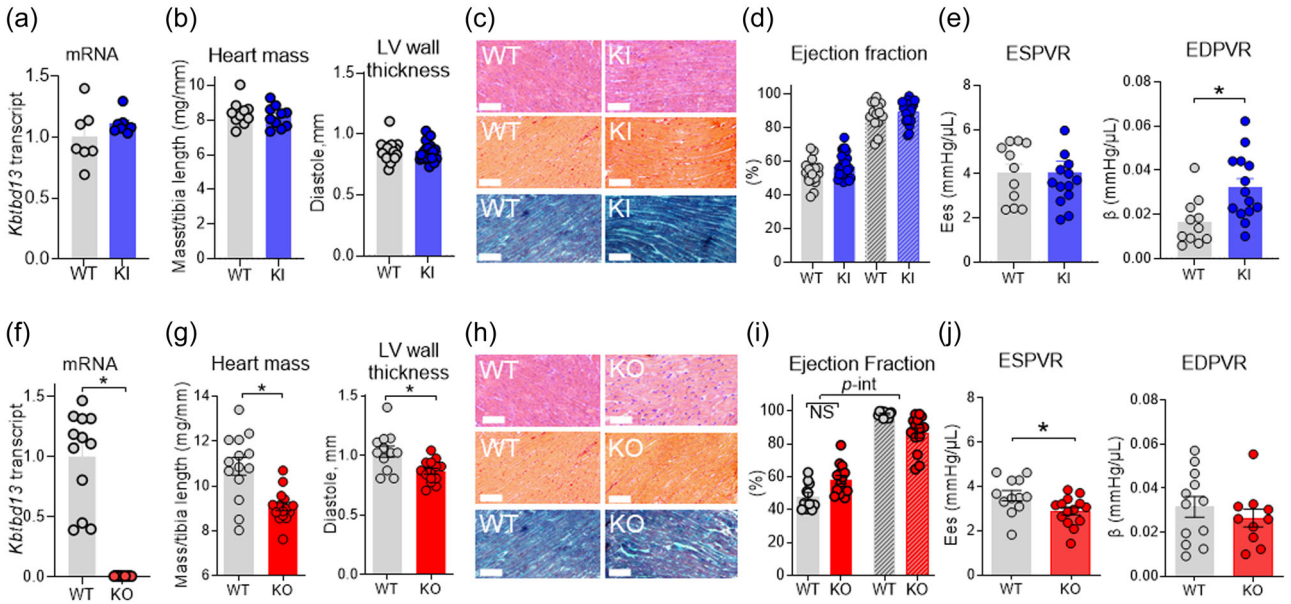

FIGURE 2 Characterization of Kbtbd13^{R408C}-knockin and Kbtbd13-knockout mice. (a) qPCR with primers that detect both WT and mutant Kbtbd13 transcript show comparable mRNA levels in *Kbtbd13*^{R408C}-WT (WT) and homozygous *Kbtbd13*^{R408C}-KI (KI) mice. Kbtbd13 transcript was normalized to housekeeping gene Csnk2a2. (b) (left) Whole heart mass and (right) left ventricle wall thickness were comparable between *Kbtbd13*^{R408C}-KI (KI) mice and *Kbtbd13*^{R408C}-WT (WT) mice. (c) (upper panel) Histological evaluation by Hematoxylin-eosin, (middle panel) Picrosirius, and (lower panel) Gomori-trichrome stainings showed normal structure of the left ventricle, no fibrosis and no protein aggregates in the cardiomyocytes of KI mice (scale bar = 50 µm). (d) Stress echocardiography revealed no changes left ventricle ejection fraction (LVEF) in KI mice, both at rest and upon dobutamine administration. (e) (left) Pressure-volume relations showed an unaffected end-systolic pressure-volume relation (ESPVR), (right) whereas the end-diastolic pressure-volume relation (EDPVR) was significantly steeper in KI mice compared with WT mice. (f) qPCR with Kbtbd13 primers show no detectable mRNA levels in KO mice. Kbtbd13 transcript was normalized to housekeeping gene Csnk2a2. (g) (left) Whole heart mass and (right) left ventricular wall thickness were lower in KO mice compared with WT mice. (h) (upper panel) Histological evaluation by Hematoxylin-eosin, (middle panel) Picrosirius, and (lower panel) Gomori-trichrome stainings showed normal structure of the left ventricle, no fibrosis and no protein aggregates in the cardiomyocytes of KO mice (scale bar = 50 µm). (i) Stress echocardiography revealed no changes in LVEF at baseline. However, upon dobutamine administration, KO mice had a blunted increase in LVEF (P-interaction < 0.01). (j) (left) Pressure-volume relations revealed a lower ESPVR in KO mice, and (right) no changes in the EDPVR compared with WT mice.

et al., 2020). The binding of mutant KBTBD13 to actin increased the rigidity of the thin filament, which slowed the kinetics of sarcomere relaxation(de Winter et al., 2020). We propose that this mechanism might contribute to the mildly impaired diastolic function, with preserved systolic function, in the *Kbtbd13*^{R408C} KI mice (Figure 2e). In our patient cohort, the cardiac phenotype was heterogeneous, with impaired cardiac relaxation kinetics observed in only five patients. Furthermore, the majority of patients with an established genotype were heterozygous for the *KBTBD13 p.R408C* variant, with the few patients potentially homozygous for the variant displaying a very severe phenotype, whereas the mice studied were homozygous for the variant and displayed only a mild cardiac phenotype. Differences in phenotype severity between mouse models and patients are not uncommon and have been reported in many previous studies. It might be a result of, for example, differences in heart rate, metabolism, and protein isoform expression (reviewed in van der Velden et al., 2022). Furthermore, the differences in cardiac phenotype in mice compared with that in patients might be related to the cross-sectional nature of this study, with mice studied at 9 months, but patients studied at ages ranging from 3 days to 81 years. Future, longitudinal studies in both patients and (heterozygous) *Kbtbd13*^{R408C} mice should establish the natural history of the cardiac phenotype. The main goal of the studies in the mouse model was to show that the variant can cause contractile dysfunction, albeit mild and not fully recapitulating the patients' phenotype.

No cardiac phenotype has been reported yet in patients harboring variants other than *KBTBD13 p.R408C*, although we cannot rule out that these patients have an undiagnosed, subtle cardiac phenotype, or will develop this at older age. Thus, whether these variants impact cardiac function is currently unknown. Importantly, we showed that, in mice, KBTBD13-deficiency blunts the response to acute cardiac stress and lowers systolic strain rate and the ESPVR (Figure 2I,J; Table S3), suggesting that loss-of-function variants in *KBTBD13* might be detrimental as well. Alternatively, variants in modifier genes in the families with the *KBTBD13 p.R408C* variant might explain the cardiac phenotype in these families, and the absence of such phenotype in families with other variants in *KBTBD13*. Variants in modifier genes can also contribute to the variability in the cardiac phenotype among the patients with the *KBTBD13 p.R408C* variant, the presence of a cardiac phenotype in the absence of a skeletal muscle phenotype in patient IV.1 of Family 3, and the different phenotype in patients compared with that

of the KI mouse model. In one patient, the index patient in Family 1 (IV.9), a cardiomyopathy gene panel was tested, but no additional variants were found (details on included genes are in the supplemental methods). In the other patients, no cardiomyopathy gene panels were tested. It should be noted, however, that clinical variability and incomplete penetrance of the cardiac phenotype among patients is common in inherited cardiomyopathies, with one sibling being asymptomatic and the other one presenting with manifest cardiomyopathy (e.g. Moolman et al., 2000). Disease manifestation is thought to be greatly influenced by lifestyle and environmental factors, and this is an important focus of current research. Note that in older patients in our cohort, also risk factors such as atrial fibrillation and hypertension could contribute to cardiac disease. However, the observed phenotypes at young and middle age, and the high LOD score (6.0) provide evidence for an association between KBTBD13 and cardiac dysfunction and cardiomyopathy. Thus, considering that (1) the *KBTBD13 p.R408C* variant causes cardiomyopathy, (2) KBTBD13-deficiency causes a loss-of-function effect, (3) for the majority of *KBTBD13* variants the functional consequences are not yet known, and (4) variants in modifier genes might be at play, detailed cardiac and genetic screening of individuals harboring *KBTBD13* variants is of utmost importance.

Limitations of our study include its partly retrospective design, and the resulting absence of a standardized protocol for cardiac diagnostics and management. To address this limitation, one of the authors (Maarten P. van den Berg, cardiologist) interpreted all cardiac data, in addition to the attending clinician. Despite the limitations, an evident cardiomyopathy phenotype was found in the *KBTDB13 p.R408C* patient cohort. Furthermore, in the mouse models we could not determine wildtype and mutant KBTBD13 protein levels as antibodies, both commercial and home-made ones, are not capable of detecting KBTBD13 protein (de Winter et al., 2020). Consequently, our conclusions on the effect of the variants warrant caution, as these are based on the expression levels of *Kbtbd13* transcript.

In summary, based on the findings of the present study, we conclude that (1) *KBTBD13* is associated with cardiac dysfunction and cardiomyopathy; (2) *KBTBD13* should be added to the cardiomyopathy gene panel to facilitate the diagnosis of patients; (3) patients harboring *KBTBD13* variants should be referred to the cardiologist for screening.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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