A unique triadin exon deletion causing a null phenotype

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Introduction

Triadin is a transmembrane protein located in the sarcoplasmic reticulum; it interacts with both ryanodine (RYR2) and calsequestrin (CASQ2) to facilitate calcium homeostasis in the human cardiac and skeletal muscle cells.[1] Pathogenic variants in the *RYR2* and *CASQ2* genes are more commonly associated with catecholaminergic polymorphic ventricular tachycardia (CPVT). Triadin is a more recently acknowledged protein, wherein genetic aberrations in triadin are responsible for a number of malignant arrhythmic syndromes, particularly in younger children.[2,3] These triadin mutations are recessively inherited, and they are associated with a prolonged QT interval and T-wave abnormalities; the term "triadin knockout syndrome" has been suggested by Altmann and colleagues.[2]

The *TRDN* gene localizes to chromosome 6, and is seen in a variety of isoforms including Trisk 32, which is expressed predominantly in cardiac muscle. These isoforms are generated through alternative splicing of the triadin gene. The protein is composed of a transmembrane domain with both cytosolic and luminal components and is 286 amino acids long. Knockout of this gene in murine cardiac muscle causes a loss of calcium regulation, impaired excitation contraction coupling, and cardiac arrhythmia, particularly during beta-adrenergic stimulation.[3–5]

KEY TEACHING POINTS

- Homozygous pathogenic *TRDN* gene mutations result in a severe phenotype with cardiac arrest often seen at a young age.
- Invariably, patients presenting with these mutations will require defibrillator placement.
- Medical therapy with beta-blockade and flecainide is also required owing to the high risk of appropriate shocks.
- Large deletions and duplications may be responsible for some of the cause of genetically elusive arrhythmia syndromes.

Case report

We present the case of an infant born to nonconsanguinous parents originating from Oman. His antenatal and postnatal course was uneventful. He had a history of glucose-6-phosphate dehydrogenase deficiency, was not on any medications, and was developmentally normal. He presented with an out-of-hospital cardiac arrest at 16 months of age, without any preceding warning. He received 18 minutes of cardiopulmonary resuscitation and following stabilization, he was transferred to a tertiary pediatric center for further management. His electrocardiogram demonstrated a prolonged rate-corrected QT (QTc: Bazett's correction) of 490 ms; it also demonstrated T-wave inversion in the anterior precordial leads (Figure 1A). Magnetic resonance imaging of the head demonstrated possible hypoxic changes in keeping with a cardiac arrest. He suffered 2 subsequent episodes of arrhythmia, 1 episode of ventricular fibrillation (VF) requiring cardiopulmonary resuscitation for 11 minutes, and torsades de pointes lasting 1 minute. His echocardiogram demonstrated a structurally normal heart. He had cardiac magnetic resonance imaging that demonstrated no clear evidence of cardiomyopathy.

He was subsequently fitted with an epicardial defibrillator (weight 9.5 kg). Medtronic (Minneapolis, MN) epicardial leads (4968; 25 cm) were placed on the atrium and ventricle, and a Medtronic endocardial shock lead (6935; 65 cm) was placed around the back of the heart and sutured into the pericardium. A Medtronic Evera XT DR implantable cardioverter-defibrillator was placed in an abdominal pocket in the right upper quadrant. The postoperative course was

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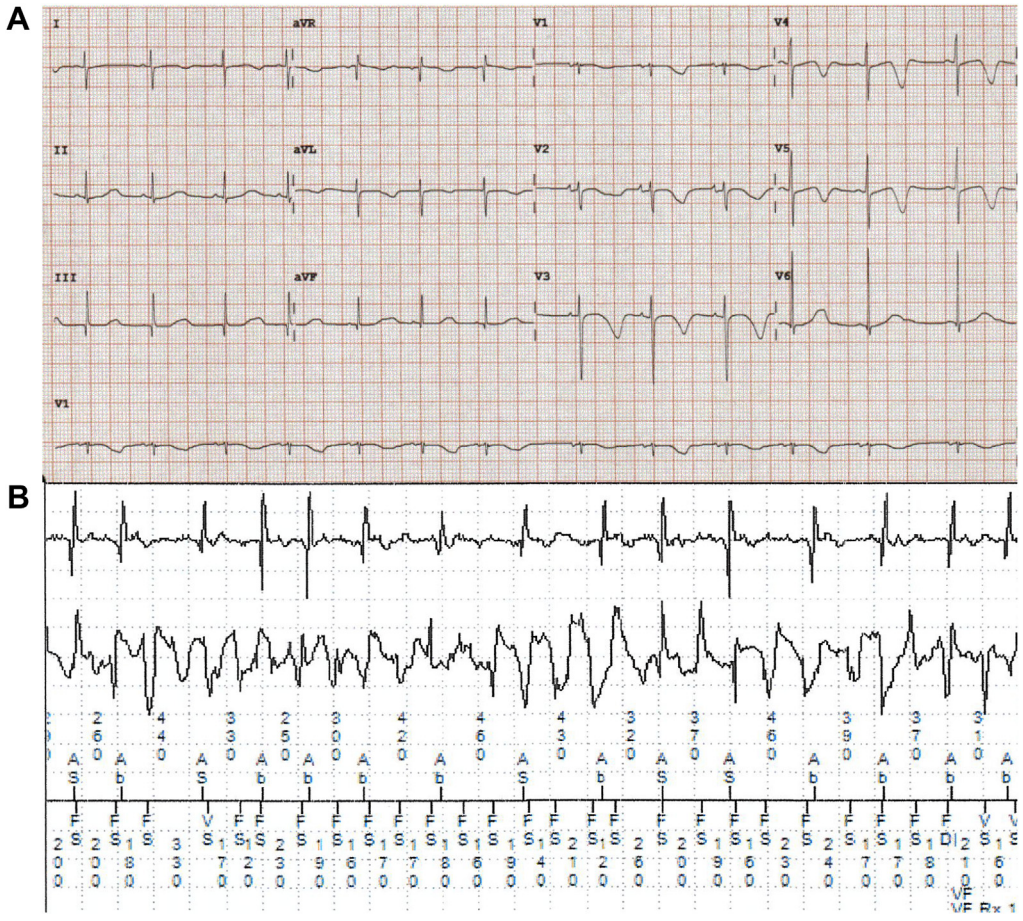

Figure 1 **A:** Electrocardiogram 6 days post cardiac arrest. There was a prolonged QTc interval of 490 ms and T-wave inversion in the anterior precordial leads from V_1 to V_5. **B:** Episode of ventricular fibrillation that was effectively converted by a single shock, 7 months postimplantation.

complicated by renal impairment, liver dysfunction, and electrolyte imbalance. The defibrillator administered 4 shocks in the immediate postoperative period for VF. A neurologic assessment prior to discharge demonstrated limb hypokinesia. He continues on nadolol 10 mg twice daily (BID) (1 mg/kg BID) and flecainide 10 mg BID (1 mg/kg BID). He had a single shock for VF 7 months after implantation (Figure 1B). This episode seemed to have been related to nonadherence with medications. The flecainide was increased (2 mg/kg BID) after this episode of VF.

Genetic analysis was performed using next-generation sequencing targeting 54 genes associated with cardiac arrhythmia using Agilent SureSelect, Custom Design sequenced on the Next Seq 2500 (Illumina, San Diego, CA). There was a change seen in the *RYR2* gene (Glu3783) that was of uncertain significance. It was a missense mutation, which was present in the asymptomatic father; predicted pathogenicity revealed mixed results. There was also a missense mutation in the *KCNE2* gene (c.170T>C, p.(Ile57Thr)), which has been previously described in long QT type 6. Copy number analysis revealed an apparently homozygous deletion of exon 2 of the *TRDN* gene. This was confirmed using multiplex ligation-dependent probe amplification analysis (Figure 2). Normal copy number was confirmed for flanking exons. Both parents were subsequently confirmed to be carriers of this deletion; both had a normal electrocardiogram and both had a normal stress test.

Discussion

We have described a case of a homozygous deletion of the *TRDN* gene, which has resulted in a more severe phenotype than those described previously.[2] The patient presented with VF at a young age and recurrence of these arrhythmias despite beta-blockade and flecainide. This deletion is predicted to have a profound effect on protein function, resulting in a triadin null phenotype. The classic features of VF, T-wave inversion, and muscle weakness are consistent with previous reports of triadin knockout syndrome.[6]

This deletion of exon 2 of the *TRDN* gene is predicted to remove amino acids 8–78. This section consists of the N-terminus and all of the transmembrane components (residues 48–68). The protein likely does not function normally in the membrane of the sarcoplasmic reticulum, and as a consequence of this, the C terminus region (KEKE repeat region) would be unavailable to interact with RYR2 and CASQ2 to form the calcium-regulating unit (Figure 3). This means that

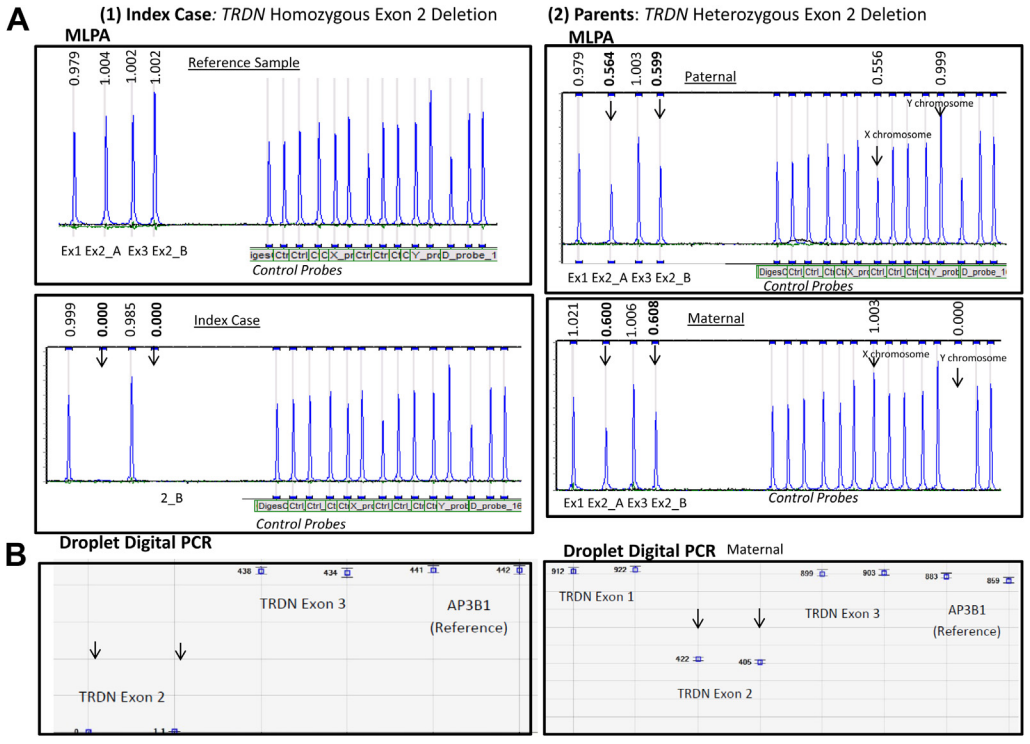

Figure 2 Quantitative analysis data showing the *TRDN* homozygous deletion of exon 2 in (1) the index case and heterozygous deletion in (2) both parents. **A:** Multiplex ligation-dependent probe amplification (MLPA) analysis using control probes targeting exons 1 and 3 in addition to standard control probes included within MLPA kit (P200). (1) The index case shows complete absence of 2 probes targeting different regions of TRDN exon 2. (2) Both parents have patient/reference ratios < 0.61 for both exon 2 probes, indicating a heterozygous deletion. **B:** Droplet digital polymerase chain reaction (ddPCR) analysis indicating absence of TRDN exon 2 in the index case and reduced signal in the maternal sample confirming heterozygous state.

the protein is essentially nonfunctional, resulting in a triadin null phenotype.[4,7] It is the large deletion of the triadin protein that makes this particular mutation novel. It is possible that deletions and copy number variations make up some inherited arrhythmias that remain genetically elusive.[8]

The term "triadin knockout syndrome" was used by Altmann and colleagues,[2] in a series of genotypically negative long QT patients. They described a group of 5 patients that all presented with a cardiac arrest at less than 3 years of age. The salient features of the syndrome were T-wave inversion in the anterior precordial leads, QT prolongation, and VF at a young age. Muscle weakness was also described in some patients. The presence of T-wave inversion in the anterior precordial leads in our patient is consistent with this part of the syndrome. Some of the patients were also described as having skeletal muscle weakness; this was seen in our patient. Other families with triadin mutations have been described by Roux-Buisson and colleagues[3]: in one, a subject with a homozygous mutation (TRDN c.53_56delACAG) died at 3 years of age following a cardiac arrest.[3] The other family had a compound heterozygous mutation (c.176C.G: a missense mutation), a substitution mutation (59 (p.T59R)), and a nonsense mutation (c.613C.T). We have also previously reported siblings with compound heterozygous triadin mutations, 1 novel and 1 as described above (TRDN c.53_56delACAG).[9]

The deletion described by Roux-Buisson and colleagues[3] and by Walsh and colleagues[9] (TRDN c.53_56delACAG), also on exon 2, is a frameshift mutation (p.D18Afs*13). It results in a premature stop codon at residue 31; this is before the bridging section of the transmembrane protein. Altmann and colleagues saw this particular mutation in homozygous form in 1 of their patients. In the 2 previously described homozygous premature stop codon mutations, cardiac arrests occurred at age 2 in the case described by Roux-Buisson and colleagues and at age 3 in the case described by Altmann and associates.[2] Given the homozygous status of the premature stop codon on residue 31 of these specific mutations, they would also be predicted to function as a null phenotype.

It is possible that our more severe phenotype is owing to chance variation; however, it is also possible that a parentally inherited novel RYR2 missense variant, c.11347G>C, p.(Glu3783Gln), has caused an increased vulnerability to arrhythmia, causing the patient to present at a younger age (ie, digenic compound heterozygous). The Ile57Thr KCNE2 mutation reduces repolarizing potassium current (I_{kr}) in vitro, and has been reported to be associated with long QT in the absence of VF or torsades de pointes.[10,11] This makes it unlikely to be the cause of the severe phenotype seen in our patient; however, it may have contributed toward the QT prolongation. Given the rarity of this *TRDN* gene deletion, it is likely that the parents shared

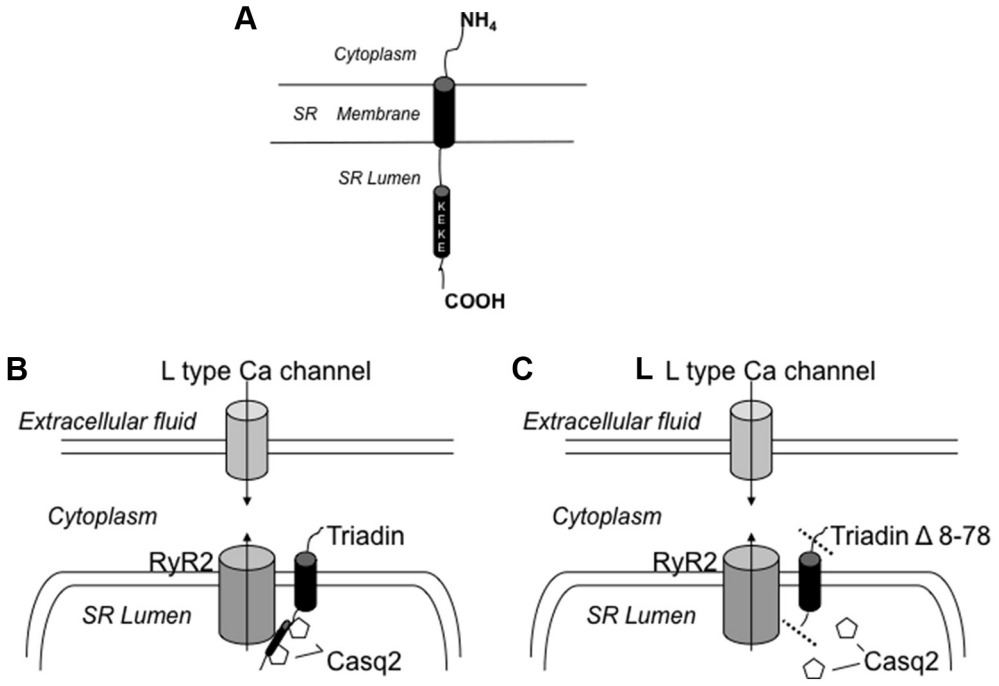

Figure 3 **A, B:** Normal functioning of the *TRDN* gene with the KEKE component close to the C-terminus, which interacts with CASQ2 and RYR2. **C:** Dashed lines show that components of *TRDN* missing in our patient, which will result in the inability of the protein to interact with CASQ2 and RYR2, and inability to anchor in the membrane of the sarcoplasmic reticulum (SR).[14]

a common ancestor; we were not, however, able to confirm this with the current genetic information.

We did deliberate extensively about placing a defibrillator in our patient, as he was returning to Oman, where they would reside permanently. After consulting with cardiologists in the patient's country of origin, we were reassured that adequate follow-up and support was available. The subsequent cardiac arrests underline the importance of placing defibrillators even in smaller patients who have clinically apparent triadin mutations.

The effectiveness of flecainide has been described previously in both CPVT and triadin mutations.[7] There is debate as to the mechanism of action of flecainide, with some reports suggesting it works via a direct effect on the ryanodine channel. Recent reports have suggested that flecainide works by altering Na^+-dependent modulation of intracellular Ca^{2+} handling.[12] Flecainide may also act by binding cytosolic proteins that modulate RYR2, such as calmodulin. Further work is needed to determine the precise mechanism of action for flecainide.[13]

Conclusion

We have described a homozygous intragenic *TRDN* gene deletion that is predicted to produce a nonfunctioning triadin protein. The case demonstrated all of the salient features of the "triadin knockout syndrome," which reinforces the phenotype. The infant presented at a very young age, and an epicardial defibrillator was placed and delivered appropriate shocks on 2 separate occasions. Patients who are genetically confirmed to have biallelic pathogenic triadin variants should be treated aggressively with antiarrhythmic medications and strong consideration should be given to defibrillator placement.

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