Minireview *Highlight article*

Dysfunctional Cav1.2 channel in Timothy syndrome, from cell to bedside

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Impact statement

The knowledge of Timothy syndrome (TS) caused by dysfunctional Cav1.2 channel due to CACNA1C mutations is rapidly evolving as novel technologies of electrophysiology are introduced and our understanding of the mechanisms of TS develops. In this review, we focus on the TS-related dysfunctional Cav1.2 and the underlying mechanisms. We update TS-related CACNA1C mutations in a precise way over the past 20 years and summarize all reported TS patients based on their clinical presentations and molecular mechanisms, respectively. We hope this review will provide a new comprehensive way to better understand the electrophysiological mechanisms underlying TS from cell to bedside, promoting the management of TS in practice.

Abstract

Timothy syndrome is a rare disorder caused by CACNA1C gene mutations and characterized by multi-organ system dysfunctions, including ventricular arrhythmias, syndactyly, dysmorphic facial features, intermittent hypoglycemia, immunodeficiency, developmental delay, and autism. Because of the low morbidity and high mortality at a young age, it remains a huge challenge to establish a diagnosis and treatment system to manage Timothy syndrome patients. Here, we aim to provide a detailed review of Timothy syndrome, discuss the mechanisms underlying dysfunctional Cav1.2 due to CACNA1C mutations, and provide some new emerging evidences in treating Timothy syndrome from cell to bedside, promoting the management of this rare disease.

Keywords: Timothy syndrome, CACNA1C gene, Cav1.2, mexiletine, cellar mechanism, disease model

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Introduction

Timothy syndrome (TS), also known as long QT syndrome 8 (LQTS8), is an unusual autosomal dominant genetic syndrome. It is caused by mutations of CACNA1C gene encoding the L-type calcium (Cav1.2) channel. A glycine substituted by arginine at position 406 (G406R) localized to DI/S6 segment in close proximity to I-II loop of exon 8A generates TS type 1 (TS1). The G406R mutation together with another substitution of glycine to serine at position 402 (G402S) of exon 8 produce a mutant of TS type 2 (TS2).[1] TS is featured by multi-organ system dysfunctions, including ventricular arrhythmias (VA), syndactyly, dysmorphic facial features, intermittent hypoglycemia, immunodeficiency, developmental delay, and autism (Figure 1). Because of obviously delayed ventricular repolarization, TS patients usually manifest with functional 2:1 atrioventricular block (AVB), T wave alternans (TWAs), and other malignant arrhythmias (Figure 2).[3]Average age of death in TS is 2.5 years, the morbidity is equally extremely low, and therefore it remains a huge challenge to establish a treatment system to manage TS patients. Here, we aim to provide a detailed review of TS, discuss the mechanisms underlying dysfunctional Cav1.2 due to CACNA1C mutations, and provide some new emerging evidences in treating TS, promoting the management of this rare disease.

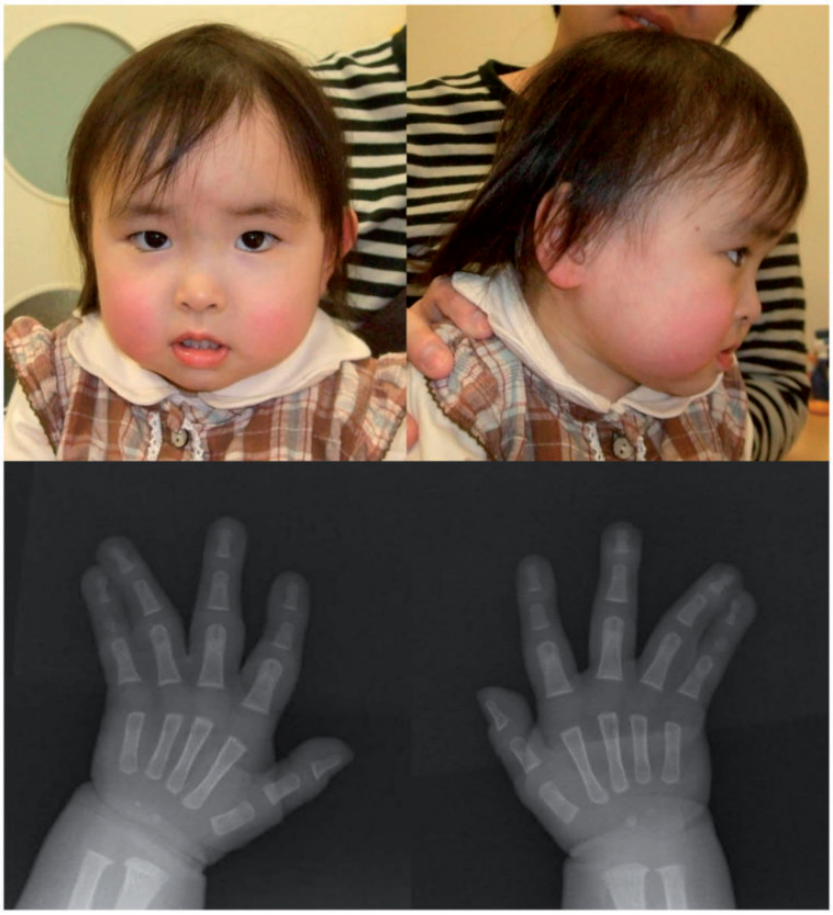

Figure 1. Clinical features of a three-year-old Japanese TS1 patient. Facial features: round face, frontal bossing, low set ears, a depressed nasal bridge, a thin upper lip, and thin scalp hair (a). Bilateral cutaneous syndactyly of four to five fingers (b). (Obtained from an open access article by Kosaki *et al.*[2]). (A color version of this figure is available in the online journal.)

History and epidemiology

TS was firstly reported by Reichenbach *et al.*[5] in 1992 as a male infant manifested with bilateral syndactyly of hands and feet as well as intrauterine bradycardia secondary to functional AVB resulted from significantly prolonged QT interval. Synostoses of the carpus were detected in his father, suggesting somatic mosaicism. The patient suddenly suffered cardiac arrest and died at five-month age. The author regarded this complex phenotype as a novel clinical entity named "heart and hand syndrome." The second case series was reported by Marks *et al.*[6,7] in 1995 that three female children were diagnosed as LQTS, AVB, and syndactyly without any affected relatives. He assumed the inheritance mode might be autosomal recessive or a *de novo* mutation. Until 2004, Splawski *et al.*[2] identified the first calcium channel mutation in this arrhythmic syndrome. He described a sporadic heterozygous mutation, G406R, in the alternatively spliced exon 8A of CACNA1C gene in 17 subjects as the cause for this multisystem disorder, namely TS. These patients were characterized by an invariant LQTS and syndactyly, as well as variable penetration of phenotypes such as autism spectrum disorders (ASD), craniofacial abnormalities, and hypoglycemia. A closely following report[1] described an analogous *de novo* G406R mutation in exon 8 of CACNA1C and another *de novo* mutation G402S also in this position in two unrelated individuals, respectively. Compared to the original TS cohort (TS1), these new patients (TS2) experienced severe arrhythmias along with an overlapping but distinct set of extra-cardiac phenotypes. Thus, mutations of CACNA1C may cause severe cardiac arrhythmias that limit survival and therefore the overall prevalence of these mutations. To date, there have been at least 60 typical or atypical TS patients, whose information are summarized in the Supplemental Table 1.[8–18] Six TS cases were followed over 24 years, suggesting that longer term survival of TS is possible; nevertheless, patients always require implantable cardioverter defibrillator (ICD) or pacemaker.[19] The most recent study of TS outcomes was conducted in 17 TS patients, whose rate corrected QT interval (QTc) was averagely 640 ms, mean longevity was 4.9 years old. β-blockers were used in all patients, ICDs were implanted in 13 patients. There were four patients suddenly died due to ventricular fibrillation (VF), two associated with anesthesia and two with hypoglycemia.[20]

Clinical manifestations

Cardiac phenotypes

TS is a physiological and developmental syndrome complex affecting multiple organ systems including the heart,

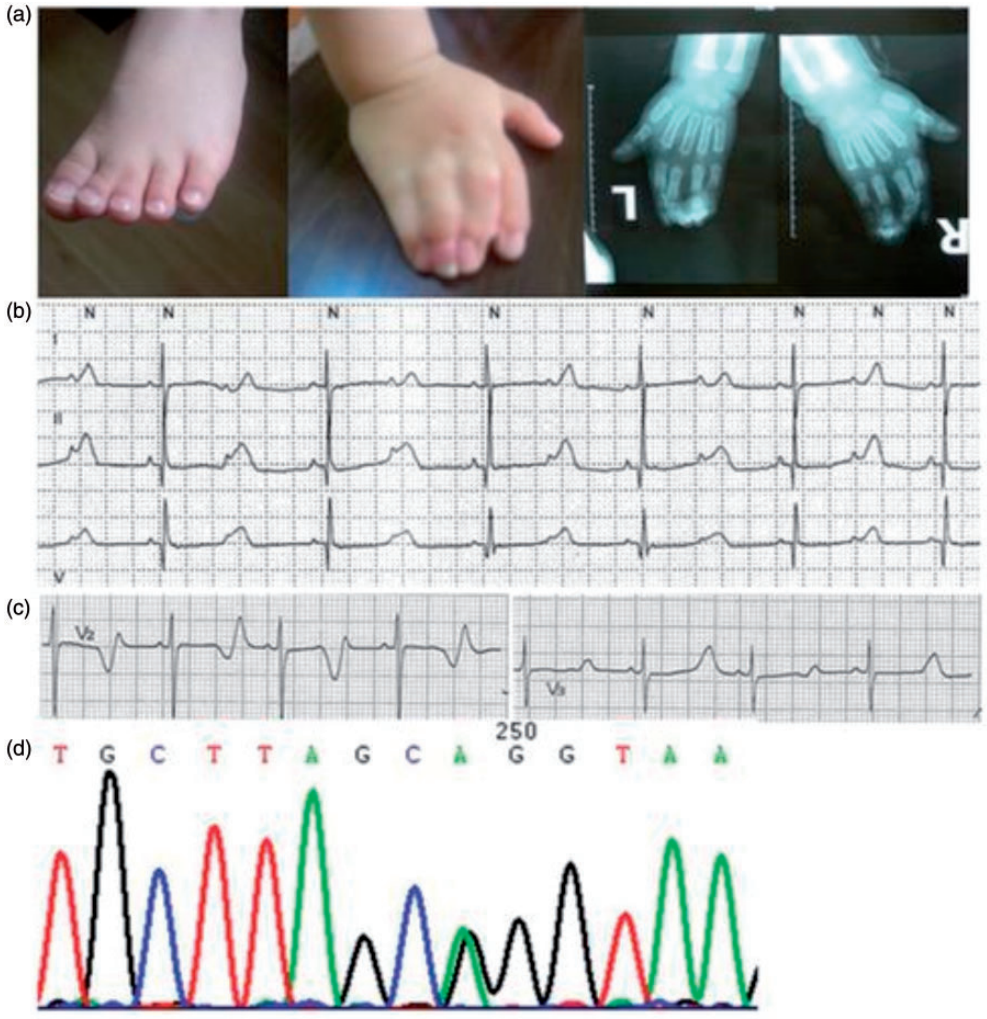

Figure 2. Phenotype and genotype of a TS1 patient. Bilateral syndactyly of two to five fingers confirmed by X-ray and cutaneous syndactyly of two to three left toes (a). Holter tracings demonstrated marked QT prolongation, functional 2:1 AVB with the blocked P wave landed on the ascending limb of T wave (b). Macro-TWA (c). DNA sequences of the TS1 patient: a heterozygous G/A at position 1216 as c.1216G>A transition leading to the p.Gly406Arg (G406R) missense mutation in exon 8A of the CACNA1C gene (d). (With permission from Xiaolin Xue *et al*.[4]). (A color version of this figure is available in the online journal.)

digits, and brain. Clinical hallmarks of TS are QT interval prolongation with QTc of 480 ms to 700 ms on electrocardiogram (ECG), cutaneous syndactyly (variably involving fingers 2 to 5 and toes 2 to 3). Most TS patients have arrhythmias, including bradycardia and functional 2:1 AVB, which are sometimes related to fetal distress,[21] TWA, torsades de pointes (TdP) and even VF, the main cause of death in TS children at the mean age of 2.5 years.[1] Approximately 70% patients suffered congenital heart diseases such as patent ductus arteriosus (PDA), patent foramen ovale (PFO), ventricular septal defect (VSD), pulmonary arterial hypertension (PAH), hypertrophic cardiomyopathy, and tetralogy of Fallot.[22,23]

Extra-cardiac phenotypes

Extra-cardiac manifestations include distinct dysmorphic facial features (born bald, flat nasal bridge, low-set ears, small upper jaw, small misplaced teeth), immunodeficiency, intermittent hypoglycemia, myopia, and hypothermia.[24] Arrhythmic deaths can be triggered by sepsis, episodic hypoglycemia, or anesthesia.[2] Most of the survival TS children show developmental delays, including stature, intelligence, language, and behavior. Some children meet criteria for ASD. The correlation between ASD and TS is significant ($P < 1.2 \times 10^{-8}$).[2]

Inheritance pattern

The inheritance pattern of TS indicates parental somatic or germ cell mosaicism. Mosaicism is that genetically different cell lines differentiating from a single spermatovum, exist in a single tissue. In view of mosaicism, tissues express more than one genotype or different degrees of mosaicism, suggesting that TS mutations represent cases of parental mosaicism. Therefore, careful genotyping of tissue rather than only peripheral blood lymphocytes should be guaranteed.[25,26]

Novel CACNA1C mutations and phenotypes

Except that classic TS patients of recurrent *de novo* missense mutation (G406R in exon 8A, G406R and G402S in exon 8) in CACNA1C, many other non-classic cases have also been

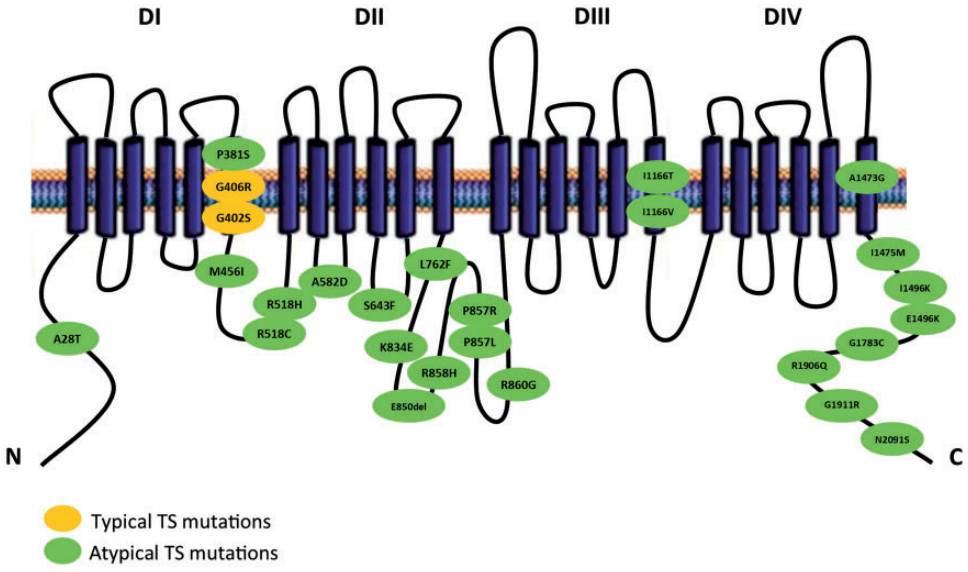

Figure 3. Updated TS-related CACNA1C mutations identified from 2004 to present. The yellow represents typical TS mutations, green represents atypical TS mutations. (A color version of this figure is available in the online journal.)

reported to expand the spectrum of TS. These novel mutations of CACNA1C and variable manifestations arouse controversy about genotypes and phenotypes of TS (Figure 3).

Patients carrying mutations of P857R/L, K834E, R1906Q, P381S, M456I, A582D, R858H, G1783C, R518C/H, N2019S, L762F, R518C, R860G, and S643F in CACNA1C gene only manifest LQTS without extracardiac phenotypes observed in TS. These identified variants might only perturb Cav1.2 channels or auxiliary subunits in the heart and hardly impact Cav1.2 in other tissues, resulting exclusively in cardiac disorder. Accordingly, these variants are suggested as "cardiac-only TS."[28–34]

Mutations A1473G and G1911R of CACNA1C can cause central and/or peripheral neurological symptoms such as stroke, seizures, cortical blindness, pathological signs in LQT8 patients.[23,35] A novel mutation R1024G and TS2 variant G402S have been reported in patients with extracardiac symptoms of TS but no QT prolongation.[2,36] As such, another typical TS2 mutation G406R can cause hearing loss which may be an unrecognized feature of TS.[37]

In conclusion, different missense mutations might exert diverse effects on excitable and non-excitable cells. Further studies of CACNA1C mutations are indispensable to better clarify the mechanisms underlying the cardiac and extracardiac features of TS.

Mechanisms underlying TS

Structure and function of Cav1.2 channels

Voltage-gated calcium channels mediate Ca^{2+} influx in various excitable cells, such as cardiomyocytes, neurons, skeletal muscle cells, sensory and endocrine cells, therefore regulating multiple physiological courses.[38] In the cardiomyocytes, Ca^{2+} entry via Cav1.2 channels forms the action potential (AP) and is crucial for excitation-contraction (EC) coupling. Alterations in Cav1.2 channel have significant effect on electrical and mechanical excitability and cardiac functions.

Cav1.2 channel contains the main pore forming α1C subunit, and auxiliary subunits including α2δ, β, and possibly γ subunits. The human α1C subunit is encoded by CACNA1C gene that locates on chromosome 12 and spans over 500 kb. The α1C subunit consists of four homologous domains (DI to DIV), each domain has six transmembrane segments (S1 to S6). Cav1.2 channels are clustered close to the ryanodine receptor 2 (RyR2) to form "Ca^{2+} release units" which permits optimal coupling for the Ca^{2+} induced Ca^{2+} release process.[39] Disruption of this relationship may give rise to Ca^{2+} overload thus dysfunction.

At least 12 alternative splicing loci that produce about 42 mutants occur in different regions of Cav1.2 channel, suggesting a wide variety of expression modes and a diversity of biophysical properties. Some alternative splicing loci are verified to modulate Ca^{2+} current and change the cardiac biophysical properties. CACNA1C mutations could have distinct functional consequences relying on which splice variant is expressed.[40]

Cav1.2 channel activation at approximately −30 mV is rapid and voltage-dependent; its inactivation is slower whereby both VDI and calcium-dependent inactivation (CDI) mechanisms.[41,42] The interdomain loop I–II of Cav1.2, where TS mutations are located, is important in controlling this process via Cav1.2 interactions. Regulation of Cav1.2 channels via trafficking or by modulating channel dynamics determines Cav1.2 activity and multiple modes of dysfunction.[43]

Three other subunits (α2δ, β, and γ) have been proved to regulate channel activity or membrane localization. Arrhythmias have also been identified in Cavα2δ-1 and Cavβ2b gene mutations. Expression of α2δ-1 to increase Cav1.2 distribution on plasma membrane was demonstrated *in vitro*, implying a positive influence on Cav1.2 trafficking.[44–46] Cavβ subunits exert pleotropic effects on Cav1.2

activities. Binding of Cavβ2 to the alpha interacting domain (AID) in Cav1.2 promotes channel trafficking to the cell surface.[41] The γ subunits modulate Cav1.2 channel function by altering both activation and inactivation characteristics when co-expressed with β subunits.[47] Because of these effects, it is important to perform exhaustive functional analyses before determining the mechanistic effects of gain or loss of function mutations.

Dysfunction of Cav1.2 channel in TS

First, Cav1.2 channels of TS are hyperactive and have severely impaired VDI. Both VDI and CDI restrict the open duration of Cav1.2 channels and thus controlling the Ca^{2+} influx, protecting cells from Ca^{2+} overload, and shortening APD.[48,49] Calcium current via Cav1.2 channel of TS mutations (G406R, G402S) is larger by causing almost complete loss of VDI.[3] Analysis of TS Cav1.2 in heterologous expression system showed that these mutant channels open much longer and more frequently than the wild-type (WT) via impaired VDI.[49–52]

Whether TS mutations impact CDI still remains contradictory as some studies indicating that TS mutation does not alter it. However, a significant defect in CDI in TS channels has been found to increase the steady state Ca^{2+} current. Other data supported that a nonlinear threshold behavior exists in inducing arrhythmias by TS channels, suggesting an essential treatment principle that a slightly negative shift in mutant channels may bring significant clinical improvement.[53]

Second, the zones and activities of "Ca^{2+} sparklets" generated by repetitive openings of Cav1.2 channels are higher in TS cells than in WT cells. The C-tails get extremely close (< 60 nm) to each other permitting the channels to physically interact more easily. Besides, C-tails dimerize via Ca^{2+}CaM bridges and these stable dimers have been isolated from cardiac Cav1.2 channels, further supporting a molecular model where these Cav1.2 clusters in TS undergo physical interactions.[52,54,55] Meanwhile, fusion of TS channels to WT channels could induce WT channels to function like TS channels thus generating a hyperactive cluster. Therefore, physical interactions among the C-tails could produce disproportionally large Ca^{2+} current, finally leading to cardiac arrhythmias.[52,55,56]

Third, the calmodulin-dependent protein kinase II (CaMKII) is a part of the proarrhythmic mechanism in TS. The loss of VDI triggers CaMKII activation. CaMKII amplifies Ca^{2+} influx that facilitates APD prolongation and causes EAD, highlighting small changes in intracellular Ca^{2+} can lead to unanticipated, unadaptive, and extensive alterations in Ca^{2+}-activated signaling. Besides, the RyR is a secondary proarrhythmic target for excessive CaMKII activity in TS, leading to Ca^{2+} overloading.[57] As such, CaMKII inhibition might be a viable alternative therapeutic approach for TS patients.[58,59]

Fourth, AKAP150 is important for the TS phenotype. Ablation of AKAP150 restores pathological Cav1.2-LQT8 channel gating, protects against arrhythmias, and prevents hypertrophy by reducing Ca^{2+} influx. Therefore, disrupting the interaction between AKAP150 and Cav1.2-LQT8 channel is a possible therapeutic target for treating the broad spectrum of TS.[50]

Additionally, there exists many other mechanisms underlying TS. TS mutations increase the frequency and opening time of Cav1.2 channels via abnormal phosphorylation of S439 in S6 helices leading to the excitotoxicity.[51] TS mutations significantly slow deactivation of Cav1.2.[60] TS mutations disrupt the coupling between activation and inactivation in Cav1.2 protein by altering tightly sealing point and perturbation of the I-II linker helical structure.[61,62]

Electrophysiological mechanisms underlying TS

Mechanisms predisposing to TS are sophisticated. Generally, the latent condition underlying malignant VA is a deficiency in ventricular repolarization. Gain-of-function TS mutations cause alterations in Cav1.2 channel kinetics and surface expression. The impaired voltage-dependent inactivation (VDI) is the dominating cause for aberrant electrical activities in TS cells.[63] Both TS1 and TS2 variants lead to a sustained inward Ca^{2+} current during AP plateau thus providing the substrate for ventricular arrhythmia. Cav1.2 channels of TS increase Ca^{2+} in the cytosol and the sarcoplasmic reticulum (SR), creating a Ca^{2+}overloaded state that increases the potential of arrhythmogenic spontaneous SR Ca^{2+} release.[64] Intracellular Ca^{2+} overloading promotes early and delayed after depolarization (EAD and DAD), triggered activities, and spontaneous diastolic depolarization, which further enhance the spatial dispersion of ventricular repolarization and cause reentrant arrhythmias.[65–69] The spatial dispersion of repolarization could be exaggerated by sympathetic influences, accounting for the great sensitivity of adrenergic stimuli in TS patients.[70]

Cellular mechanisms of different genotypes

TS mutations present gain of channel functions, they usually localize to the specific regions of highly conservation, cause conformational changes of subunits, and therefore Cav1.2 channel dysfunction. As stated, different genotypes might influence Cav1.2 functions in different manners. Gain of Cav1.2 channel function is an essential mechanism underlying TS arrhythmia. Classic mutations G406R and G402S cause impaired VDI via perturbing DI–II linker, leading to significant AP prolongation in accordance with TS phenotypes.[3] Mutations K834E, P857R/L may disrupt the functional PEST sequence (rich in proline (P), glutamic acid (E), serine (S), and threonine (T), regulate rapid protein degradation), lead to increased channel protein stability to produce a gain-of-function effect via an increase in peak I_{Ca} and surface membrane channels expression.[28] G1911R and L762F variant disturb the highly conserved region in Cav1.2 channel to produce a gain-of-function effect by generating a sustained window current, therefore leading to arrhythmia.[32,35] Mutations R518C/H, N2091S and S643F of CACNA1C exert both gain- and loss-of-function effects. The overall consequence of these variants accelerates activities of Cav1.2 channel, thus providing a pro-arrhythmic substrate.[30,31,34]

To sum up, there exists regions resemble genetic hotspots leading to cardiac disorders within CACNA1C gene. These regions can produce distinct clinical phenotypes ranging the spectrum of TS, cardiac-only TS, and LQTS. Future studies are needed to reveal the different mechanisms among these variants.

Models mimicking TS mutations

Cell models

Most of TS mutation functional studies were conducted in the heterogeneous system such as human embryonic kidney 293 (HEK293) cells, Chinese hamster ovary (CHO) cells, Xenopus oocytes, and human embryonic kidney tsA-201 cells.[1,3,29,51,61,71] These studies demonstrated that TS mutations altered VDI and CDI, leading to APD prolongation, EAD, and DAD. Models mimicking TS mutations are summarized in Table 1.

Tissue models

Tissue models mimic TS phenotypes have been applied to investigate mechanisms and drug effects on TS mutations. Coronary-perfused left ventricular wedge models of dog and rabbit have successfully mimicked the gain-of-function Cav1.2 by using BayK8644. BayK8644 significantly prolonged APD of the M cells, inducing QT prolongation and increased transmural dispersion of repolarization (TDR). Ranolazine and mexiletine restored TS dysfunctions by inhibiting all actions of BayK8644 in this model.[4,84]

Animal models

Mutant animal models have been established to study TS and the related disorders.[72] In a transgenic TS mice model controlled by a cardiac promoter, the similar phenotypes as human patients, including QT prolongation, TdP and hypertrophy, were detected.[50] A knock-in TS2 (G406R) heterozygous mouse model with an inverted neomycin cassette (TS2-neo) can survive to adulthood. This kind of TS2-neo mice developed ASD without any cardiac abnormalities.[73] Since then, TS2-neo mice model has become widely used to investigate mechanisms underlying ASD in which microscopic organization and cellular function are affected by dysfunctional Cav1.2 channel.[74] TS2-neo mice showed altered serotonin level and axon innervation in different brain areas suggesting that serotonin system is affected by Cav1.2 channels and has important implications for ASD.[75] Stercobilin is decreased in TS2-neo mice, which may act as a new clinical ASD biomarker.[76] Impaired VDI of Cav1.2 channel was also observed to significantly affect neuropsychic behavioral development in TS2-neo model.[77] Accordingly, this kind of model will persistently enrich our knowledge of this complex disorder.

iPSC models

Cardiac phenotypes differ between mice and humans. Developing human models of TS is imperative to better understand human-specific mechanisms of QT prolongation and severe arrhythmias. Induced pluripotent stem cell (iPSC) technology opens new avenues for studying the cellular mechanisms of cardiac arrhythmias, providing a stable platform for developing new drugs to treat these conditions.[85] The first TS iPSC derived from dermal fibroblasts of two TS patients revealed excess Ca^{2+} influx, abnormal calcium transients, irregular electrical activities, prolonged APD and irregular contraction in ventricular-like cells, and demonstrated the potential of verapamil, β-blockers, ranolazine, nifedipine, and roscovitine to inhibit abnormal Ca^{2+} overloading and restore Cav1.2 channel functions.[82,86] iPSC-induced neurons of TS showed phenotypes of defects in Ca^{2+} signaling, decreased gene expressions in callosal projection neurons and lower cortical layers, increased norepinephrine and dopamine, and abnormal levels of tyrosine hydroxylase, all these abnormalities can be reversed by roscovitine.[83] TS-related baldness was investigated by using hair follicle stem cells. Mutant Cav1.2 channels inhibited the bulge-derived BMP inhibitor follistatin-like1 (Fstl1) to delay anagen markedly. This study revealed the effect of channels on non-excitable cells and suggested novel therapies for tissue regeneration.[81]

Recently, some new techniques emerge and improve the property of iPSC. *In silico* interface used on electronically expressed $I^{(K1)}$ is an effective approach to improve the electrophysiological stability of AP of iPSC-induced cardiaomyocytes.[80] Locus-specific endonucleases increase homology-directed repair and generate a syngenic TS cell line in a scarless pattern with high efficiency.[87] Using genetically encoded fluorescent indicators, ArcLight and R-GECO1 can observe the prolonged APD and calcium overloading simultaneously and improve recording efficiency.[88] These new approaches will boost iPSC applicability to illustrate mechanisms underlying cardiac diseases and to develop novel therapeutics.

Computer models

Application of computer simulations or programs provides a more convenient method to TS studies.[78] PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (http://sift.jcvi.org/), and the multi-scale computational models of the human ventricle have been used to assess and predict pathogenicity of TS mutations.[23,79] However, the accuracy of computer simulation remains to be certificated.

Treatment and management

In TS patients, SCD is the most prevalent cause of mortality. Long-term administration of β-blockers is recommended in TS patients. Additional treatment strategies are usually dependent on TS genotypes and clinical phenotypes including late sodium current (I_{NaL}) blocker, mexiletine,[1,4,24,25,37,89–94] other antiarrhythmic drugs such as verapamil and nicorandil, potassium and/or magnesium supplementation, ICD, pacemakers, and left cardiac sympathetic denervation.

Verapamil, a synthetic papaverin derivative, is a Cav1.2 channel antagonist. It reduces the plateau height of the AP, shortens muscle AP slightly, and prolongs Purkinje fiber AP mildly. Gain-of-function mutations of the Cav1.2

Table 1. Models of TS due to different mutations.

Models	Mimic mutants	Modeling method	Mechanisms	Drug interference	References
Transgenic mice	G406R of exon 8 in CACNA1C	Transfection of the wild-type- and TS2-Cav1.2 mutant plasmids	Greater L-type Ca^{2+} influx	No	72
TS2-Neo mice	G406R or G402S of exon 8 in CACNA1C	A Neo cassette was inserted at the end of exon 8 of the Cav1.2 channel locus; all vectors were constructed and introduced into mouse stem cells	Loss of Cav1.2 inactivation	No	73–77
Ten Tusscher-Panfilov (TP06) human ventricular cell models	R858H mutation in CACNA1C	Computational cardiac modeling	Augmented sarcoplasmic reticulum calcium content, leading to the development of after depolarizations	No	78
Multi-scale computational models of the human ventricle	G1911R mutation in CACNA1C	Mathematical method	Increased calcium influx by changing voltage-dependence of activation, voltage-dependence of inactivation and the time constant of inactivation	No	79
iPSC	CACNA1C G406R mutation	Dual optical recordings	Prolonged action potentials and abnormal calcium handling	Roscovitine	79
iPSC	CACNA1C G406R mutation	Locus-specific endonucleases	Nearly complete loss of voltage-dependent channel inactivation, producing sustained inward Ca^{2+} currents	No	72
iPSC	CACNA1C G406R mutation	Use a genome-wide weighted co-expression network analysis	Loss of voltage-dependent channel inactivation	No	80
TS mouse model	CACNA1C G406R mutation	Transgene	Increase Ca^{2+} in the cytosol and the SR, creating a Ca^{2+} overloaded state	No	51
iPSC	CACNA1C G406R mutation	Using in silico interface to develop electronically expressed I(K1) and BayK-8644 to induce gain of function of ICa	Improve the stability of mutant ICa	No	80
Hair follicle stem cells	CACNA1C G406R mutation	Transgenic mice	Lack detectable voltage-dependent calcium currents	Verapamil	81
TS ventricular wedge model	CACNA1C G406R mutation	Using BayK-8644 on rabbit ventricular wedges.	Inhibit late INa	Mexiletine	4
iPSC	CACNA1C G406R mutation	Generate cardiomyocytes from TS patients	A significantly reduced voltage-dependent inactivation, abnormal calcium handling	Verapamil, beta-blockers, ranolazine, nifedipine, roscovitine	82
iPSC	CACNA1C G406R mutation	Derive from TS individuals	Defects in Ca^{2+} signaling and activity-dependent gene expression	Roscovitine	83
iPSC	CACNA1C G406R mutation	Reprogramme human skin cells from TS patients	Irregular contraction, excess Ca^{2+} influx, abnormal calcium transients	Roscovitine	73

TS: Timothy syndrome; iPSC: induced pluripotent stem cells.

channel suggest that calcium channel blockers may help in treating the disorder. A TS2 patient was treated with verapamil and responded well. Although verapamil did not shorten QT interval, it decreased the number of VF episodes and also improved the patient's neuropsychiatric function.[95]

Mexiletine has been reported to be effective in many TS patients to reduce their burden of VA.[1,4,24,25,37,89–94] Mexiletine is a class IB antiarrhythmic agent that blocks I_{NaL} preferentially without an obvious effect on I_{CaL}.[27,96] In addition to its effect to shorten the QT interval, a blunted QT–RR relationship by mexiletine also played an important role in termination of 2:1 AVB in TS patient (Figure 4). Mexiletine shortened the QT interval more markedly at slower pacing rates in the rabbit ventricular wedge model and, therefore, reducing ΔQT during a wide range of pacing rates. 2:1 AVB in LQTS might result from significant alterations in ventricular repolarization during transitions in heart rates. A blunted QT–RR relationship by mexiletine would be expected to produce a more gradual change in QT during transition in heart rates to reduce functional 2:1 AVB. Mexiletine might decrease intracellular Ca^{2+} overloading via Na^{+}–Ca^{2+} exchange, which is supported by the decreased contractility observed in the rabbit ventricular wedge model. This explains why mexiletine could prevent VA and attenuate TWA and phasic T wave, which is associated with intracellular Ca^{2+} overloading in TS patients.[97,98]

The potential of ranolazine to reduce the burden of atrial and ventricular fibrillation has been reported in a TS2 patient who responded unfavorably to verapamil.[99] Ranolazine has successfully terminated TdP in TS models using BayK8644. Multipotent inhibition of various ion channels, particularly I_{NaL} and I_{CaL}, could contribute to eliminate EAD- and DAD-induced triggered activities for reentry actions for TdP.[84]

Dihydropyridine Ca^{2+} channel antagonists (DHPs), such as nifedipine, block Cav1.2 channels by resuming and stabilizing their VDI *in vitro*. Therefore, DHPs might ameliorate the intricate excitotoxicity of the TS mutations and the resultant physiological and developmental abnormalities in TS patients.[71]

Tri-substituted purine roscovitine, a cyclin-dependent kinase (CDK) inhibitor, affects Cav1.2 by suppressing current amplitude, slowing activation and enhancing inactivation, without affecting deactivation or CDI.[100] Roscovitine restores open state VDI of TS mutant channels and normalized the Ca^{2+} influx during cardiac AP. Drugs that enhance open state VDI can normalize AP-induced Ca^{2+} influx, suggesting that these drugs may be useful in treating the multiple disorders associated with the TS mutation.[60] Other study also demonstrated that roscovitine exerts its therapeutic effects partly by inhibiting CDK5, providing new evidences in regulating cardiac Cav1.2 channels and developing therapeutics for managing TS patients.[101]

Anesthesia-induced cardiac arrest

Cases of TS children suffering SCD during anesthesia induction have been reported.[24,102] A nine-month-old TS

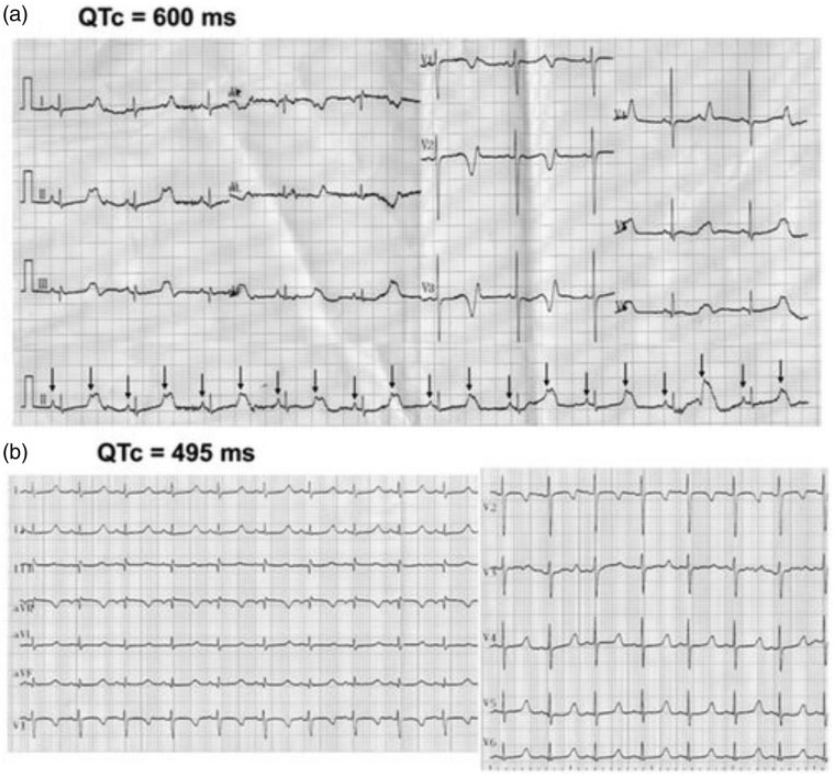

Figure 4. ECG of the TS1 patient obtained immediately after mexiletine administration showed marked QT prolongation (QTc: 600 ms) accompanied by 2:1 AVB (P waves marked by arrows) (a). Mexiletine (12.5 mg/kg per d) significantly shortend the QT interval (QTc: 495 ms) and abolished 2:1 AVB (b). (With permission from Xiaolin Xue *et al*.[4]).

boy underwent life-threatening arrhythmias resulted from excessive sympathetic stimulation due to an arterial tourniquet, suggesting that adequate block of sympathetic responses and a comprehensive anesthesia plan are necessary.[70] Anesthetic drugs appear to be genotype-specific in terms of QT prolongation, implicating that genotype-specific anesthetic plans to improve perioperative safety are imperative.[103] Anesthetic drugs act on diverse ion channels to impair cardiac repolarization reserve. Halothane, sevoflurane, and isoflurane reduce cardiac contractility, shorten APD and refractory time by inhibiting I_{CaL}. Halothane interferences with I_{CaL} also through β-adrenergic regulation. Halothane and isoflurane can shorten and mismatch APD via inhibition of voltage-dependent transient outward K^+ current (I_{to}). Isoflurane and sevoflurane increase ATP-dependent K^+ current for myocardial preconditioning. Halothane, isoflurane, and sevoflurane inhibit voltage-dependent sustained outward K^+ current and fast Na^+ current to induce delayed repolarization and tachyarrhythmia, respectively.[104]

PROSPECTIVES

To date, an increasing number of CACNA1C mutations has been discovered and expanded the spectrum of TS. Controversy is existing concerning the genotypes and phenotypes of TS patients. New technologies and models open new avenues to help better understand the mechanisms underlying TS. Though emerging agents and cardiac assist technologies can improve the survival in some cases of TS children, more supporting evidences are necessary and a remarkable effort should be carried out to assemble these data to promote the management of TS patients in clinic.

Authors' contributions: Dr. Dan Han and Dr. Guoliang Li collected the data and drafted the manuscript. Dr. Guoliang Li, Yang Yan and Xiaolin Xue revised it. All of the authors reviewed and approved the final manuscript.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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