

## **Timothy Syndrome 1.1: A Timothy Syndrome 1 Genotype without Syndactyly and Major Extracardiac Manifestations**

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**ABSTRACT**

Timothy syndrome 1 (TS1) is a rare genetic disorder characterized by multisystem abnormalities including QT prolongation, congenital heart defects, facial dysmorphism, episodic hypoglycemia and neurological symptoms. A morphological hallmark of TS1 is syndactyly, present in all cases. TS1 is caused by the canonical p.Gly406Arg mutation in the alternatively spliced exon 8A in the *CACNA1C* gene, encoding for the main cardiac L-type calcium channel. A variant case of TS1 is reported. The proband had intermittent fetal bradycardia with heart rate of 72 bpm. On the first day of life bradycardia due to 2:1 atrioventricular (AV) block and marked QTc prolongation of 600 ms was noted. On medical therapy with propranolol and mexiletine 1:1 AV conduction returned with QTc prolongation of 470-580 ms. The patient lacked other extracardiac manifestations, most importantly syndactyly, neurological complications or autism. On genetic analysis, the canonical TS1 causing mutation, p.Gly406Arg in exon 8A of the *CACNA1C* gene was detected. The *CACNA1C* p.Gly406Arg variant was not present in the parents, but was detected in the buccal DNA sample of the index patient. Our case highlights further phenotypic variability in TS. Most importantly, it underlines that the lack of syndactyly does not exclude the presence of a TS1 genotype.

**KEY WORDS**

Timothy syndrome

*CACNA1C* gene

AV block

QTc prolongation

syndactyly

## INTRODUCTION

Timothy syndrome (TS) is a very rare genetic disorder which is characterized by numerous multisystem abnormalities leading to clinically two distinct forms of the syndrome (reviewed in [Liao and Soong 2010]). Timothy syndrome 1 (TS1) is characterized by QT prolongation, congenital heart defects (including patent ductus arteriosus, patent foramen ovale, and ventricular septal defect), facial dysmorphisms (including a flat nasal bridge, small upper jaw, low-set ears, or small and misplaced teeth), episodic hypoglycemia and neurologic symptoms including developmental delays, autism, seizures, and intellectual disability [Splawski et al. 2004]. A morphological hallmark of TS1 is syndactyly, which is reported to be present in 100% of the cases (*Table 1*) [An et al. 2013; Corona-Rivera et al. 2015; Dufendach et al. 2013; Ergul et al. 2015; Etheridge et al. 2011; Krause et al. 2011; Lo et al. 2005; Splawski et al. 2004]. Timothy syndrome 2 (TS2) patients have no syndactyly but carry the other multisystem manifestations of the disease [Diep and Seaver 2015; Frohler et al. 2014; Hiippala et al. 2015; Philipp and Rodriguez 2016; Splawski et al. 2005]. Because of extreme QT prolongation, individuals with TS can experience ventricular tachycardia/fibrillation (VT/VF) and cardiac arrest, and the overall complications of multisystem abnormalities often lead to early death.

The predominant genetic cause of TS1, identified in 2004 [Splawski et al. 2004], was a recurrent, canonical ‘*de novo*’ heterozygous missense mutation, p.Gly406Arg, in the alternatively spliced exon 8A in the *CACNA1C* gene, encoding for the main cardiac L-type calcium channel, Ca<sub>v</sub>1.2. In cases with TS2, 2 additional mutations, p.Gly406Arg and p.Gly402Ser, were identified in exon 8 of the *CACNA1C* gene [Splawski et al. 2005]. Exons 8 and 8A of the *CACNA1C* gene undergo alternative splicing in a mutually exclusive manner, with exon 8 being the predominantly expressed isoform. Recently some other mutations, all

affecting the *CACNA1C* gene, were reported in TS patients [Boczek et al. 2015; Gillis et al. 2012; Wemhoner et al. 2015].

Here we report a variant case of Timothy syndrome, named Timothy syndrome 1.1, manifested as neonatal 2:1 AV block and marked QT prolongation. Genetic analysis revealed a *CACNA1C* gene exon 8A p.Gly406Arg mutation, thereby defining the case as Timothy syndrome 1 on genetic grounds. However, the patient lacks many of the other hallmarks of TS1, most notably syndactyly, and also other major extracardiac manifestations.

## **PATIENT AND METHODS**

### **Clinical report**

The proband was born as a second child to a healthy 31-year-old woman and 31-year-old man, both of Caucasian descent. There was no report of consanguinity, and the family histories were non-contributory. Especially, family history was negative for premature sudden cardiac death, arrhythmic disorders, syndactyly, facial dysmorphism or autism. The pregnancy was uncomplicated. Intermittent fetal bradycardia (heart rate of 72 bpm) was noted at 37 weeks gestation. The baby was born via normal uncomplicated vaginal delivery at 38 weeks gestation. Apgar scores were 9 and 10 at one and 5 minutes, respectively. The baby's birth weight was 2950 g (18th centile), length was 54 cm (98th centile), and head circumference measured 34 cm (35th centile). On the second day of life cardiac evaluation revealed bradycardia due to 2:1 atrioventricular (AV) conduction and marked QTc-interval prolongation of 600 ms (*Figure 1, Panel A*). On medical therapy (propranolol and mexiletine) QTc duration stabilized in the range of 470-580 ms; 2:1 AV conduction resolved, and there was consistent 1:1 AV conduction with T-wave alternans (*Figure 1, Panel B*). Initial imaging with echocardiography demonstrated a patent foramen ovale, with a diameter of 2.5 mm with left-to-right shunt, and a mildly dilated right ventricle. The Botall duct was closed and there were no signs of ventricular septal defect, cardiomyopathy or ventricular dysfunction.

Laboratory findings did not reveal hypoglycemia or hypocalcaemia. The patient did not have facial dysmorphisms (hypertelorism, flattened nasal bridge, prominent forehead, etc.) and was not bald. He did not have joint hypermobility. Most importantly no syndactyly, either on the hands or on the feet, were present (*Figure 1, Panel C and D*). No hypoplastic teeth and decay was noted. During his first three years of life he has made steady developmental progress. He sat unassisted at age 7.5 months, crawled at age 8 months, walked with support at age 8.5 months and walked independently at age 12 months. First words were at age 14 months. At age 2 years an M-CHAT (Modified Checklist for Autism in Toddlers) test was done which did not indicate autism or autism spectrum disorder. He does not have frequent or serious infections. As the patient's father is a board certified cardiologist, he is under continuous bedside ECG monitoring during nights with frequent traditional Holter monitoring performed in every 3-6 month. Only at one occasion a 2:1 AV conduction was detected for 12 hours, without symptoms, but no sustained or non-sustained ventricular tachyarrhythmias were ever encountered.

At age 2 years he was hospitalized because of an episode of severe hypoglycemia (blood glucose level 1.4 mmol/l) associated with convulsions. As head trauma could not be excluded, a cranial CT was performed under general anesthesia. No VT or VF was observed under medication with propofol, midazolam, fentanyl and muscle relaxants. The only observed rhythm disturbance was an intermittent 2:1 AV block which returned to normal conduction after the correction of serum glucose levels. The episode resolved without any further consequence. Glucose monitoring with tissue glucose sensor did not reveal any further episode of hypoglycemia in the subsequent days. At present, at age 3 years, his mental and physical development seems to be undisturbed. He is in a good general health and attends kindergarten without assistance. He is able to express feelings, understands jokes and sadness.

### **Molecular genetic analysis**

The investigations were approved by the Institutional Ethics Committee of the University of Szeged, and conducted according to the Helsinki declaration. Family members and the patient's care-givers gave prior written informed consent to the molecular genetic analysis. Coding sequences and exon-intron boundaries of all 13 causative long QT genes were analyzed by next-generation sequencing using Agilent's SureSelect technology with custom-designed 120-mer RNA baits specific to target region (Agilent Technologies, Santa Clara, CA, United States). Targeted resequencing was carried out on a SOLiD 5500xl System (Life Technologies, Grand Island, NY, United States). Variants, identified by targeted resequencing, were validated by standard capillary sequencing using custom-designed primers. Exon 37 of *ANK2* gene, exon 8A of *CACNA1C* gene, and exon 1 of *KCNQ1* gene were direct-sequenced on a ABI Prism 310 Genetic Analyzer (Applied Biosystems by Life Technologies, Grand Island, NY, United States). Genetic mosaicism was assessed in the index patient and both parents using DNA extracted from buccal cells and from sperm, in case of father.

Mapping of the SOLiD reads were accomplished by Genomic Workbench ver 7.0.3 (CLC Bio, now part of Qiagen) using the human genome assembly hg19 as reference sequence. Variant calling and variant annotation were performed by the same software. The functional impact of amino acid changes caused by missense mutations was predicted by SIFT and PROVEAN programs. Nucleotide and amino acid changes are reported according to the Ensembl database (release: 85), based on reference sequences ENST00000155840.9 for *KCNQ1*, ENST00000399591.5 for *CACNA1C* and ENST00000264366.10 for *ANK2*.

## RESULTS

### Mutation data

Three different heterozygous genetic variants in three genes have been detected using targeted resequencing by next-generation sequencing: p.Gly406Arg (c.1216G>A, rs79891110) in exon

8A of the *CACNA1C* gene (*Figure 2*); p.Tyr94Cys (c.281A>G, rs781717051) in exon 1 of the *KCNQ1* gene; and p.Ile3252Thr (c.9755T>C, rs36210417) in exon 37 of the *ANK2* gene. All three variants were previously reported. The *KCNQ1* p.Tyr94Cys variant was predicted to be deleterious by PROVEAN and to be tolerant by SIFT. The *ANK2* p.Ile3252Thr variant was predicted to be neutral/tolerant by both prediction methods. The identified *CACNA1C* gene exon 8A p.Gly406Arg variant is the canonical Timothy syndrome 1 causing mutation. Although sequence homology is high between exons 8 and 8A of the *CACNA1C* gene, the presence of several “exon specific” nucleotides made possible to distinguish between the two (*Figure 2*). Sequence comparison with all available published sequences of *CACNA1C* exon 8 and 8A in previous reports proved that our patient indeed carried the p.Gly406Arg variant affecting exon 8A of the *CACNA1C* gene. The *CACNA1C* p.Gly406Arg variant was also present in the DNA sample of the index patient extracted from buccal cells. The height of the mutant nucleotide peak was similar in both samples and were equal to the normal nucleotide peak (*Figure 2*).

### **Family screening**

First degree family members of the index patient (mother, father and sister) underwent genetic screening for all three variants. The proband’s father was proved to be carrier of the *KCNQ1* p.Tyr94Cys variant, while mother and sister were non-carriers for both the *KCNQ1* and *ANK2* variants. Neither the parents nor the sister of the index patient carried the *CACNA1C* p.Gly406Arg variant (*Figure 2*), therefore this variant must have arisen ‘*de novo*’ (paternity was proven). The *KCNQ1* p.Tyr94Cys variant-carrying father has no signs or symptoms of long QT syndrome and has a completely normal ECG and echocardiogram (data not shown), suggesting that the *KCNQ1* p.Tyr94Cys variant has no major influence on the disease phenotype.



In order to assess whether the *CACNA1C* Gly406Arg mutation is indeed arisen ‘*de novo*’ in the family, or one of the parents has mosaicism for the mutation, we performed genetic analysis of the DNA extracted from buccal cells (in case of both parents) and sperm (in case of father). The *CACNA1C* Gly406Arg variant was not present in any sample extracted from different tissues, therefore no proof for genetic mosaicism was found (data not shown).

## DISCUSSION

Here we report a variant case of Timothy syndrome, referred to as Timothy syndrome 1.1, caused by a TS1-specific *CACNA1C* exon 8A p.Gly406Arg mutation. The clinical phenotype was characterized by neonatal 2:1 AV block and marked QT prolongation. However, the patient lacked many of the other hallmarks of TS1, most notably syndactyly, and no other major extracardiac manifestations were present.

Syndactyly is reported in 100% of patients with *CACNA1C* exon 8A p.Gly406Arg mutation (*Table 1*). Moreover, syndactyly was also present in patients proved to be somatic or germline mosaic carriers of the mutation [Dufendach et al. 2013; Etheridge et al. 2011]. In one of these cases the direct presence of the *CACNA1C* exon 8A p.Gly406Arg mutation was demonstrated in left/right arm skin biopsy samples with an estimated percent mosaicism of ~6.5% [Dufendach et al. 2013]. As Ca<sub>v</sub>1.2 was highly expressed in apical ectodermal ridge cells of developing digits, syndactyly is thought to be caused in TS by Ca<sup>2+</sup>-induced cell death in the apical ectodermal ridge [Splawski et al. 2004].

The most probable explanation for the lack of syndactyly and major extracardiac manifestations in our case is the presence of somatic mosaicism in our patient. The mutant Ca<sub>v</sub>1.2 channel must be absent from unaffected organs (limbs, brain) and might be present in low levels in probably affected organs (heart, pancreas) in our patient. However, as we had only two sources of tissue (lymphocytes, buccal cells), and both were shown to harbor the mutation, direct evidence for the above explanation is lacking.

Reports on mosaic patients with TS1 describe a partial phenotype of the disease, similar to ours. These patients are usually parents of a more severely affected proband with genetically confirmed mosaicism with the potential to transmit the TS1-causative mutation to their offspring, resulting in fully expressive TS1. Dufendach et al reported a mosaic female patient who had right-sided syndactyly and was the mother of a newborn boy who presented with syndactyly, extreme QT prolongation and 2:1 atrioventricular block during syndactyly-corrective surgery [Dufendach et al. 2013]. The mother didn't have evidence of QT prolongation or other TS1 manifestation. In a report by Etheridge et al. two TS1 mosaic patients were described [Etheridge et al. 2011]. One was the father of a patient with TS1 in whom mosaicism was detected. The father had complete cutaneous syndactyly of the right and left feet, but had no other dysmorphic features. He was cognitively normal and never had syncope or seizure. ECG demonstrated a prolonged QTc of 480 ms. The other patient was a 14-year-old girl who had a cardiac arrest with documented ventricular fibrillation that occurred while making a phone call. She had a prolonged QTc interval of 560 ms and bilateral syndactyly involving the hands and the feet, but no other manifestation of TS1. The possible presence of somatic mosaicism was also reported with regard to TS2 [Hiippala et al. 2015; Splawski et al. 2005].

In conclusion, we described a Timothy syndrome 1 genotype without syndactyly and major extracardiac manifestations. The case highlight further phenotypic variability in Timothy syndrome. Most importantly, it underlines that the lack of syndactyly doesn't exclude the presence of Timothy syndrome 1 genotype.

## **ACKNOWLEDGEMENTS**

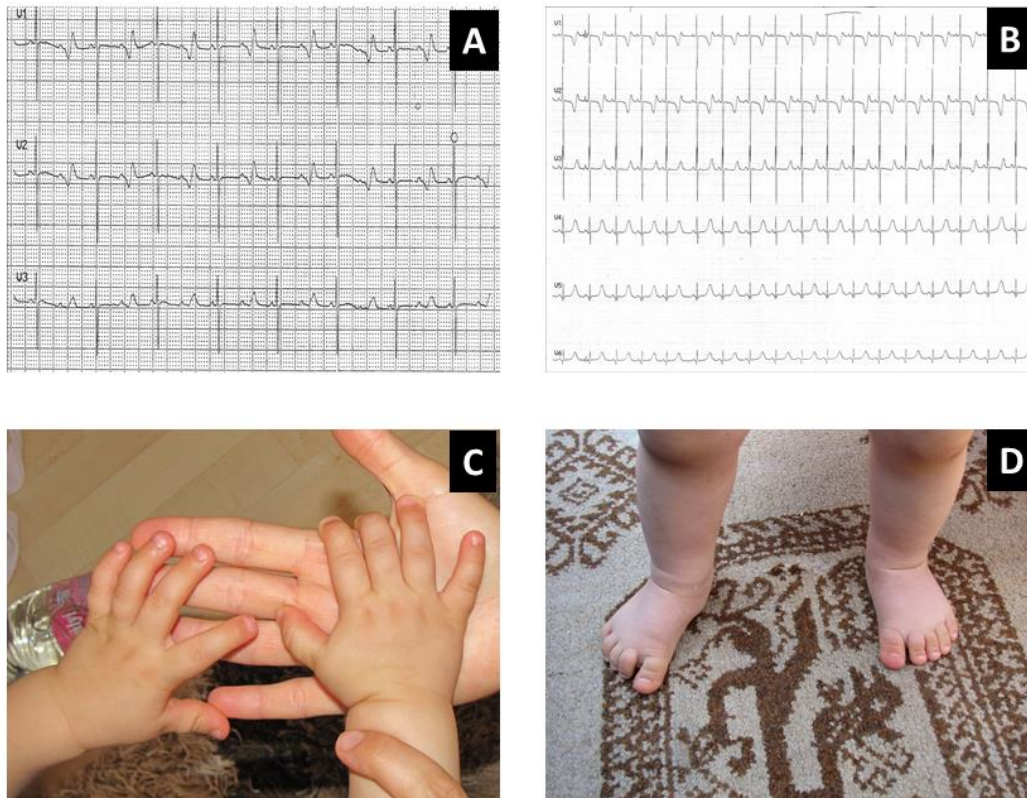
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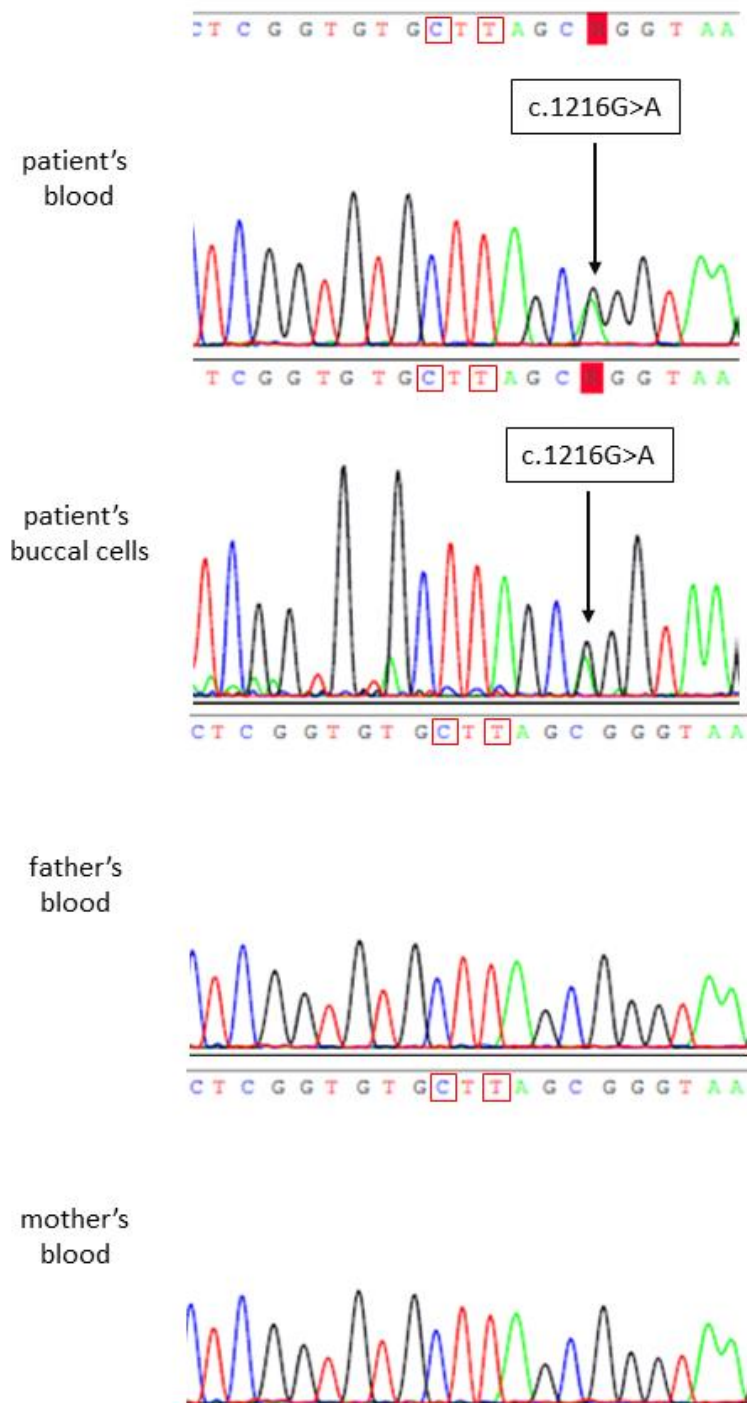
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**Figure 1.** 12-lead ECG of the proband illustrating 2:1 AV block and marked QTc-interval prolongation of 600 ms at a heart rate of 72 bpm (Panel A). On medical therapy (propranolol and mexiletine) 2:1 AV conduction resolved, and there is a consistent 1:1 AV conduction with QTc prolongation of 516 ms with T-wave alternans at a heart rate of 123 bpm (Panel B). Paper speed 25 mm/s, calibration 10 mm/mV. Panel C and D: Photograph of the proband's hands and feet, at age 12 months, demonstrating no syndactyly of the fingers or toes.



**Figure 2.** Sequence analysis of exon 8A of the *CACNA1C* gene illustrating the c.1216G>A mutation. The mutation changes the GGA triplet at codon 406, encoding for glycine, to AGA, encoding for arginine (p.Gly406Arg). Sequencing result is shown from DNA isolated from

blood (patient, father, mother) and from buccal cells (patient). Testing of parents suggested that the mutation is *'de novo'*. Nucleotides framed in red are unique for exon 8A when compared to homologous exon 8.



Table 1. Clinical and genetic characteristics of Timothy syndrome patients with proven genetic diagnosis reported in the literature.

Report	reported pts. (n)	TS type	affected <i>CACNA1C</i> exon	<i>CACNA1C</i> mutation	QT interval and arrhythmia	additional cardiac phenotype	main additional symptoms
Splawski et al, 2004 <sup>2</sup>	13	TS1	8A	Gly406Arg	QT prolongation (100%), VT (71%), bradycardia, AV block (94%)	PDA (59%), PFO (29%), VSD (18%), cardiomegaly (35%)	autism/autism spectrum disorder (60-80%), bald at birth (100%), small teeth (100%), recurrent infections (43%), facial dysmorphism (53%)
Lo-A-Njoe et al, 2005 <sup>9</sup>	2	TS1	8A	Gly406Arg	QT prolongation, bradycardia, AV block, VT	HCM, PDA, HF	fetal hydrops
Krause et al, 2011 <sup>8</sup>	1	TS1	8A	Gly406Arg	QT prolongation, bradycardia, AV block, VT	HCM, VSD, PDA	lower set ears and bald head
Etheridge et al, 2011 <sup>7</sup>	2	TS1	8A	Gly406Arg	QT prolongation, bradycardia, AV block, VT	VSD	widely spaced eyes, broad forehead, wide nasal bridge, broad nasal tip
Dufendach et al, 2013 <sup>5</sup>	1	TS1	8A	Gly406Arg	QT prolongation, bradycardia, AV block	none	
An et al, 2013 <sup>3</sup>	1	TS1	8A (9)*	Gly406Arg	QT prolongation, bradycardia, AV block, CA	none	round face, small upper jaw and teeth, nearly bald head
Corona-Rivera et al, 2015 <sup>4</sup>	1	TS1	8A	Gly406Arg	QT prolongation, AV block	PDA, PFO	fetal hydrops
Ergül et al, 2015 <sup>6</sup>	1	TS1	8A	Gly406Arg	QT prolongation, bradycardia, AV block, CA	PDA, PFO	neuro-motor development retardation, facial dysmorphism
Splawski et al, 2005 <sup>14</sup>	1	TS2	8	Gly406Arg	QT prolongation, bradycardia, AV block, VT, CA	HCM, biventricular dysfunction	mental retardation, nemaline myopathy, facial dysmorphism, seizures
Splawski et al, 2005 <sup>14</sup>	1	TS2	8	Gly402Ser	QT prolongation, VT, CA	HCM	facial dysmorphism, recurrent infections
Fröhler et al, 2014 <sup>11</sup>	2	TS2	8	Gly402Ser	QT prolongation, VT, CA	none	no

Hiippala et al, 2014 <sup>12</sup>	1	TS2	8	Gly402Ser	QT prolongation (mild), CA	none	no
Diep et al, 2015 <sup>10</sup>	1	TS2	8	Gly406Arg	QT prolongation, AV block, VT	PDA, PFO	hypotension, metabolic acidosis, recurrent apnea, seizure-like activity, poor growth, facial dysmorphism
Philipp et al, 2016 <sup>13</sup>	1	TS2	8	Gly406Arg	QT prolongation, AV block, VT, CA	PDA, biventricular systolic dysfunction	intermittent hypoglycemia, hypocalcemia
Gillis et al, 2012 <sup>17</sup>	1	TS	38	Ala1473Gly	QT prolongation, AV block, VT	PDA, PFO	facial dysmorphism, contractures, episodes of hypoglycemia, hypocalcemia, seizures, cortical blindness, developmental delay
Boczek et al, 2015 <sup>16</sup>	1	TS	27	Ile1166Thr	QT prolongation, AV block	PDA, biventricular dysfunction	seizures, cerebral and cerebellar atrophy, facial dysmorphism, clinodactyly, respiratory failure
Wemhöner et al, 2015 <sup>18</sup>	1	TS	27	Ile1166Thr	QT prolongation, CA	PDA, HCM	developmental delay

TS: Timothy syndrome; PDA: patent ductus arteriosus; PFO: patent foramen ovale; VSD: ventricular septal defect; HCM: hypertrophic cardiomyopathy; VT: ventricular tachycardia; AV: atrio-ventricular; CA: cardiac arrest

\*reported as exon 9, but based on direct sequence comparison it corresponds to exon 8A