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REVIEW

Mutations in voltage-gated L-type calcium channel: implications in cardiac arrhythmia

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ABSTRACT

The voltage-gated L-type calcium channel (LTCC) is essential for multiple cellular processes. In the heart, calcium influx through LTCC plays an important role in cardiac electrical excitation. Mutations in LTCC genes, including *CACNA1C, CACNA1D, CACNB2* and *CACNA2D*, will induce the dysfunctions of calcium channels, which result in the abnormal excitations of cardiomyocytes, and finally lead to cardiac arrhythmias. Nevertheless, the newly found mutations in LTCC and their functions are continuously being elucidated. This review summarizes recent findings on the mutations of LTCC, which are associated with long QT syndromes, Timothy syndromes, Brugada syndromes, short QT syndromes, and some other cardiac arrhythmias. Indeed, we describe the gain/loss-of-functions of these mutations in LTCC, which can give an explanation for the phenotypes of cardiac arrhythmias. Moreover, we present several challenges in the field at present, and propose some diagnostic or therapeutic approaches to these mutation-associated cardiac diseases in the future.

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Introduction

Cardiac arrhythmia is one of the major causes for sudden cardiac death (SCD) [1,2]. Over the past decades, some arrhythmic susceptible genes, including voltage-gated L-type calcium channel (LTCC), have been identified many arrhythmia-associated mutations [3–5]. LTCC is the main pathway for calcium ion influx into excitable cells in response to the membrane depolarization [6,7], which forms one part of cardiomyocyte action potentials (APs). In the heart, LTCC is a multi-subunit protein complex composed by four subunits: α_1 subunit and auxiliary β, $\alpha_2\delta$ and γ subunits, encoded by *CACNA1C* or *CACNA1D*, *CACNB2*, *CACNA2D* and *CACNG*, respectively [8,9]. It has been known that the mutations in these LTCC genes induce the dysfunctions of calcium channels, which result in the abnormal excitations of cardiomyocytes, and finally lead to cardiac arrhythmias.

In this work, we summarize recent findings on the mutations in the different subunits of LTCC, which are associated with cardiac arrhythmias (Figure 1), and describe the functional roles of these mutations in channel properties (Table 1), which can shed a light in understanding of cardiac electrophysiological characteristics in these diseases.

Molecular basis of L-type calcium channel

The pore-forming α_1 subunit, encoded by *CACNA1C* or *CACNA1D* gene, is the dominant voltage-gated calcium channels expressed in the working cardiomyocytes or sinoatrial nodal cells (SANCs), respectively. This α_1 subunit determines the main pharmacologic and biophysical properties of the channel [8,10], it contains four repeated homologous domains (DI-DIV), and each domain is comprised of six transmembrane segments (S1-S6) (Figure 1) [10]. $Ca_V1.2$ α_{1C} (*CACNA1C*) plays a critical role in the cardiovascular function. Deletion of α_{1C} subunit in mice resulted in embryonic lethality [11], and conditional knockout of smooth muscle α_{1C}

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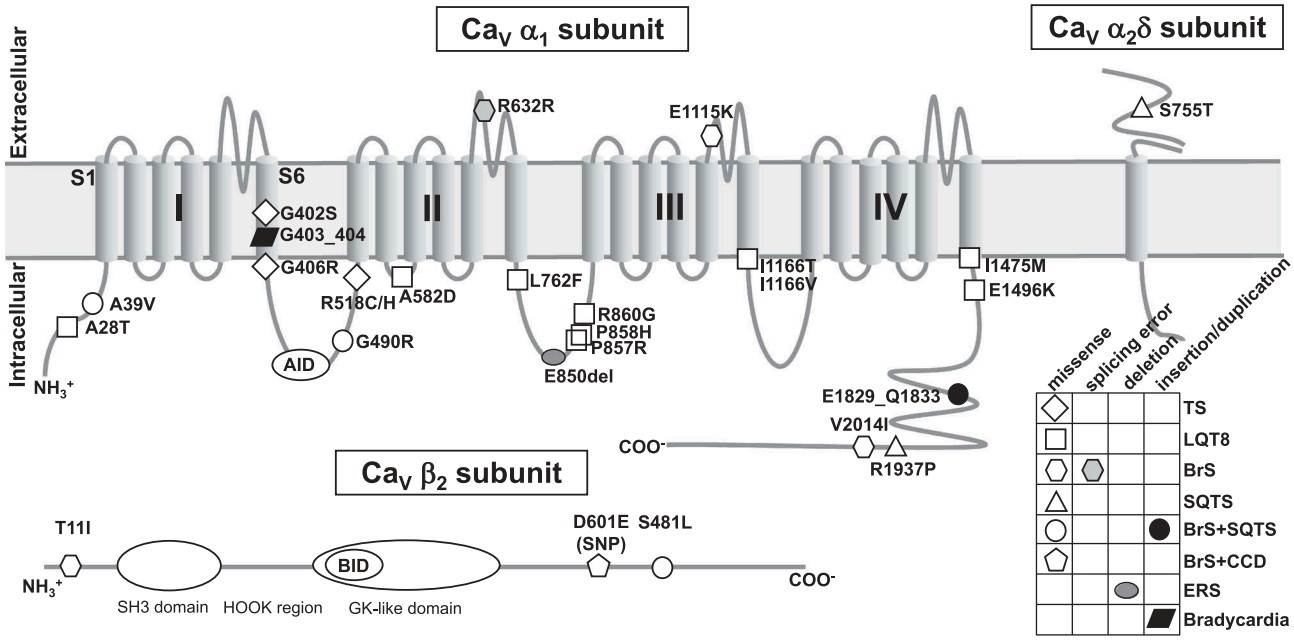

Figure 1. Predicted topology of Ca_V α_1 subunit with associated β_2 and $\alpha_2\delta$ subunits shows the location of functional mutations. All mutations in α_1 subunit are derived from α_{1C} ($Ca_V1.2$), except one mutation G403_404ins is come from α_{1D} ($Ca_V1.3$). AID = α-subunit interaction domain; BID = β-subunit interaction domain; BrS = Brugada syndrome; CCD = cardiac conduction disease; ERS = early repolarization syndrome; GK = guanylate kinase; LQTS = long QT syndrome; SH3 = Src homology 3; SQTS = short QT syndrome; SNP = single nucleotide polymorphism; TS = Timothy syndrome.

(SMAKO) lowered the arterial blood pressure in mice [12]. Recently, multiply alternative splicing events have been found in *CACNA1C*, which optimize the functions of $Ca_V1.2$ channel [13,14]. Moreover, alternative splicing in $Ca_V1.2$ channels make some roles in several cardiovascular diseases, including cardiac arrhythmia [15–17]. $Ca_V1.3$ α_{1D} (*CACNA1D*) is highly expressed in both SANCs and cochlear inner hair cells [18–21]. Targeted deletion of α_{1D} caused deafness, pronounced bradycardia, and nonfatal sinoatrial arrhythmia in mice [22,23].

The auxiliary β subunit modifies the gating property of α_1 subunit, it can increase the calcium currents by regulating the expression of α_1 subunit in the cell membrane [6,24,25]. Mechanistically, $Ca_V\beta$ works as a chaperone for the α subunit, and its β-interaction domain (BID) binds with the reserved α-interaction domain (AID) located at I-II loop of $Ca_V\alpha_1$ subunit, enhancing the trafficking of the channels from endoplasmic/sarcoplasmic reticulum (ER/SR) to cell membrane [24,26]. To date, 4 genes encoding β subunits ($\beta_{1\text{-}4}$) have been identified [27,28]; of which, $Ca_V\beta_2$ subunit, encoded by *CACNB2* gene, is the dominant variant expressed in the heart [29], which has at least 8 distinct splice variants ($\beta_{2a\text{-}h}$) [30,31].

The extracellular α_2 and transmembrane δ subunit, linked with each other via disulfide bonds, are encoded by the gene *CACNA2D* [32]. The $\alpha_2\delta$-1 subunit, encoded by *CACNA2D1*, is abundant in skeletal and cardiac muscles [33,34]. The $\alpha_2\delta$-2 and $\alpha_2\delta$-3 subunits, encoded by *CACNA2D2* and *CACNA2D3* respectively, express in neurons and some other tissues [33,35]. In addition, the expression of $\alpha_2\delta$-4 subunit, encoded by *CACNA2D4*, is mostly copious in non-neuronal cells apart from retinal neurons [36,37]. By bioinformatics, the proteins encoded by several other genes have been identified, and they share the similar structure with $\alpha_2\delta$ subunits [38], but have not been proved to function as calcium $\alpha_2\delta$ subunits. The $\alpha_2\delta$-1 subunit plays a vital role in hypertensive vascular $Ca_V1.2$ channel properties [39,40]. In the heart, the atrium is characterized by increased $\alpha_2\delta$-1 protein expression as compared with the ventricle, which might lead to the $Ca_V1.2$ electrophysiological differentiation between atrial and ventricular cardiomyocytes [41]. Furthermore,

Table 1. Summary of mutations or polymorphisms in *CACNA1C, CACNA1D, CACNB2b* and *CACNA2D1*.

No.	Amino acid change	Nucleotide change	Exon	Location	Mutation type	Gain/loss of function	Main effects on LTCC	Diagnosis	References
Mutations in *CACNA1C*									
1	p.A28T	c.82 G > A	2	N-terminus	Missense	Gain	$I_{Ca,L}$ ↑, positively shift of $V_{0.5,inact}$	LQT8	Wemhoner et al. 2015[82]
2	p.A39V	c.116 C > T	2	N-terminus	Missense	Loss	$I_{Ca,L}$ ↓, LTCC trafficking ↓	BrS3/SQT4	Antzelevitch et al. 2007[98]
3	p.P381S	c.1141 C > T	8A	DI-S6	Missense	-	N.S.	LQT8	Fukuyama et al. 2014[88]
4	p.G402S p.G406R	c.1204 G > A c.1216 G > A	8	DI-S6	Missense	Gain	VDI ↓	TS2	Splawski et al. 2005[62]
5	p.G406R	c.1216 G > A	8A	DI-S6	Missense	Gain	VDI ↓↓, CDI ↑	TS1	Splawski et al. 2004[61]; Barrett et al. 2008[66]
6	p.M456I	c.1368 G > A	9	I-II loop	Missense	-	N.S.	LQT8	Fukuyama et al. 2014[88]
7	p.G490R	c.1468 G > A	10	I-II loop	Missense	Loss	$I_{Ca,L}$ ↓	BrS3/SQT4	Antzelevitch et al. 2007[98]
8	p.R518C/H	c.1552 C > T/ c.1553 G > A	12	I-II loop	Missense	Loss/gain	$I_{Ca,L}$ ↓, $Ca_V1.2$ inactivation ↓, window current ↑, late current ↑	Cardiac only TS	Boczek et al. 2015[83]
9	p.A582D	c.1745 C > A	13	DII-S2/S3	Missense	Gain	$Ca_V1.2$ inactivation ↓	LQT8	Fukuyama et al. 2014[88]
10	p.R632R	c.1896 G > A	14	DII-S5/S6	Splicing error	Loss	LTCC mRNA ↓	BrS3	Fukuyama et al. 2014[101]
11	p.L762F	c.2284 C > T	16	II-III loop	Missense	Gain	$Ca_V1.2$ inactivation ↓, window current ↑	LQT8	Landstrom et al. 2016[89]
12	p.E850del	c.2548_2550 delGAG	19	II-III loop	Deletion	Loss	$I_{Ca,L}$ ↓↓	ERS2	Burashnikov et al. 2010[102] Sutphin et al, 2016[90]
13	p.P857R	c.2570 C > G	19	II-III loop	Missense	Gain	$I_{Ca,L}$ ↑, surface expression ↑	LQT8	Boczek et al. 2013[84]
14	p.R858H	c.2573 G > A	19	II-III loop	Missense	Gain	$I_{Ca,L}$ ↑	LQT8	Fukuyama et al. 2014[88]
15	p.R860G	c.2578 C > G	19	II-III loop	Missense	Gain	SSC ↑, positively shift of $V_{0.5,inact}$	LQT8	Wemhoner et al. 2015[82]
16	p.E1115K	c.3343 G > A	26	DIII-S5/S6	Missense	Loss	Single channel conductance ↓	BrS3	Burashnikov et al. 2010[102] Simms. 2014[103]
17	p.I1166V	c.3496 A > G	28	DIII-S6	Missense	Gain	$I_{Ca,L}$ ↑	LQT8	Wemhoner et al. 2015[82]
18	p.I1166T	c.3497 T > C	28	DIII-S6	Missense	Gain	$I_{Ca,L}$ ↑, negatively shift of $V_{0.5,act}$	LQT8	Boczek et al. 2015[81] Wemhoner et al. 2015[82]
19	p.I1475M	c.4425 C > G	38	DIV-S6	Missense	Gain	SSC ↑, Negatively shift of $V_{0.5,act}$	LQT8	Wemhoner et al. 2015[82]
20	p.E1496K	c.4486 G > A	38	C-terminus	Missense	Gain	Negatively shift of $V_{0.5,act}$, SSC ↑, current decay ↓	LQT8	Wemhoner et al. 2015[82]
21	p.G1783C	c.5347 G > T	44	C-terminus	Missense	-	N.S.	LQT8	Fukuyama et al. 2014[88]
22	p. E1829_Q1833dup	c.5485_5499 dup15	43	C-terminus	Duplication	Loss	$I_{Ca,L}$ ↓↓	BrS3/SQT4	Burashnikov et al. 2010[102]
23	p.R1937P	c.5918 G > C	46	C-terminus	Missense	Loss	$I_{Ca,L}$ ↓↓, negatively shift of $V_{0.5,inact}$	SQT4	Chen et al. 2017[116]
24	p.V2014I	c.6040 G > A	46	C-terminus	Missense	Loss	$I_{Ca,L}$ ↓, negatively shift of $V_{0.5,inact}$	BrS3	Burashnikov et al. 2010[102]

(*Continued*)

Table 1. (Continued).

No.	Amino acid change	Nucleotide change	Exon	Location	Mutation type	Gain/loss of function	Main effects on LTCC	Diagnosis	References
25	p.N2091S	c.6272A> G	47	C-terminus	Missense	Gain	$I_{Ca,L}$ ↑, negatively shift of $V_{0.5,act}$	-	Sutphin et al, 2016[90]
26	p.K834E	c.2500 A > G	19	II-III loop	Missense	Unknown	Unknown	LQT8	Boczek et al. 2013[84]
27	p.P857L	c.2570 C > T	19	II-III loop	Missense	Unknown	Unknown	LQT8	Boczek et al. 2013[84]
28	p.A1473G	c.4418 C > G	38	DIV-S6	Missense	Unknown	Unknown	TS	Gillis et al. 2012[80]
29	p.R1880Q	c.5639 G > A	44	C-terminus	Missense	Unknown	Unknown	BrS3	Burashnikov et al. 2010[102]
30	p.C1873Y	c.5510 G > A	45	C-terminus	Missense	Unknown	Unknown	BrS3/SQT4	Burashnikov et al. 2010[102]
31	p.R1906Q	c.5717 G > A	44	C-terminus	Missense	Unknown	Unknown	LQT8	Boczek et al. 2013[84]
32	p.R1977Q	c.6167 G > A	47	C-terminus	Missense	Unknown	Unknown	SQT4	Mazzanti et al. 2014[115]
33	p.D2130N	c.6388 G > A	47	C-terminus	Missense	Unknown	Unknown	BrS3	Burashnikov et al. 2010[102]
Mutations in *CACNA1D*									
1	p.G403_404ins	c.1028_1029 insGGG	8B	DI-S6	Insertion	Loss	Conduction of $Ca_V1.3$ ↓	Bradycardia	Baig et al. 2011[126]
Mutations in *CACNB2b*									
1	p.T11I	c.32 C > T	2	N-terminus	Missense	Loss	Fast and slow decay ↑	BrS4	Cordeiro et al. 2009[104]
2	p.S481L	c.1442 C > T	14	C-terminus	Missense	Loss	$I_{Ca,L}$ ↓	BrS4/SQT5	Antzelevitch et al. 2007[98]
3	p.D601E	c.1803 T > G (polymorphism)	13b	C-terminus	Missense	Gain	Late $I_{Ca,L}$ ↑, $Ca_V1.2$ inactivation ↓	BrS4/CCD	Burashnikov et al. 2010[102] Hu et al. 2010[119]
4	p.A73V	c.218 C > T	4	SH3 domain	Missense	Unknown	Unknown	IVF	Burashnikov et al. 2010[102]
5	p.S143F	c.428 C > T	5	HOOK region	Missense	Unknown	Unknown	BrS4	Burashnikov et al. 2010[102] Risgaard et al. 2013[107]
6	p.S160T	c.479 G > C	6	HOOK region	Missense	Unknown	Unknown	BrS4	Burashnikov et al. 2010[102]
7	p.K170N	c.510 G > T	7b	HOOK region	Missense	Unknown	Unknown	BrS4/CCD	Burashnikov et al. 2010[102] Kanter et al. 2012[110]
8	p.L399F	c.1195 C > T	12	GK-like domain	Missense	Unknown	Unknown	BrS4/CCD	Burashnikov et al. 2010[95]; Kanter et al. 2012[110]
9	p.T450I	c.1349 C > T	14	C-terminus	Missense	Unknown	Unknown	BrS4	Burashnikov et al. 2010[95]; Risgaard et al. 2013[107]
10	p.D538E	c.1614 C > A	13	C-terminus	Missense	Unknown	Unknown	BrS4/CCD	Kanter et al. 2012[118]
11	p.R571C	c.1711 C > T	14	C-terminus	Missense	Unknown	Unknown	BrS4	Burashnikov et al. 2010[102]
Mutations in *CACNA2D1*									
1	p.S755T	c.2264 G > C	28	Extracellular	Missense	Loss	$I_{Ca,L}$ ↓	SQT6	Templin et al. 2011[117]

(*Continued*)

Table 1. (Continued).

No.	Amino acid change	Nucleotide change	Exon	Location	Mutation type	Gain/loss of function	Main effects on LTCC	Diagnosis	References
2	p.D550Y	c.1648 G > T	19	Cache domain	Missense	Unknown	Unknown	BrS9	Burashnikov et al. 2010[102]
3	p.S709N	c.2126 G > A	26	Extracellular	Missense	Unknown	Unknown	BrS9	Burashnikov et al. 2010[102] Risgaard et al. 2013[107]
4	p.Q917H	c.2751 A > T	34	Extracellular	Missense	Unknown	Unknown	BrS9	Burashnikov et al. 2010[102] Risgaard et al. 2013[107]
5	p.S956T	c.2867 C > A	36	Extracellular	Missense	Unknown	Unknown	ERS4	Burashnikov et al. 2010[102]

BrS = Brugada syndrome; CDI = calcium-dependent inactivation; CCD = cardiac conduction disease; ERS = early repolarization syndrome; $I_{Ca,L}$ = calcium currents of L-type channel; IVF = idiopathic ventricular fibrillation; LQT = long-QT syndrome; LTCC = L-type calcium channel; SQT = short-QT syndrome; SSC = steady-state current; TS = Timothy syndrome; $V_{0.5,act}$ = half-activation potential; $V_{0.5,inact}$ = half-inactivation potential; VDI = voltage-dependent inactivation.
N.S. = no significant differences vs. wild type channel.

deletion of $\alpha_2\delta$-1 in mice induced the decreased $Ca_V1.2$ Ca^{2+} currents of cardiomyocytes, and also decreased the basal myocardial contractility and relaxation [42]. These observations imply the potential roles of $\alpha_2\delta$ subunits in cardiac arrhythmias.

The γ subunit, which is encoded by *CACNG*, has 8 isoforms and consists of 4 transmembrane domains [43]. The first γ_1 subunit was cloned from skeletal muscle [44,45], whereas it was not expressed in cardiac muscle [46]. The γ subunit distinguishingly modulates the functions by altering both activation and inactivation properties of LTCC [46]. For example, targeted disruption of γ_1 subunit increased the amplitude of peak calcium current in isolated myotubes [47]; whereas coexpression with γ_2 subunit could depolarize the activation and inactivation curve of $Ca_V1.2$ channels [48]. In the heart tissue, four different γ subunits, γ_4, γ_6, γ_7 and γ_8, are expressed, and they all physically interact with the $Ca_V1.2$ complex, which diversify the functions of $Ca_V1.2$ channels [46]. To date, no evidences indicate the association of γ subunits and cardiac arrhythmias, thus it is of value to investigate their possible links.

Long QT and Timothy syndromes

Long QT syndrome (LQTS) is one of the most common cardiac electrophysiological diseases with an estimated prevalence of 1 in every 2,534 persons [49]. It is a typical cardiac repolarization abnormality defined by heart rate-corrected QT interval (QTc) prolongation on resting electrocardiogram (ECG) [4,50], and characterized by an increased trend for ventricular tachycardia with *torsade de pointes* (TdP). LQTS exists as a congenital genetic disease (cLQTS) with mutations described in different genes, which cause different types of LQTS. LQTS also can be acquired (aLQTS) by drug-intake [51] or structural heart disease [52], which may be more prevalent than cLQTS [53]. To date, 16 genes were identified to responsible for LQTS, of which, mutations in *CACNA1C* is accounted for long QT syndrome 8 (LQT8) [54]. Two canonical mutations in *CACNA1C* (p.G406R and p.G402S) are associated with a severe LQT8, namely Timothy syndrome (TS).

Timothy syndrome 1

In the 1990s, a severe syndrome which was called as the heart-hand syndrome, now known as TS or TS1, was observed in young children with significant clinical phenotypes: syndactyly, severe cardiac arrhythmia, congenital heart disease, developmental abnormalities, autism, and neurological dysfunction [55–57]. Most patients were diagnosed during neonatal period or rarely in late infancy, only two cases reported the fetal hydrops as antenatal expression of TS1 [58,59].

Splawski et al. discovered a *de novo* G-to-A mutation, which caused a p.G406R substitution in translational sequence at position 1216 (c. G1216A) of *CACNA1C* exon 8A (also known as exon 8 [60]) in all 13 individuals with TS. However, the p.G406R mutation was not identified in 180 ethnically matched controls [61], indicating that p.G406R mutation is associated with TS. This amino acid p.G406 is completely conserved in $Ca_V\alpha_{1C}$ subunit of other mammalian species, and is located at the C-terminal portion of the S6 of domain I [61,62].

The inactivation of the voltage-gated calcium channel has two mechanisms: voltage-dependent inactivation (VDI) and calcium-dependent inactivation (CDI), and they play an essential role in controlling excitation-contraction coupling in cardiomyocytes [6,63–65]. Functional analysis revealed that p.G406R mutation caused the maintained inward calcium currents by impairing the two types of channel inactivation [61]. By measuring whole-cell ionic currents in transfected human embryonic kidney-293 (HEK293) cells, the p. G406R mutation slowed the VDI of $Ca_V1.2$ calcium channels, while it possibly accelerated the kinetics of CDI [66]. It is known that the small glycine residues offer flexibility to allow for the I-II loop to interact with the intracellular pore of the channel [67–69], and G406 is located wiαthin a short stretch of nonhelical motif at the start of the α_{1C} I-II loop [70]. Therefore, p.G406R mutation would produce a more bulky arginine residue to impede the movement of the I-II loop, leading to

the slower channel inactivation. As a result of abnormally huge calcium ion influx, the action potential duration (APD) can be prolonged. Thus, the patients' ECG showed a prolonged QT interval leading to death from cardiac arrhythmia [66]. Interestingly, this mutant $Ca_V1.2$ channel function could be modulated by one anchoring protein AKAP150, elimination of which could abrogate the prolonged QT interval [71], implying AKAP150 may be a promising target for TS.

To explore the effects of the p.G406R mutation on the electrical activity and contraction of living cardiomyocytes, human skin cells from TS patients were reprogrammed to generate induced pluripotent stem cells (iPSC), and differentiated into cardiomyocytes [72]. The electrophysiological recording and calcium imaging studies revealed excessive calcium ion influx, irregular electrical activity and contraction, prolonged APs, and abnormal calcium transients in these ventricular-like cells [72,73]. Roscovitine, both an atypical LTCC blocker and a cyclin-dependent kinase inhibitor, which can enhance the VDI of $Ca_V1.2$, restored the electrical and calcium signaling properties of cardiomyocyte from TS patients [72–75]. Recently, TS mouse model had been generated and proved to have more arrhythmogenic events, which might be due to the increase of cytosol calcium concentration ($[Ca^{2+}]_i$) by sarcolemmal Ca^{2+} leak and the impairment of VDI [76]. Syndactyly or craniofacial abnormalities may also be caused by irregular $Ca_V1.2^{TS}$ channels, because $Ca_V1.2^{TS}$ channels affected the development of jaw and mandibular in the mouse and zebrafish models [77].

Timothy syndrome 2

Splawski et al. also identified two mutations, c.G1204A and c.G1216A, in exon 8 (also known as exon 8a [60]) of the *CACNA1C* gene, resulting in the p.G402S and p.G406R substitution, respectively [62]. Owing to that the exon 8 splice variant was found to highly express in human heart and brain, the patients who carried this mutation had a longer QT interval and more severe arrhythmia than patients with a mutation in exon 8A. Since the symptoms caused by the mutations in exon 8 were different from previous TS, this disease was named as TS2. The patients suffered from multiple arrhythmias and SCD due to the extreme prolongation of QT interval, but syndactyly might not be manifested [62,78], and these patients were more likely died of TdP and ventricular fibrillation [62]. Structurally, the glycine residue at 402 position of IS6, together with the same positions in the IIS6, IIIS6 and IVS6 segments, form the G/A/G/A motif, which is near the inner channel mouth of $Ca_V1.2$. These four residues interact with larger bulky residue from neighboring S6 helices to stabilize the inactivation gate [68]. In TS2, in addition to p.G406R mutation, p.G402S will also introduce a more bulky serine residue to disrupt the tightly sealing of the S6 helices, resulting in the slower deactivation [68]. Although neither heterozygous nor homozygous p.G402S transgenic mice can survive to weaning because of the high expression of exon 8 and a fatal extremely high level of calcium channels mutation, TS2-like mouse model with p.G406R had been generated by heterozygously keeping an inverted neomycin cassette in exon 8A [79], these mice could survive through adulthood due to the lowered expression of p.G406R LTCCs. The survived mice displayed the behavioral abnormalities, corresponding to the core aspects of autism spectrum disorder [79].

Timothy syndrome with novel CACNA1C mutations

A newborn, with healthy parents, had the similar symptoms with TS, he presented with prolonged QT interval and associated polymorphic ventricular tachycardia, dysmorphic facial features, syndactyly of the hands and feet, and joint contractures. By full gene sequencing, the patient had no p.G402S and/or p.G406R mutations, but a novel *CACNA1C* mutation p.A1473G had been detected in exon 38 [80]. This case expanded the molecular basis of TS, however the electrophysiological properties of this mutant channel need further investigations. Recently, another novel *CACNA1C* mutation p.I1166T in exon 28 was identified in a young male with diagnosed TS, and this mutation induced an overall loss of current density and a shift of activation of $Ca_V1.2$ channels, which lead to an increased window current [81]. However, Wemhoner et al. found this p.I1166T mutation

could induce a leftward shift of activation curve, but an increased peak current density [82]. These different results maybe attribute to different clones of $Ca_V1.2$ subunits, or even different ratios of subunits expressed in HEK293 cells.

Long QT syndromes without extracardiac signs

Some LQT8, with typical long QT interval, have no extracardiac phenotypes. A novel mutation p.R518C/H in *CACNA1C* was just identified in patients with a complex phenotype including LQTS, hypertrophic cardiomyopathy and congenital heart defects, which was annotated as "cardiac only TS" [83]. This mutation in $Ca_V1.2$ also revealed a complex channel phenotypes, including loss of current density and inactivation in combination with increased window and late currents [83]. By whole-exome sequencing and bioinformatics/systemic biology, Boczek et al. further identified 4 novel mutations of *CACNA1C* in LQTS, including p.K834E, p.P857R, p.P857L, and p.R1906Q [84], 3 of which were located at the conserved proline, glutamic acid, serine and threonine rich (PEST) domain of $Ca_V\alpha_1$ II-III loop, this domain acts to proteolytic signaling through the cellular quality control system [85]. Functionally, p.P857R mutation, cosegregated with the disease within the pedigree, significantly increased calcium currents and surface membrane expression of the channel as compared with wild type (WT) $Ca_V1.2$ channel [84]. Interestingly, p.R1906Q locates one amino acid away from α_{1C} C-terminal STIM1 (stromal-interacting molecule 1, a calcium store sensor) binding domain, which may affect STIM1-mediated $Ca_V1.2$ channel gating inhibition and channel internalization [86,87]. The identification of these *CACNA1C* mutations co-segregating with disease indicated that *CACNA1C* genetic perturbations might underscore autosomal dominant LQT8 in the absence of TS.

Additionally, five novel *CACNA1C* mutations (p.P381S, p.M456I, p.A582D, p.R858H, and p.G1783C) were identified in the patients with LQT8, but without typical TS phenotypes [88]. Of significance, p.R858H mutant channel had a larger I_{Ca}, and p.A582D mutant channel displayed a slower inactivation [88], which could partially explain the phenotype of long QT interval. By screening 540 patients with LQTS, 6 more *CACNA1C* mutations were identified recently, including p.A28T, p.R860G, p.I1166T, p.I1166V, p.I1475M and p.E1496K [82]. These mutations affected the different properties of channel. Briefly, p.A28T increased calcium current of L-type channel ($I_{Ca,L}$) and positively shifted steady-state inactivation (SSI). p.R860G positively shifted SSI and increased steady-state current (SSC). Both p.I1166T and p.I1166V mutations showed an increased $I_{Ca,L}$, but p.I1166T negatively shifted the steady-state activation (SSA) and increased SSC. p.I1475M and p.E1496K negatively shifted the SSA, moreover p.E1496K increased SSC and slowed the current decay [82]. Using computational cardiac AP model, the researchers found these gain-of-function mutations delayed repolarization of the cardiac myocytes, which induced prolongation of APD [82]. Recently, a novel *CACNA1C* mutation p.L762F was identified to associate with the development of LQTS. This mutation slowed the channel inactivation and increased persistent and window current, which attributed to the gain-of-function of $Ca_V1.2$ channels [89]. Furthermore, a novel mutation of *CACNA1C* (p.N2091S) is also found in autopsy-negative sudden unexplained death of a 24-year-old white female. No premortem ECGs were available and neither decedent had any documented family history of arrhythmia-related cardiac events. However, this p.N2091S mutation induced a dramatic increase of I_{Ca} and a minor hyperpolarization of $V_{0.5}$ of channel activation, indicating a LQTS-like gain-of-function electrophysiological phenotype [90].

Brugada syndromes

Brugada syndrome (BrS), first described in 1992, is an inherited cardiac arrhythmic syndrome associated with a high risk of ventricular fibrillation without structural heart disease [91]. The ECG of the Brugada patient is characterized by right bundle branch block and ST segment elevation in precordial leads V1-V3.

The symptom of BrS typically manifests during the adulthood, but the youngest individual diagnosed with BrS is only 2 days old, while the oldest is 84 years old [92,93]. Generally, BrS is more

prevalent among male patients owing to the gender differences in the expression of cardiac transient outward potassium channel (I_{to}). Because of the presence of a more predominant I_{to} in males compared with females, male patients are more likely to induce the loss of the AP dome and the development of phase 2 reentry and polymorphic ventricular tachycardia [94,95]. Mutations in *CACNA1C, CACNB2* and *CACNA2D1* can cause BrS3, BrS4 and BrS9, respectively [96,97].

Brugada syndrome 3

A male European descent with BrS and a shortened QTc interval showed a heterozygous mutation in exon 2 of the *CACNA1C* gene, which made a p.A39V substitution, near the N-terminus within a highly conserved region of the $Ca_V\alpha_{1C}$ [98]. In another case, a male Turkish patient with BrS and a shortened QT interval, was detected a novel heterozygous mutation in exon 10 of the *CACNA1C* gene was predicted to result in a p.G490R substitution in I-II loop [98]. Patch-clamp analysis in Chinese hamster ovary (CHO) cells, which transfected with p.A39V or p.G490R mutant $Ca_V1.2$ channels, showed the current amplitude was dramatically reduced as compared with WT channels, although voltage at peak current remained unchanged [98]. These results gave an sound explanation for the shortened QT interval. Moreover, p.A39V mutant channel had a defect in trafficking of mature LTCCs, however, confocal imaging revealed the normal trafficking of p.G490R mutant $Ca_V1.2$ channels from ER to cell membrane [98]. Therefore, it is reasonable to conclude that p.G490R mutation may affect the channel open probability (P_o), but not cell surface expression. Interestingly, this p.A39V mutation in N-terminus also affected the calmodulin-dependent activation of $Ca_V1.2$ channel [99], but didn't affect the surface expression of neuronal $Ca_V1.2$ channels [100], which might be involved the mechanisms of BrS. Recently, a p.R632R in $Ca_V1.2$ α_{1C} due to c.G1869A mutation in exon 14 of *CACNA1C* gene was identified in a patient with BrS, this mutation caused an aberrant splicing, which in turn made a premature stop codon in the downstream. This type of mutation might lead to nonsense-mediated mRNA decay, which induced the loss-of-function of $Ca_V1.2$ calcium channel [101].

By screening 162 probands with BrS or BrS combined with short QT syndrome (SQTS) (BrS + SQTS), there were 7 newly identified mutations in *CACNA1C*, including p.E1115K, p.R1880Q, p.V2014I, p.D2130N, p.E1829_Q1833dup, p.C1873Y and p.E850del [102]. Functionally, $Ca_V1.2$ channel with p.E1115K had a reduced calcium influx. Mechanistically, this mutation strictly reduced single channel conductance, but did not change the voltage or calcium-dependent gating [103]. p.V2014I mutation in $Ca_V1.2$ channel reduced peak current density and shifted half-inactivation voltage to more negative potentials, whereas voltage at the maximum peak current remained unchanged. In another mutation, the duplication of five amino acids in exon 43 of *CACNA1C* (p.E1829_Q1833dup) resulted in nearly compete suppression of calcium current (I_{Ca}) [102]. These loss-of-function mutations may help to explain the shortening of QT interval and other features of ECG.

Brugada syndrome 4

In two right precordial ECG leads, the patient displayed a type I ST-segment elevation, and a novel p.T11I mutation in *CACNB2b* was identified. This mutant $Ca_V\beta_{2b}$ had a faster fast and slow decay I_{Ca} and a reduced total charge compared to WT $Ca_V\beta_{2b}$, but had almost same peak calcium current density, SSI and recovery from inactivation [104]. In 6 affected family members with BrS and a shortened QTc interval, a c.C1442T heterozygous mutation in exon 13 of the *CACNB2b* gene resulted in p.S481L substitution downstream of the BID of β_{2b} subunit [98]. This mutation was not found in either 4 phenotype-negative family members or in 400 ethnically matched control alleles. The calcium current in the cells coexpressing with p.S481L $Ca_V\beta_{2b}$ was dramatically smaller than WT $Ca_V\beta_{2b}$, but the surface expression was almost the same, which indicated that the p.S481L channel traffics normally [98]. Due to the fact that the mutation is located at the position nearby the BID domain binding with α_{1C} subunits, the mechanism of dysfunctions of calcium channel

could be interfered by the stimulatory role of β_2 subunit on LTCC [105].

A patient with BrS, who has a p.V2014I mutation in *CACNA1C*, also had a p.D601E polymorphism in *CACNB2b*, and this polymorphism dramatically augmented the late calcium current of LTCC, which prolonged the QT interval, the modulatory effect of this single nucleotide polymorphism probably interprets the fact that QTc in this proband is not associated with SQTS [102]. Other mutations were also identified [102,106], including p.S143F and p.T450I, which were reported in the prevailing BrS-associated variants of NHLBI GO Exome Sequencing Project population [107]. However, the functions of these mutations on the $Ca_V\beta_2$ subunit remain unknown.

Brugada syndrome 9

Human mutations in *CACNA2D1* have been identified to be associated with several kinds of cardiac dysfunctions, including BrS [97]. It has been found 3 different missense mutations in *CACNA2D1* (p.S709N, p.D550Y, and p.Q917H) in 3 BrS patients from a cohort made up of 205 patients with BrS [102]. Among these mutations, p.S709N and p.Q917H were also identified in Exome Sequencing Project population by Risgaard et al. [107]. Nevertheless, more works are needed to explore the possible functions of these mutations on Ca_V channels.

Short QT syndromes

The short QT syndrome (SQTS), first described in 2000 [108], was defined by an abnormally shortened QT intervals and a propensity for cardiac arrest (CA) [109,110]. SQTS has been associated with the gain-of-function mutations in 3 distinct potassium channels, *KCNH2, KCNQ1* and *KCNJ2*, which cause SQT1, SQT2 and SQT3, respectively [111–114]. Nowadays, more evidences indicate the mutations in LTCC are also linked to SQTS. Some cardiac arrhythmias have the combined phenotypes of SQTS and BrS, since the shortened QT interval is also one of the manifestations of BrS. Thus, BrS3, caused by the mutation in *CACNA1C*, such as p.A39V, p.G490R, p.E1829_Q1833 duplication, and p.E850 deletion, also known as SQT4; and BrS4, caused by *CACNB2* p.S481L mutation, is also known as SQT5 [98].

Short QT syndrome 4

One proband's ECG showed that the QTc interval of the patient was shorter than that of healthy controls and characterized with aborted CA, and a novel *CACNA1C* mutation (p.R1977Q) was first identified in this SQTS patient, but the parents didn't carry this mutation [115]. However, the roles of p.R1977Q mutation in SQTS are still unknown. Recently, a novel *CACNA1C* mutation p.R1937P was reported in a Chinese family of hypertrophic cardiomyopathy with early repolarization and SQTS, p.R1937P induced the loss-of-function of $Ca_V1.2$ channels, which dramatically decreased the I_{Ca} and hyperpolarized the SSI [116].

Short QT syndrome 6

A novel type of SQTS (SQT6) caused by a mutation p.S755T in the *CACNA2D1* was reported in 2011 [117]. The ECG of the patient revealed a shortened QT interval. Templin et al. took advantage to analyze the functional roles of the p.S755T mutation in transfected HEK293 cells. Compared with the WT variant, p.S755T $\alpha_2\delta$-1 subunit extremely reduced the barium currents under the whole-cell patch clamp recording [117]. Western blotting showed that the membrane expression of α_{1C} subunit with mutant $\alpha_2\delta$-1 was similar to WT $\alpha_2\delta$-1 subunit, thus this mutation might only alter the open probability (P_o) of the $Ca_V1.2$ channels without modulating the membrane expression of the α_{1C} subunit of LTCC.

Other cardiac arrhythmias

Cardiac conduction disease

Combined mutations in *CACNB2b* (p.K170N/L399F) in one infant displaying BrS phenotype had a severe intraventricular conduction delay, and another case with conduction delay had identified p.D538E mutation in *CACNB2b* [118]. A novel polymorphism (p.D601E) was also identified in *CACNB2b* in the family displaying first-degree atrioventricular block, which induced the slowed

inactivation of $Ca_V1.2$ channels by strongly increasing the total charges [119]. Together with the mutation of *SCN5A* ($Na_V1.5$ sodium channel) in this case, the sodium current reduction can cause loss of the right ventricular epicardial AP dome absent from the slowed inactivation of calcium current and slowed conduction, whereas p. D601E polymorphism could restore the dome of APs [119]. These results suggest the gain-of-function mutation in calcium channel can rescue the loss-of-function of sodium channel mutation in the process of cardiac conduction disease without BrS. It is notable that the p.D601E polymorphism in *CACNB2b* was also found in the BrS, and this polymorphism could increase the late calcium current [102], indicating the multiply functions of p. D601E polymorphism.

Idiopathic ventricular fibrillation and early repolarization syndrome

Early repolarization has been found in patients with idiopathic ventricular fibrillation (IVF) [120,121], these IVF associated with early repolarization was also called early repolarization syndrome (ERS). Mutations in the LTCC genes, including *CACNA1C* and *CACNB2b*, have been found to associate with both diseases. Burashnikov et al. reported *CACNB2b* p.A73V mutation was associated with IVF; and *CACNB2b* p.S160T or p.R571C, *CACNA2D1* p.S956T, and *CACNA1C* p.E850 deletion had possible roles in ERS [102]. The glutamic acid at 850 position is located within the PEST domain of $Ca_V\alpha_1$ II-III loop. The mutations in the PEST domain are proved to affect the surface membrane expression of $Ca_V1.2$ channels [84]. Functionally, p.E850 deletion in *CACNA1C* is found to lead to an almost complete loss-of-function in I_{Ca} [90]. Thus, the p. E850 deletion might decrease the surface expression of LTCC presumably because of an aberrant protein degradation, which induces a dramatic reduction of I_{Ca}.

Bradycardia and sinoatrial arrhythmia

Autorhythmicity of sinoatrial nodal pacemaker cells results from the slow auto-depolarization as a result of $Ca_V1.3$ calcium influx [122,123]. The inactivated $Ca_V1.3$ channels in mice induced strong reduction of calcium current in pacemaker cells and profoundly affected its pacemaking effect, which showed predominant sinoatrial nodal dysfunction [124]. With the inactivated $Ca_V1.3$ calcium currents, both sinoatrial arrhythmia and bradycardia have been observed [23,125]. A homozygous 3-bp insertion in *CACNA1D*, inducing p. G403_404ins, was first screened from a Pakistani family with pronounced bradycardia. This p. G403_404ins, located at alternative spliced exon 8b of $Ca_V\alpha_{1D}$, resulted in nonconducting $Ca_V1.3$ channels [126], which may explain the phenotype of bradycardia.

Challenges and future diagnostic or therapeutic approaches

Mosaicism implies that in an organism from a single zygote that is the presence of genetically distinct cell line [127]. Taken TS as an example, the p.G406R mutation in exon 8 could be detected in the oral mucosa sample but not in the blood sample of the patients' mother [61], whereas in the case of a Chinese girl with a typical TS phenotype, the G406R mutation also expressed in her father's oral mucosa, sperm and white blood cell [128]. Additionally, cardiac arrhythmias also have phenotypic heterogeneity. The genotype-negative or even phenotype-negative LQT8 patients had been identified several novel mutations in *CACNA1C* [82,88]. Hence, careful screening of parental tissue in family with incidence of congenital cardiac arrhythmias, prenatal DNA screening, and monitoring of the fetal echocardiogram are extremely significant for the congenital cardiac arrhythmia. Remarkably, whole-genome or whole-exome sequencing [129] may be necessary for the identification of potential mutations of LTCC in cardiac arrhythmias.

LTCC has many variants because of transcriptional and post-transcriptional modification, which is complicate and may be the future challenges of the field. For example, alternative splicing, one of most important mechanisms of post-transcriptional modification [130,131], generates more than 20 alternative spliced exons in α_{1C} mRNA, which forms many variants of α_{1C} [14,60,132]. It has been indicated the mutations

in these alternative spliced exons of LTCC can also attribute to the abnormal cardiac excitation [61,62,126]. Therefore, it is noteworthy to screen the potential mutations in these alternative spliced exons of LTCC in the patients with cardiac arrhythmias.

To date, most functional studies utilized heterologous systems expressing WT or mutant calcium channels in the cultured cells, which do not fully recapitulate the phenotypes of cardiomyocytes. With the advent of iPSC, the concept of cellular reprogramming has been revolutionized, which makes producing unique human iPSC from somatic cells possible [133], then induced to specific cells. TS patient-specific iPSC-derived cardiomyocyte (iPSC-CM) carrying a missense mutation p.G406R in the $Ca_V1.2$ had been generated by traditional method or genetically encoded fluorescent indicators, which increases the inward calcium currents leading to prolonged QT interval [72,134]. Recently, genetic mutations could be directly introduced into generic embryonic stem cell or non-diseased iPSCs by genome editing techniques, to produce the phenotypes of cardiac arrhythmia [135], providing an exciting approach to drug screening in the iPSC-CM-based disease model. However, iPSC techniques have several limitations when being used as a treatment strategy for the diseased heart, e.g. the stem cell-derived cardiomyocytes being cocultured with native cardiomyocytes showed an slow conduction velocity [136] and inadequate excitation contraction coupling [137]. Nevertheless, the iPSC approach could potentially be a powerful tool for diagnosis and prognosis of cardiac arrhythmias and provide a robust assay for developing new drugs to treat these diseases [138–140]. Moreover, isogenetic control of iPSCs provides a method to reveal the pathogenic mechanism underlying the specific disease phenotype. In brief, the iPSC-based technology will play a central role in specific aspects of translational medicine [141].

It has been understood that major LTCC mutation-associated cardiac arrhythmias are attributed to the over-activities of calcium channel functions, which are due to the gain-of-function mutations in LTCC. However, the treatment of cardiac arrhythmias by calcium channel blockers meets a lot of challenges, e.g. limited range of available choices, and insufficient efficacy [2,142]. Moreover, the LTCC channel blockers, which have been used in clinic for decades, have none or very little selectivity for the different LTCC variants to some extent. At the same time, these drugs induce a lot of adverse effect risks because of off-target effects. Therefore, developing highly selective calcium channel blockers targeting different variants of LTCC, or even mutant LTCC will be much attractive in the management of these mutation-associated cardiac arrhythmias.

Conclusions

Taken together, the mutations of the LTCC genes can cause different cardiac arrhythmias, including LQTS, TS, BrS, SQTS and other cardiac arrhythmias, which are critically associated with increased risks of cardiovascular diseases, syncope and even SCD. The mechanisms how these mutations cause the distinct cardiac arrhythmias are dependent on their different roles in the channel functions (structure-function mechanisms), thus more efforts on the crystal structure analysis of the LTCC are appreciated to understand these mutation-associated cardiac arrhythmias. Nevertheless, there are some challenges in the detection, diagnosis and treatment of cardiac arrhythmias at present, the future studies will be necessary to get a better understanding of the roles of LTCC in the context of cardiac arrhythmias, which will be much valuable for the diagnosis and even management of these diseases.

Disclosure statement

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Author Contributions

Q.Z., J.C., Y.Q. and J.W. drafted the manuscript. J.W. prepared the figures. J.W. edited the manuscript. L.Z. read and gave the comments to this work.

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