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TBX5-encoded T-box transcription factor 5 variant T223M is associated with long QT syndrome and pediatric sudden cardiac death

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Abstract

Long QT syndrome (LQTS) is a genetic disease resulting in a prolonged QT interval on a resting electrocardiogram (ECG), predisposing affected individuals to polymorphic ventricular tachycardia and sudden death. Although a number of genes have been implicated in this disease, nearly one in four individuals exhibiting the LQTS phenotype are genotype-negative. Whole-exome sequencing identified a missense T223M variant in *TBX5* that cosegregates with prolonged QT interval in a family with otherwise genotype-negative LQTS and sudden death. The TBX5-T223M variant was absent among large ostensibly healthy populations (gnomAD) and predicted to be pathogenic by *in silico* modeling based on Panther, PolyPhen-2, Provean, SIFT, SNAP, and

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Author Contribution Statement:

Authors HAD, JJK, and APL conceived and designed the study. HAD, JJK, and APL obtained IRB approval, recruited/consented the family, performed and interpretated the clinical evaluation. AMM, PKRM, WE, KA, and APL performed genotyping, Sanger sequencing, expression vector design and mutagenesis, and the luciferase assay. AMM, PKRM, AA, GW, and APL performed the RNAseq and analysis. AMM, PKRM, and JEE wrote the initial manuscript. All authors revised the manuscript. XHTW and APL provided funding and equipment for studies.

Data Availability

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

Conflicts of Interest

X.H.T.W. is a founding partner of Elex Biotech, a start-up company that developed drug molecules that target ryanodine receptors for the treatment of cardiac arrhythmia disorders. This is not directly relevant to this study. All other authors have no conflicts of interest or disclosures.

PredictSNP prediction tools. The variant was located in a highly conserved region of TBX5 predicted to be part of the DNA-binding interface. A luciferase assay identified a 57.5% reduction in the ability of TBX5-T223M to drive expression at the ANF promotor compared to wildtype TBX5 *in vitro*. We conclude that the variant is pathogenic in this family, and we put TBX5 forward as a disease susceptibility allele for genotype-negative LQTS. The identification of this familial variant may serve as a basis for the identification of previously unknown mechanisms of LQTS with broader implications for cardiac electrophysiology.

Keywords

Long QT syndrome; repolarization abnormality

Long QT syndrome (LQTS) is a heritable cardiac channelopathy defined by a prolonged QT interval on a resting electrocardiogram (ECG), signifying a delay in cardiac repolarization following the depolarization that causes contraction (Nakano & Shimizu, 2016; Tester & Ackerman, 2014). LQTS is associated with syncope and sudden death secondary to potentially fatal arrhythmias that occur in the setting of delayed repolarization (Nakano & Shimizu, 2016; Singh, Morin, & Link, 2019). The prevalence of LQTS has been estimated to be 1:2,000 live births (Schwartz et al., 2009). Currently, there are 17 genes associated with LQTS that have varying strengths of association with the disease (Ackerman et al., 2011; Adler et al., 2020). The most common causal variants in LQTS are found within the cardiac ion channel-encoding genes *KCNQ1, KCNH2*, and *SCN5A*, altogether accounting for 75% of causes of LQTS (Tester & Ackerman, 2014). However, the strength of association between these 17 genes and LQTS causation has been the subject of evidence-based genetic reappraisal initiatives, demonstrating that 9 of the 17 genes deemed to be causal variants in LQTS have insufficient or inconsistent evidence supporting their causal role (Adler et al., 2020). Despite the diagnostic role of whole genome sequencing and the identification of causal variants in congenital LQTS, nearly 20% of clinical cases exhibiting prolonged cardiac repolarization are genotype negative (Ackerman et al., 2011; Nakano & Shimizu, 2016). Further investigation into the genetic and molecular mechanisms underlying these genotype negative cases is therefore of great importance (Fonseca & Vaz da Silva, 2018). The study of lesser-known familial variants associated with LQTS may allow for the identification of novel mechanisms implicated in LQTS as well as inform our broader understanding of cardiac electrophysiology (Giudicessi, Wilde, & Ackerman, 2018).

TBX5-encoded transcription box factor 5 (TBX5), which is part of the T-Box gene family, is crucial in limb and cardiac development (Lichiardopol, Militaru, Popescu, Hila, & Mixich, 2007; Steimle & Moskowitz, 2017). The gene encoding TBX5 is located on 12q24.1 (Lichiardopol et al., 2007). Variants in the gene encoding TBX5 are primarily associated with being causal variants in Holt-Oram Syndrome (HOS), characterized by cardiac and limb structural deformities (Li et al., 1997). When inherited, HOS follows an autosomal dominant inheritance pattern, but *de novo* pathogenic variants are implicated in 85% of HOS cases (McDermott, Fong, & Basson, 1993). Variants in *TBX5* are found in nearly 74% of individuals with HOS, often manifesting as cardiac conduction abnormalities and congenital heart defects (CHDs) including atrial septal defects (ASDs) and ventricular septal defects

(VSDs) (McDermott et al., 2005; Spiridon et al., 2018). Of note, TBX5 modulates the cardiac conduction system through driving expression of the *SCN5A* gene, which encodes the cardiac sodium channel NaV1.5 (Arnolds et al., 2012). In particular, the expression of NaV1.5 and connexin 40 in the ventricular conduction system (VCS), both of which facilitate rapid cardiac conduction, is directly dependent on TBX5 expression (Arnolds et al., 2012). To this end, there are no prior reports of a *TBX5* variant associated with LQTS.

We identified a genotype-negative family with a multigenerational history of LQTS and sudden death. Following receipt of informed consent from the Duke University and Baylor College of Medicine Institutional Review Boards, the proband and relatives underwent a full clinical evaluation by pediatric cardiology specialists. The proband (IV.3) is a 5-year-old Caucasian-Hispanic male found to exhibit cardiac structural, conduction, and repolarization abnormalities, with a family history of sudden death being the impetus for clinical evaluation (Table S1). His ECG demonstrated a prolonged QT interval of 558 ms and QTc of 540 ms, leading to the diagnosis of LQTS (Figure 1A). He also had a prolonged PR interval of 226 ms and right ventricular hypertrophy. His echocardiogram demonstrated the presence of a large secundum atrial septal defect (ASD) and two small muscular ventricular septal defects (VSDs) (Figure 1B and 1C, respectively). The proband was started on nadolol and underwent surgical closure of the ASD along with placement of an epicardial implantable cardioverter-defibrillator (ICD). Of note, the proband exhibits a normal upper extremity bone structure upon X-ray (Figure 1D).

Given the history of sudden cardiac death in this family from a small, rural town in Mexico, siblings and parents of the proband underwent clinical evaluation. On clinical evaluation, the proband's mother (III.2), a 58-year-old Hispanic female with a history of fetal demise, presented with a prolonged PR interval of 208 ms, nonspecific ST and T wave abnormalities, and sinus bradycardia on an ECG (Figure S1). Individual III.2 reports a prior consanguineous relationship with individual III.3, stating that the relationship was due to the small population size of the locale. Individual III.3 later experienced a sudden death event attributed to an unspecified heart condition. Additionally, both children of III.2 and III.3 (individuals IV.5 and IV.6) experienced a sudden death event attributed to LQTS. In particular, IV.6 experienced the sudden death event while dancing. However, the proband's biological father (III.1), a 39-year-old Caucasian male, exhibited a normal QT of 420 ms and QTc of 401 with no significant cardiac abnormalities (Figure S2). An ECG of the proband's 11-year-old brother (IV.2) met criteria for a prolonged QT interval of 468 ms and QTc of 475 ms in addition to a prolonged PR interval of 212 ms and left ventricular hypertrophy with a repolarization abnormality (Figure S3). The echocardiogram of IV.2 indicated the presence of at least four apical and mid-muscular septal defects, along with a small patent foramen ovale. The proband's 5-year-old sister (IV.4) underwent an ECG that yielded normal findings with a QT interval of 348 ms and QTc of 425 ms (Figure S4).

The proband underwent standard-of-care clinical genetic testing using the Familion LQTS gene panel test. The LQTS gene panel test was negative for pathogenic or likely pathogenic variants in the examined genes, necessitating the use of whole exome sequencing (detailed further in the Supplemental Methods). Whole exome sequencing was conducted utilizing the Illumina HiSeq 2000 platform along with cascade screening on the proband, yielding the

identification of the p.T223M c.C668T variant (Figure 2A). Candidate variants were then confirmed by Sanger sequencing, and sequence conservation across species was demonstrated (Figure 2B and 2C, respectively). In addition to the proband (IV.3), four kindred were consented and underwent genetic testing. The presence of the heterozygous T223M variant was also confirmed in the mother of the proband (III.2) along with the brother of the proband (IV.2), suggesting an autosomal dominant mode of inheritance with reduced penetrance.

To confirm the absence of the identified variant in ostensibly healthy individuals, the publicly available Genome Aggregation Database (gnomAD) was used as a control cohort. The T223M variant was absent in this control cohort. This variant localized to a highly conserved DNA-binding domain of the protein (Figure 2D). *In silico* prediction modeling was performed to predict the pathogenicity of the T223M variant in the *TBX5* gene. This variant was predicted to be either “damaging,” “probably damaging,” “deleterious,” or “effect-inducing” in all five models, with an average confidence of 77% (Table S2) (Adzhubei et al., 2010; Bendl et al., 2014; Choi & Chan, 2015; Korf, 2004; Mi, Muruganujan, Ebert, Huang, & Thomas, 2019; Sim et al., 2012).

To test the ability of TBX5 to drive expression of the atrial natriuretic factor (ANF) promoter, a known transcriptional target of TBX5, a luciferase assay was conducted in which luciferase was placed under the expression control of the ANF promoter (Fan, Liu, & Wang, 2003). We identified a 57.5% reduction in the ability of TBX5-T223M to drive expression at the ANF promotor compared to wildtype TBX5 (P=0.005) and an empty vector control (P=0.001), indicating a loss of function in TBX5 due to the T223M variant (Figure 2E). This suppression of ANF transcription in the presence of the T223M variant was seen regardless of presence of N-terminal GFP and FLAG fusion (Figure S5). There was no significant difference in expression levels of the TBX5-T223M variant and ANF-Luciferase (P=0.1). Furthermore, chromatin immunoprecipitation sequencing (ChIP-seq) analysis demonstrated Tbx5 binding either upstream or downstream of 11 genes currently included in the Familion LQTS Gene Panel Test, particularly including *Scn5a, Kcnh2*, and *Kcnq1* (Table S3, Supplemental Materials). With ChIP-Seq analysis, we identified 64 peaks common to the two independent ChIP-Seq experiments, 16 of which were proximal to the LQTS-associated genes *Scn4b* and *Snta1* (Table S4). T-box enrichment analysis was performed using the canonical T-box sequence of AGGTG (Waldron et al., 2016). We identified an 18.75% enrichment of the T-box binding motif within these examined loci.

As nearly 20% of clinical cases exhibiting prolonged cardiac repolarization remain genetically unexplained, studying rare familial variants with a whole-genome sequencing approach can facilitate the identification of novel mechanisms of LQTS with broader implications for cardiac electrophysiology (Ackerman et al., 2011; Giudicessi et al., 2018). Currently, 17 genes are linked to LQTS, with variants in the last 14 genes identified being extremely rare (Ackerman et al., 2011). Here, we report the identification of a variant that cosegregates in a family with a history of both LQTS and sudden death that also exhibits cardiac features of HOS.

Variants in the TBX5-encoding gene have been classically associated with HOS, a genetic condition characterized by with congenital skeletal malformations of the upper limb, particularly the radial ray (Basson et al., 1994; Li et al., 1997). While upper extremity malformations are the most typical finding of HOS, no upper extremity malformations were present in the proband (Li et al., 1997). Additionally, variants within the *TBX5* gene are found in nearly 74% of individuals with HOS, but the location of the variant and type of variant within the *TBX5* gene does not appear to be predictive of malformation severity, ultimately suggesting broad variation in disease mechanisms (Basson et al., 1999; Brassington et al., 2003; McDermott et al., 2005). Nevertheless, variants in the *TBX5*-encoding gene manifest as congenital heart defects (CHDs) in 75% of HOS cases, commonly as atrial septal defects (ASDs) and ventricular septal defects (VSDs) (Spiridon et al., 2018; Zhu et al., 2008). These cardiac features common to HOS are consistent with the cardiac findings of the proband heterozygous for the T223M variant in TBX5 in this study, despite the lack of presence of upper limb malformations typically associated with TBX5 variants. Therefore, this finding supports a broadening of the phenotype associated with variants in the *TBX5* gene with the use of whole exome sequencing (Spiridon et al., 2018). These findings function to further support the variable presence and severity of upper limb malformations that has been observed in individuals with a variant in the *TBX5* gene, as most described variants in TBX5 are associated with some degree of upper-limb malformation (Brassington et al., 2003; Vanlerberghe et al., 2019). Missense variants in the *TBX5* gene have been previously described in reports of congenital heart disease associated with cardiac conditions other than HOS (Heinritz, Shou, Moschik, & Froster, 2005; Iwanicka-Pronicka, Socha, Jędrzejowska, Krajewska-Walasek, & Jamsheer, 2016; Lin et al., 2015; Yoshida et al., 2016). In particular, variants in *TBX5* have been linked to individuals exhibiting the cardiac phenotypes of dilated cardiomyopathy (DCM), atrial fibrillation (AF), and acute myocardial infarction (AMI) (Laforest et al., 2019; Lin et al., 2015; Patterson, Coats, & McGowan, 2020; S. Wang et al., 2019). In a comparable manner, our finding of a LQTS phenotype in an individual with a T223M variant in the *TBX5* gene further expands the disease phenotype associated with variants in *TBX5*.

Numerous studies have demonstrated a molecular link between *TBX5* and *SCN5A*, as multiple enhancers capable of regulating the ventricular conduction system expression associated with the *Scn5a/Scn10a* locus in mice have demonstrated regulation by Tbx5 (Arnolds et al., 2012; van den Boogaard et al., 2012). Our ChIP-seq analysis suggests wild-type Tbx5 binding both upstream and downstream of *Scn5a, Kcnh2*, and *Kcnq1*, supporting the possibility that impaired Tbx5 binding, such as in the presence of the T223M variant, may result in prolonged QT due to transcriptional alteration of known LQTS-associated loci (Arnolds et al., 2012). Moreover, the expression of NaV1.5 in addition to connexin 40 in the ventricular conduction system, both of which facilitate rapid cardiac conduction, is directly dependent on *TBX5* gene expression (Bruneau et al., 2001). Considering the pleomorphic role of *SCN5A*, its direct regulation by TBX5, and the variable expressivity of *SCN5A* in cardiac disease manifestation, it stands to reason that both genes have a ubiquitous and diverse role in the mechanism and manifestation of this case of LQTS associated with the T223M variant (Verkerk, Amin, & Remme, 2018).

Additionally, we identified a 57.5% reduction in the ability of TBX5-T223M to drive expression at the ANF promotor compared to wildtype TBX5 (P=0.005). Considering that *ANF* and *SCN5A* are downstream transcriptional targets of TBX5, a loss of function in TBX5 would appear to yield a loss of SCN5A expression (C. Wang, Cao, Wang, & Wang, 2011). However, loss-of-function mutations in *SCN5A* are traditionally associated with Brugada Syndrome, characterized by ST elevation on a resting ECG, specifically within the right precordial leads (Hedley et al., 2009). Therefore, the lack of alignment between the observed LQTS phenotype in the T223M proband compared to the Brugada Syndrome phenotype, maintaining that both are associated with a loss of function in *SCN5A* expression, suggests a complex mechanism associated with *TBX5* variants in producing the disease phenotype (Hedley et al., 2009).

The identification of TBX5-T223M in multiple families may implicate this locus as a disease-susceptibility allele that may have an informative role in the diagnostic test of LQTS (Brassington et al., 2003; Vanlerberghe et al., 2019). Inclusion of TBX5 on the LQTS gene panel should be considered only after additional independent studies corroborate this association. Of note, previously identified families hosting the TBX5-T223M variant do not appear to exhibit the LQTS phenotype (Brassington et al., 2003; Vanlerberghe et al., 2019). In addition, investigation into the mechanism of delayed repolarization of TBX5 disease-associated variants, as well as the basis for incomplete penetrance and the marked variability expressivity of disease is needed (Brassington et al., 2003; Vanlerberghe et al., 2019). While it is certainly possible that this locus may constitute a disease susceptibility locus, formal evaluation of this locus in larger disease- and population-based cohorts is needed to understand the prevalence of rare genetic variation that be tolerated physiologically (Brassington et al., 2003; Vanlerberghe et al., 2019).

Although this novel genetic cause of LQTS is a rare familial variant, our findings yield pertinent additional insight into the development of LQTS. While there was no overt evidence of cardiac conduction disease or ventricular hypertrophy, both causes of secondary QT prolongation, we cannot exclude the possibility that QT prolongation is secondary to other underlying pathology and not a primary electrophysiologic property of the myocardium. Further studies are needed to identify the precise mechanisms by which the T223M variant, or other variants within *TBX5*, are able to alter repolarization of the heart, demonstrated by a prolonged QT interval and prolonged PR interval. These may include studying the electrophysiological effect of the T223M variant in induced pluripotent stem cell-derived cardiomyocytes or examining gene expression utilizing neonatal rat cardiomyocytes. Therefore, additional studies are needed to confirm the role of TBX5-mediated transcriptional changes in arrhythmias including long QT. Despite these limitations, we report the novel identification of an individual heterozygous for a T223M variant in the *TBX5*-encoding gene who exhibits elongated cardiac repolarization, characteristic of LQTS, but who does not exhibit the characteristic skeletal abnormalities of HOS. A T223M variant within the *TBX5* gene has never before been implicated in cardiac repolarization abnormalities. Given previous reports of TBX5 variants associated with QT prolongation, these findings support the conclusion that TBX5 can be a long QT disease susceptibility allele.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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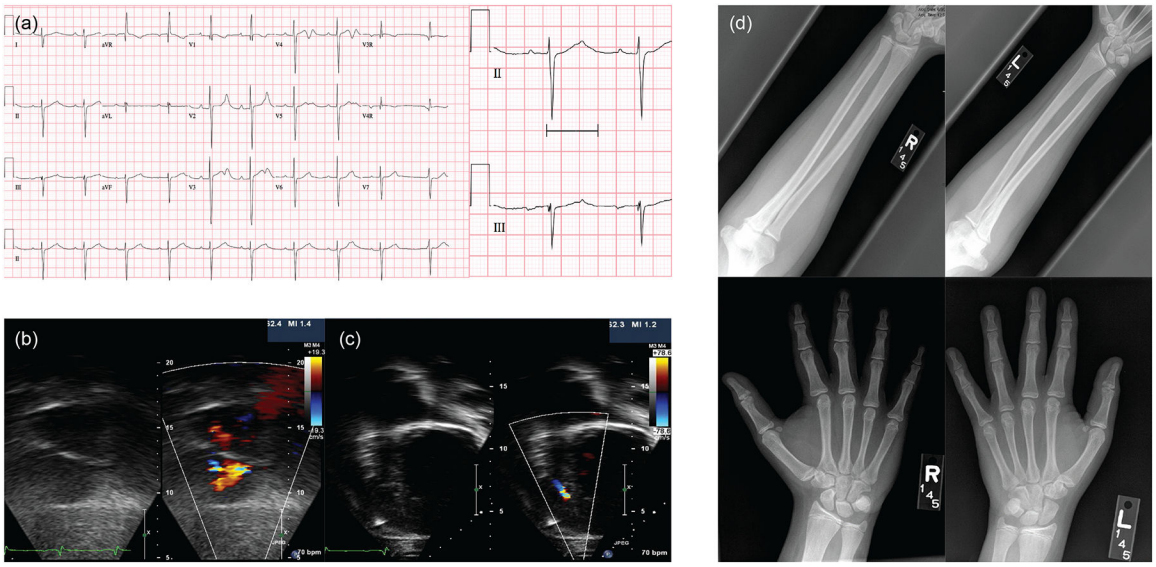

Figure 1. Clinical Evaluation of TBX5-T223M Proband

(A) Representative ECG obtained from the proband demonstrating QT/QTc prolongation, PR interval prolongation, and right ventricular hypertrophy. (B) Representative echocardiographic images with color flow Doppler in the subcostal view demonstrating the presence of a secundum atrial septal defect (ASD). (C) Representative echocardiographic images with color flow Doppler in the apical four chamber view demonstrating the presence of one of the two small muscular ventricular septal defects (VSDs) in the proband. (D) Representative X-ray images of the forelimb, wrist, and hand of the proband depicting normal bone structure.

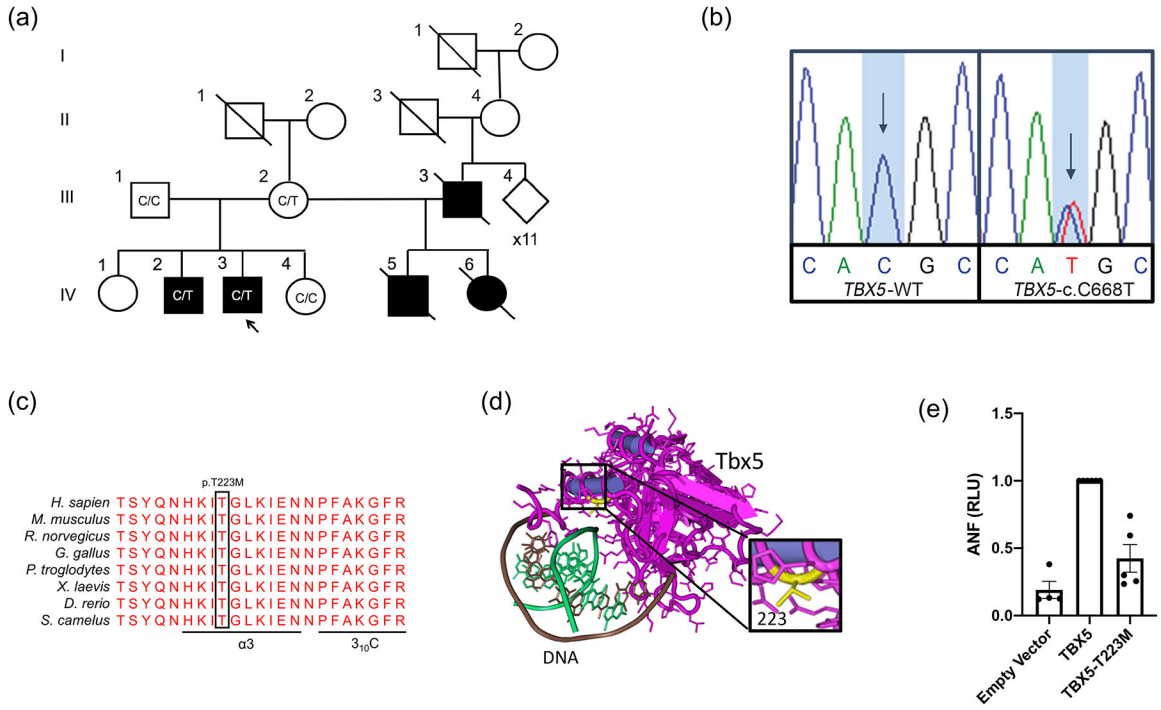

Figure 2. T223M Variant Presence in Pedigree, Site Conservation, and Variant Localization

(A) Pedigree of T223M proband and kindred. A total of four generations underwent clinical evaluation (denoted by roman numerals). Black fill denotes LQTS phenotype or a sudden death. Arrow denotes the proband (IV.3). C/C denotes wildtype, while C/T denotes heterozygosity for the T223M variant. (B) Sanger sequencing chromatogram of *TBX5* wildtype genotype and heterozygous variant *TBX5*-T223M genotype. (C) Amino acid sequence at residue 223 of TBX5 across divergent species demonstrating conservation. (D) Visual depiction of the T223M mutation localized to the DNA-binding domain of TBX5. (E) Bar graph of a luciferase assay quantifying ability of TBX5, TBX5-T223M, and empty vector to drive the ANF promoter. Each dot represents an experimental replicate comprised of a technical triplicate.