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Cardiac L-type calcium channel regulation by Leucine-Rich Repeat-Containing Protein 10

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ABSTRACT

L-type calcium channels (LTCCs), the major portal for Ca^{2+} entry into cardiomyocytes, are essential for excitation-contraction coupling and thus play a central role in regulating overall cardiac function. LTCC function is finely tuned by multiple signaling pathways and accessory proteins. Leucine-rich repeat-containing protein 10 (LRRC10) is a little studied cardiomyocyte-specific protein recently identified as a modulator of LTCCs. LRRC10 exerts a remarkable effect on LTCC function, more than doubling L-type Ca^{2+} current ($I_{Ca,L}$) amplitude in a heterologous expression system by altering the gating of the channels without changing their surface membrane expression. Genetic ablation of LRRC10 expression in mouse and zebrafish hearts leads to a significant reduction in $I_{Ca,L}$ density and a slowly progressive dilated cardiomyopathy in mice. Rare sequence variants of LRRC10 have been identified in dilated cardiomyopathy and sudden unexplained nocturnal cardiac death syndrome, but these variants have not been clearly linked to disease. Nevertheless, the DCM-associated variant, I195T, converted LRRC10 from a $I_{Ca,L}$ potentiator to a $I_{Ca,L}$ suppressor, thus illustrating the wide dynamic range of LRRC10-mediated $I_{Ca,L}$ regulation. This review focuses on the contemporary knowledge of LTCC modulation by LRRC10 and discusses potential directions for future investigations.

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Introduction

Calcium ions (Ca^{2+}) are indispensable for cardiac function. With every heartbeat, Ca^{2+} from the extracellular space enters the cardiomyocyte via the voltage-gated L-type Ca^{2+} channel (LTCC) [1,2]. This Ca^{2+} influx ($I_{Ca,L}$) triggers intracellular Ca^{2+} release from the sarcoplasmic reticulum (SR) through the ryanodine receptor (RyR) [3,4]. The consequence of this Ca^{2+}-induced Ca^{2+} release (CICR) is a temporary increase in cytosolic Ca^{2+} concentration known as the Ca^{2+} transient (CaT). During the CaT, cytosolic Ca^{2+} binds to the contractile machinery (myofilaments) of the cardiomyocyte and initiates contraction [5]. Cellular relaxation ensues as Ca^{2+} is sequestered back into the SR and extruded out of the cell, lowering the cytosolic Ca^{2+} to submicromolar concentrations at rest [6]. Given the absolute reliance of cardiac excitation-contraction (EC) coupling on Ca^{2+}, it is not surprising that LTCC is a major effector of physiological processes that regulate cardiac function, including β-adrenergic signaling [7]. In addition, aberrancies of $I_{Ca,L}$ and intracellular Ca^{2+} homeostasis have been implicated in a wide range of cardiac diseases, including multiple forms of heart failure and arrhythmias [8,9].

The cardiac LTCC is a hetero-oligomeric protein complex consisting of the pore-forming subunit (either $Ca_V1.2$ encoded by the *CACNA1C* gene or $Ca_V1.3$ encoded by the *CACNA1D* gene) and two auxiliary subunits (an intracellular β_2 subunit encoded by the *CACNB2* gene and an extracellular $\alpha_2\delta$-1 subunit encoded by the *CACNA2D1* gene). $Ca_V1.2$ is expressed in cardiomyocytes throughout the heart, whereas $Ca_V1.3$ expression is limited to cardiomyocytes in the atria and conduction system (sinoatrial node, atrioventricular node, and Purkinje fibers) [10]. Thus, in adult ventricular cardiomyocytes, the $Ca_V1.2$ subunit is responsible for voltage-dependent gating and selective permeation of Ca^{2+}. The β_2 and $\alpha_2\delta$-1 subunits are important for efficient trafficking and functional modulation of $Ca_V1.2$.

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(See recent reviews by Catterall [11] and Yao et al. [12] for detailed discussion on the structural biology of voltage-gated Ca^{2+} channels). All three subunits are subjected to alternative splicing and posttranslational modifications, which lead to the combinatorial diversification of LTCCs [13–16]. Moreover, interactions between these classical subunits and other proteins add another layer of complexity to LTCC regulation. New LTCC-interacting accessory proteins that contribute to $I_{Ca,L}$ modulation continue to be discovered. Rad, for example, has been identified as an LTCC inhibitor that interacts with the β_2 subunit to suppress $I_{Ca,L}$, and dissociation of Rad from the LTCC complex allows $I_{Ca,L}$ to increase in response to β-adrenergic stimulation [17].

Recently, leucine-rich repeat-containing protein 10 (LRRC10), a protein specifically expressed in cardiomyocytes has been identified as a potent LTCC regulator capable of augmenting both $I_{Ca,L}$ in heterologous expression systems and native $I_{Ca,L}$ in cardiomyocytes [18]. This contemporary review summarizes how the research trajectories of LRRC10 and LTCC converged, discusses the knowns and unknowns of cardiac LTCC regulation by LRRC10, and suggests potential avenues for future investigations.

Discovery of LRRC10 and its roles in cardiac biology and disease

LRRC10, also known as heart-restricted leucine-rich repeat protein (HRLRRP) and Serdin1, was discovered in the early 2000s through *in silico* screenings of multispecies cDNA libraries for cardiac-specific expressed sequence tags (ESTs) [19–21]. It is a member of the leucine-rich repeat (LRR)-containing protein superfamily, which includes more than 300 proteins that mediate a wide range of physiological processes, such as cell adhesion, innate immune signaling, and neuronal development [22,23]. The role of LRR-containing proteins as ion channel regulators is exemplified by LRRC26 and its paralogs (LRRC38, LRRC52, and LRRC55), which have been identified as transmembrane γ subunits of the large-conductance Ca^{2+}-activated K^+ channel ($K_{Ca}1.1$, also known as BK channel) [24,25]. Unlike these K_{Ca}-γ subunits and many other LRR-containing proteins, LRRC10 does not contain any predicted transmembrane or catalytic domain apart from the LRR motifs. Almost 60% of the amino acid residues of LRRC10 are devoted to seven LRRs at the center of the protein, which fold into a solenoid-shaped structure that commonly mediates protein–protein interactions (Figure 1) [22,26], suggesting that LRRC10 functions by binding to its target protein and/or by participating in macromolecular complex formation as an adaptor protein.

The cardiomyocyte-specific expression of LRRC10 is regulated by cardiac transcription factors, including NK2 homeobox 5 (NKX2–5), GATA binding protein 4 (GATA4), serum response factor (SRF), and myocyte enhancer factor 2C (MEF2C) [27,28]. Cardiac specificity of LRRC10 expression is conserved in vertebrates, including zebrafish, mice, rats, and humans [19–21]. Knockout and knockdown studies in various animal models have revealed insights into the biological importance of LRRC10. Morpholino-mediated knockdown of LRRC10 in zebrafish embryos led to cardiac looping defects, decreased number of cardiomyocytes, systolic dysfunction (impaired cardiac contractility), and embryonic lethality [20]. However, later genetic knockout studies in zebrafish and mice reported less severe phenotypes that permit survival to adulthood [29–31], suggesting that the lethal cardiac development failure might be unique to the morpholino knockdown approach. Germline knockout of LRRC10 in mice did not cause looping defects in the developing heart but still resulted in prenatal systolic dysfunction, which progressed to postnatal dilated cardiomyopathy (DCM) [30]. *Lrrc10*$^{-/-}$ mice also showed more severe systolic dysfunction compared to wild-type mice following transverse aortic constriction (TAC), which is a commonly used experimental procedure to induce pressure overload to the left ventricle [29]. In addition, LRRC10 has recently been implicated in cardiac regeneration in Mexican tetra fish (*Astyanax mexicanus*) [31], zebrafish [31,32], and neonatal mice [33].

LRRC10 variants have been associated with cardiac diseases in humans. Two missense variants, L41V and L163I were detected in two Chinese Han families with familial dilated cardiomyopathy (DCM) inherited as an autosomal dominant trait [34]. Two other missense variants, P69L and E129K were identified in cases of sudden unexplained nocturnal cardiac death syndrome

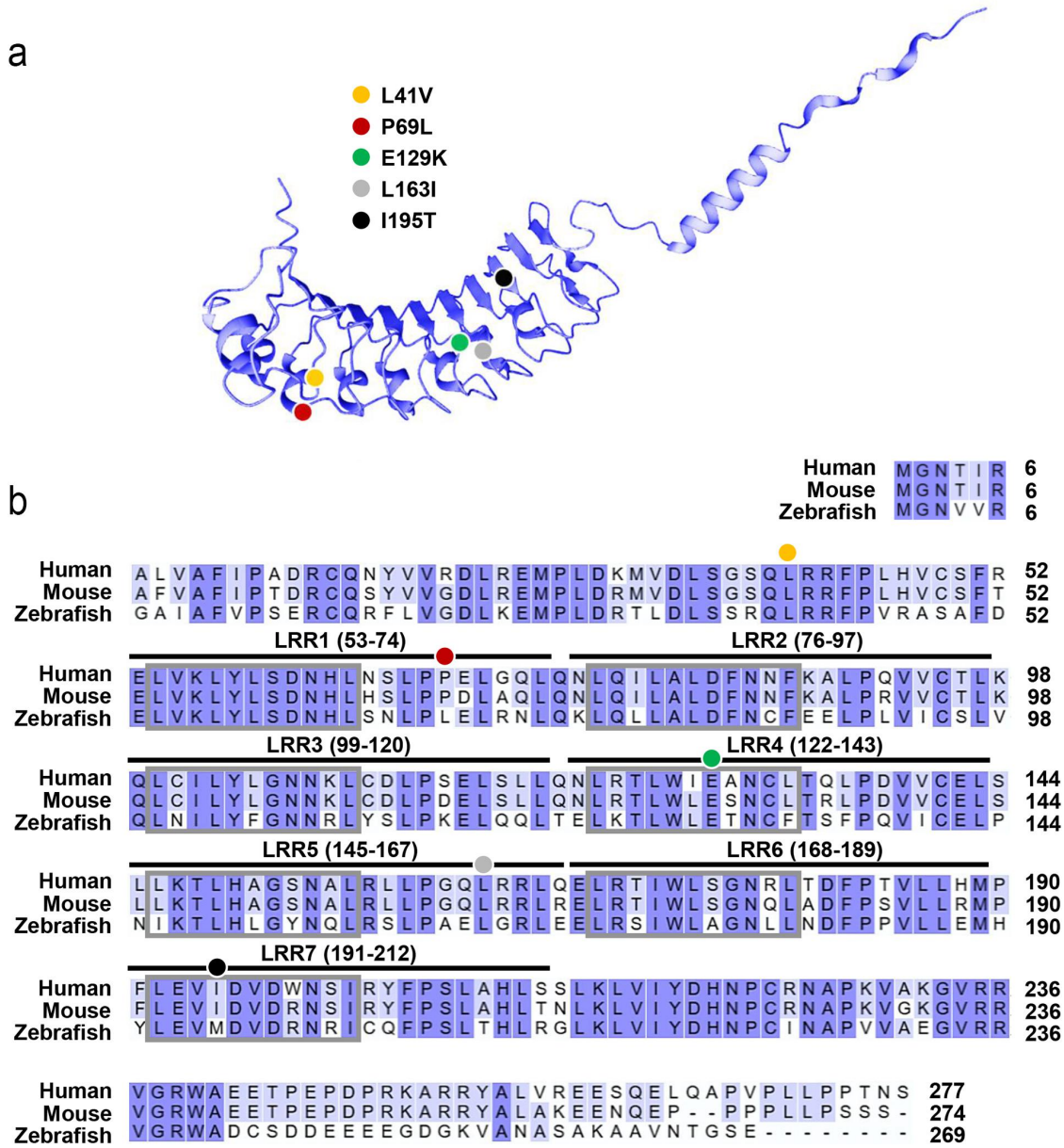

Figure 1. LRRC10. (a) Tertiary structure of human LRRC10 as predicted by AlphaFold [26]. (b) Alignment of amino acid sequences of LRRC10 in humans, mice, and zebrafish. Blue and light blue highlights denote amino acid residues that are conserved between three and two species, respectively. Black lines indicate the seven leucine-rich repeats (LRR) containing the highly conserved segments (HCS) "LxxLxLxxNxL" in gray boxes. Colored dots in both panels indicate the positions of variant amino acid residues discussed in this review.

(SUNDS) [35]. Another DCM-associated variant, I195T, was found by trio-based whole-exome sequencing in a sporadic case of fulminant pediatric DCM [18]. However, the evidence to date does not identify LRRC10 variants as pathogenic given the limited pedigrees and mechanistic understanding. For example, the I195T variant showed a potential autosomal recessive inheritance pattern, but the proband's unaffected brother also inherited I195T on both alleles of LRRC10. Nevertheless, the dramatically distinct functional effects of wild type and I195T LRRC10 on $I_{Ca,L}$ described later suggest that this variant can potentially act as a disease modifier.

Subcellular localization of LRRC10

Although LRRC10 contains a putative nuclear localizing sequence [19,21], its localization varies depending on the model system and maturation

Table 1. Evidence supporting the association between LRRC10 and cardiac LTCC.

Models	Detection methods	Major findings	Refs
Adult rat cardiomyocytes	IF	LRRC10 localized to the Z-disc/dyad region.	[19]
Adult mouse ventricular cardiomyocytes	IF	LRRC10 localized to the Z-disc/dyad region.	[29,30,36]
Adult zebrafish cardiomyocytes	IF, patch clamp	LRRC10 co-localized with $Ca_V1.2$ and modulated $I_{Ca,L}$.	[32]
Adult mouse ventricular cardiomyocytes	Co-IP, patch clamp	LRRC10 co-precipitated with $Ca_V1.2$ and modulated $I_{Ca,L}$.	[18]
Transient expression of rabbit $Ca_V1.2$ in HEK-293 cells (without auxiliary subunits)	Co-IP, patch clamp	LRRC10 co-precipitated with $Ca_V1.2$ and modulated $I_{Ca,L}$.	[18]
Transient expression of rabbit $Ca_V1.2$ in HEK-293 cells (with auxiliary subunits)	FRET, patch clamp	LRRC10 interacted with $Ca_V1.2$ and modulated $I_{Ca,L}$.	[38]

Co-IP, co-immunoprecipitation; FRET, Förster/fluorescence resonance energy transfer; IF, immunofluorescence.

state. Heterologously expressed LRRC10 accumulated in the nuclei and mitochondria of COS7 cells [21]. However, in HEK-293 and NIH/3T3 cells, the heterologous expression pattern of LRRC10 was diffuse [19]. Nuclear localization of native LRRC10 was also reported in immature, proliferating cardiac cells, such as HL-1 (an immortalized cell line derived from the AT-1 mouse atrial cardiomyocyte tumor lineage) [19], cardiomyocytes differentiated from P19 embryonal carcinoma cells [19], and embryonic and neonatal mouse cardiomyocytes [19,36]. On the contrary, in adult zebrafish and adult mammalian cardiomyocytes, LRRC10 is predominantly localized to the Z-disc/dyadic region [19,29,30,32,36]. The cardiac dyad is a subcellular domain near the Z-disc (sarcomeric boundary) of the cardiomyocyte, where the T-tubule interfaces with the sarcoplasmic reticulum (SR). The two juxtaposing membranes and the narrow dyadic space between them are enriched with proteins involved in Ca^{2+} signaling, including LTCC, RyR, and their modulators [37]. Indeed, confocal immunofluorescence imaging of adult mammalian cardiomyocytes has demonstrated co-localization of LRRC10 with $Ca_V1.2$ as well as other components of the Z-disc/dyad, including RyR, actin, and actinin [19,29,30,32,36].

Association of LRRC10 with $Ca_V1.2$ LTCC complex

The localization of LRRC10 to the Z-line along with the identification of I195T LRRC10 in a case of infantile DCM led to the initial study investigating whether LRRC10 associated with and regulated LTCCs as well as played a potential role in DCM [18]. Co-immunoprecipitation (co-IP) experiments provided evidence of the association of LRRC10 and $Ca_V1.2$. LRRC10 coprecipitated with $Ca_V1.2$ in mouse heart lysates [18]. Interestingly, when lysates of HEK-293 cells expressing LRRC10 and $Ca_V1.2$ (without the auxiliary subunits) were used for co-IP, LRRC10 still coprecipitated with $Ca_V1.2$ [18]. Therefore, the interaction between LRRC10 and $Ca_V1.2$ does not require the β_2 and $\alpha_2\delta$-1 subunits of LTCC. This finding is recently supported by consistent results from a Förster/fluorescence resonance energy transfer (FRET) assay [38]. These data, however, do not prove a direct interaction between LRRC10 and $Ca_V1.2$ but do at least suggest LRRC10 and $Ca_V1.2$ are associated in a macromolecular complex.

The aforementioned findings from confocal immunofluorescence imaging, co-IP, and FRET that support the association between LRRC10 and LTCC are summarized in Table 1. The following section discusses functional modulation of cardiac LTCC/$I_{Ca,L}$ by LRRC10.

Modulation of $I_{Ca,L}$ by LRRC10

The functional impact of LRRC10 on $I_{Ca,L}$ was first studied in a heterologous expression system (HEK-293 cells), which offers control over LTCC subunit composition and the presence or absence of LRRC10. Co-expression of LRRC10 with the LTCC complex ($Ca_V1.2$, β_2, and $\alpha_2\delta$-1 subunits) in HEK-293 cells resulted in an approximately 1.4-fold increase in the maximal magnitude of $I_{Ca,L}$ (Figure 2). In addition, LRRC10 co-expression shifted the voltage of half-maximal $I_{Ca,L}$ activation ($V_{1/2}$) ≈4 mV in the hyperpolarizing direction and increased the slope (i.e. decreased the Boltzmann distribution

k parameter) of the $I_{Ca,L}$ activation curve. LRRC10 also increased the slope (decreased k) of the steady-state $I_{Ca,L}$ inactivation curve and suppressed the residual (slowly inactivating) current, but the $V_{1/2}$ of inactivation remained unchanged. These effects were preserved even when the β_2, and $\alpha_2\delta$-1 subunits were omitted, corroborating the co-IP finding that LRRC10 was associated with $Ca_V1.2$ in an auxiliary subunit-independent manner. Consistent with the observations in the heterologous systems, $I_{Ca,L}$ was smaller in ventricular cardiomyocytes isolated from *Lrrc10*$^{-/-}$ mice compared to wild-type [18]. Likewise, LRRC10 knockout in zebrafish resulted in cardiac $I_{Ca,L}$ that was smaller, decayed faster, and inactivated less completely (leaving a larger residual current) [32]. Of note, the plasma membrane expression of $Ca_V1.2$ in HEK-293 cells was not altered by LRRC10, and the total amounts of $Ca_V1.2$ in wild-type and *Lrrc10*$^{-/-}$ mouse ventricular cardiomyocytes were not different [18], suggesting that the increased macroscopic (whole-cell) $I_{Ca,L}$ in the presence of LRRC10 is due to changes in the gating of the channels (increased P_o) although an impact on unitary conductance cannot be excluded. These findings are summarized in Table 2.

So far, the effects of genetic polymorphism of LRRC10 on its $I_{Ca,L}$ modulating function have been characterized only in the DCM-associated I195T variant. The variant residue is a highly conserved isoleucine in the seventh LRR. In contrast to wild-type LRRC10, the I195T variant markedly reduced $I_{Ca,L}$ magnitude, shifted the activation curve in the depolarizing direction and increased the residual current [18]. The exact pathogenetic mechanism of DCM in the patient harboring the I195T variant is still not known. The increased slowly inactivating (late) $I_{Ca,L}$ might contribute to DCM development and progression by producing chronic Ca^{2+} overload in the cardiomyocyte, while the reduction of peak $I_{Ca,L}$ and the depolarized voltage dependence of

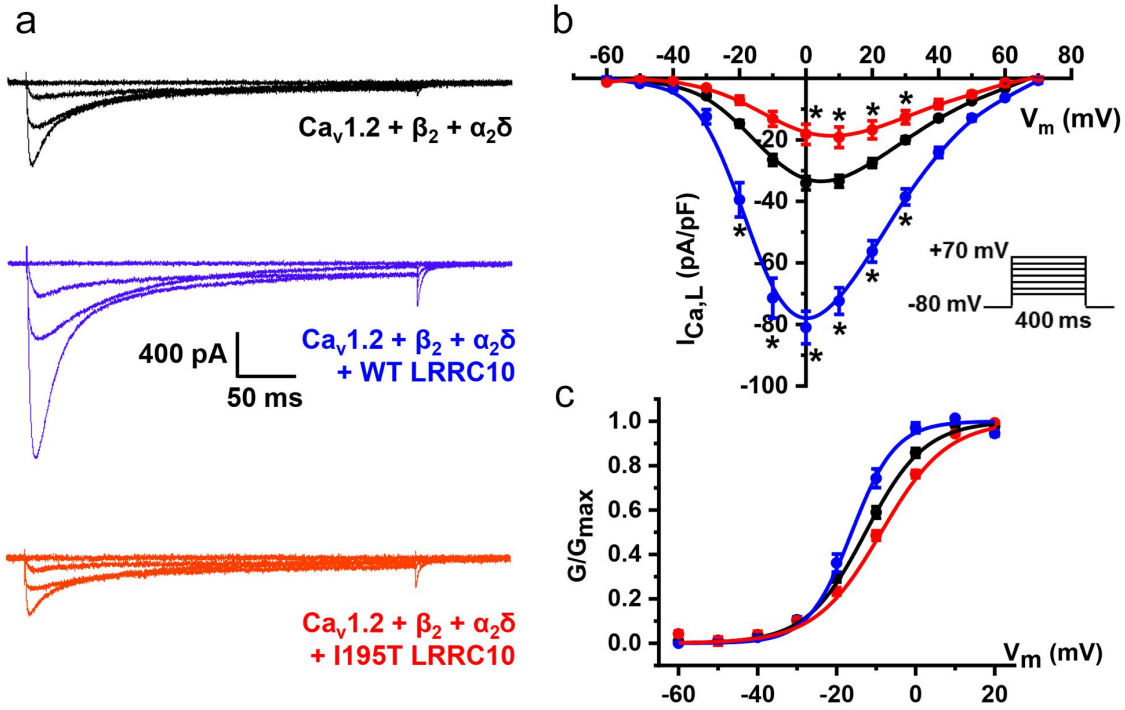

Figure 2. Modulation of $I_{Ca,L}$ by LRRC10. (a) Representative $I_{Ca,L}$ traces (at −60, −30, 0, and +30 mV) recorded from HEK-293 cells expressing the LTCC complex ($Ca_V1.2$, β_2, and $\alpha_2\delta$) without LRRC10 (black), with wild-type (WT) LRRC10 (blue), or with I195T LRRC10 (red). (b) Current-voltage relationship of $I_{Ca,L}$ in HEK-293 cells under the three different conditions, plotted in colors corresponding to (a). Asterisks (*) denote statistically significant differences ($p < 0.05$) compared to $I_{Ca,L}$ in HEK-293 cells expressing LTCC without LRRC10. The voltage clamp protocol used to elicit $I_{Ca,L}$ is shown in the inset. (c) Voltage-dependent activation curves of $I_{Ca,L}$ under the three different conditions, plotted in colors corresponding to (a). Note that the activation curve is shifted to the left with co-expression of WT LRRC10, whereas I195T LRRC10 shifts the activation curve to the right. Reproduced with modification from [18].

Table 2. Modulation of $I_{Ca,L}$ by LRRC10.

	Effects of LRRC10 on $I_{Ca,L}$								
			Voltage dependence of activation		Voltage dependence of steady-state inactivation				
Models	Current magnitude	Kinetics of inactivation (decay rate)	$V_{1/2}$	k	$V_{1/2}$	k	Residual current	Other major findings	Refs
Transient expression of rabbit $Ca_V1.2$ in HEK-293 cells (without auxiliary subunits)	↑	N/A	HP	↓	↔	↓	↓ (at 20 mV)	N/A	[18]
Transient expression of rabbit $Ca_V1.2$ in HEK-293 cells (with human β_{2cN2} and rabbit $\alpha_2\delta$-1)	↑	N/A	HP	↓	↔	↓	↓ (at 20 mV)	↔ Surface membrane$Ca_V1.2$ protein	[18]
Adult mouse ventricular cardiomyocytes (WT vs. *Lrrc10*$^{-/-}$) *	↑	N/A	↔	↓	HP	↔	↔ (at 20 mV)	↔ Total $Ca_V1.2$ protein	[18]
Adult zebrafish cardiomyocytes(WT vs. *lrrc10*$^{-/-}$) *	↑	↑	↔	↔	↔	↔	↓ (at −10 to 20 mV)	↔ Recovery from inactivation	[32]
Transient expression of rabbit $Ca_V1.2$, human β_{2b} and rat brain $\alpha_2\delta$ in HEK-293 cells	↑	N/A	N/A	N/A	N/A	N/A	N/A	N/A	[38]

*For knockout and knockdown models, the findings summarized here are those in the *presence* of LRRC10 compared to the *absence/reduction* of LRRC10. DP, depolarized; HP, hyperpolarized; N/A, not assessed or not applicable; WT, wild-type.

$I_{Ca,L}$ activation could impair cardiac contractility. Further studies are required to test this hypothesis.

A recent study [38] demonstrated that N-terminus-truncated LRRC10 still robustly interacted with $Ca_V1.2$, but the $I_{Ca,L}$-potentiating effect was markedly reduced. The N-terminus of LRRC10 alone hardly interacted with $Ca_V1.2$. However, the ability to augment $I_{Ca,L}$ was rescued when the LRRC10's N-terminus was fused with a nanobody that targeted the β subunit of LTCC (in order to bring the LRRC10's N-terminus into close proximity with $Ca_V1.2$). This fusion protein construct (named "genetically encoded enhancer of Ca^{2+} current" or "GeeC") was capable of increasing $I_{Ca,L}$ in a heterologous expression system (HEK-293 cells), mouse ventricular cardiomyocytes, and mouse cortical and hippocampal neurons. Thus, it was proposed that the N-terminus is the "minimal effector domain" of LRRC10, whereas the rest of the molecule (the LRRs and the C-terminus) serves as the "high-affinity binding domain." GeeC did not alter plasma membrane expression of LTCC, and single-channel recordings revealed that GeeC upregulated $I_{Ca,L}$ by increasing P_O without changing the unitary conductance [38].

Perspective and future directions

Although accumulating evidence suggests that LRRC10 interacts (directly or indirectly) with $Ca_V1.2$ and modulates cardiac $I_{Ca,L}$, the role of this regulation in normal cardiac physiology and pathophysiology remains largely unknown. Furthermore, the molecular nature of this regulation remains to be more fully defined. For example, it is still not known how $Ca_V1.2$ and LRRC10 associate. Since LRRC10 is a cytosolic protein, it may bind to one or more segments of $Ca_V1.2$'s N-terminus, the intracellular loops that link the four transmembrane domains, or the C-terminus. The N-terminus of $Ca_V1.2$ is an auto-inhibitory module and contains a Ca^{2+}/calmodulin-dependent kinase II (CaMKII)-binding site that offsets the intrinsic inhibition [39,40]. The C-terminus undergoes proteolytic processing, which produces the distal-C-terminus segment that re-associates with the truncated $Ca_V1.2$ and exerts an auto-inhibitory control [41]. In addition, certain amino acid residues on the cytosolic aspect of the $Ca_V1.2$ pore, collectively called the annular determinant of slow inactivation (ADSI), are important for the voltage-dependent slow inactivation of $I_{Ca,L}$ [42]. Thus, there are many possible molecular mechanisms by which LRRC10 may exert its $I_{Ca,L}$-potentiating effects.

Intriguingly, the I195T variant did not merely attenuate or neutralize LRRC10's effect on LTCC (loss of function). Rather, it reversed the directions of LRRC10's influence on multiple $I_{Ca,L}$ parameters (the effect on $I_{Ca,L}$ magnitude, for example, changed from ≈140% increase with wild-type LRRC10 to ≈40% decrease with I195T LRRC10). Therefore, the dynamic range of LTCC modulation by LRRC10 and its variants is wide. It would be interesting to test how other potentially clinically relevant LRRC10 variants (e.g. L41V, P69L, E129K, and L163I) affect LTCC function. Perhaps one or more of these could be a gain-of-function variant, which would extend the dynamic range of LTCC modulation even further. Do these variants suggest that LRRC10 may be the target of post-translational modifications or second messenger regulation that may dynamically regulate LTCC function? Precedence for accessory protein regulation of LTCCs is present with the remarkable regulation of LTCCs by RAD signaling [17].

Since LRRC10 predominantly localizes to the dyads in mature cardiomyocytes, dyadic LTCCs (≈75% of total LTCCs), which mediate CICR, are presumably the principal LTCC subpopulation regulated by it. However, it is possible that LRRC10 also interacts with extradyadic LTCCs (≈25% of total LTCCs), including those in caveolae associated with caveolin-3 scaffolds [43]. Caveolar and caveolin-3 scaffold-associated LTCCs are parts of distinct macromolecular signaling complexes instrumental in the β_2-adrenergic response (as opposed to dyadic LTCCs being coupled to the β_1-adrenergic signaling) [44]. Ca^{2+} influx through caveolar/caveolin-3-associated LTCCs has also been linked to the activation of calcineurin/nuclear factor of activated T-cell (NFAT) hypertrophic signaling cascade [45]. In mice subjected to TAC, calcineurin inhibition not only attenuated cardiac hypertrophy but also resulted in decompensation and heart failure [46], which is reminiscent of the intolerance to TAC-induced pressure overload seen in *Lrrc10*$^{-/-}$ mice [29]. Scaffolding by non-caveolar caveolin-3 also enables LTCC-CaMKII coupling, which has been shown to be disrupted by heart failure [47]. It is tempting to speculate that LRRC10 also tunes these signaling pathways via its $I_{Ca,L}$-modulating effect. Future studies are needed to test this hypothesis as well as to define other roles of LRRC10 in microdomain-specific signalosomes.

LRRC10 plays an essential role in cardiac regeneration in zebrafish and neonatal mouse hearts, but how this may be related to LRRC10's regulation of LTTCs remains incompletely understood. In a zebrafish cardiac cryoinjury model, whole-heart optical mapping revealed aberrant Ca^{2+} transient dynamics in the injury border zone of *lrrc10*$^{-/-}$ hearts [32]. Specifically, in WT zebrafish hearts, Ca^{2+} transient durations in the border zone shortened only temporarily during the early regeneration phase (≈14 days) following the cryoinjury. In contrast, *lrrc10*$^{-/-}$ zebrafish hearts, which lost the regenerative capacity, exhibited persistent shortening of Ca^{2+} transient durations in the border zone. Subsequent cytoarchitectural changes in injured *lrrc10*$^{-/-}$ cardiomyocytes include sarcomeric disarray and impaired dyadic formation [32]. The mechanistic links among these phenomena are yet to be elucidated.

The role of LRRC10 in disease states impacting the heart remains largely unknown. However, some evidence from the literature demonstrates that LRRC10 may contribute to the pathophysiology. For instance, in a mouse model of Lodder-Merla syndrome, a multisystem disorder caused by pathogenic variants of the *GNB5* gene encoding the guanine nucleotide-binding protein subunit β_5, *Lrrc10* mRNA is upregulated in the heart along with other genes related to cardiomyocyte excitation and contraction, such as *Scn10a*, *Myh7*, and *Tnnt2* [48,49]. This is another interesting area to explore to gain more insights into the biology of LRRC10.

Lastly, it is worth noting that LTCC complexes consisting of $Ca_V1.3$ contribute to $I_{Ca,L}$ in the sinoatrial node, atrioventricular node, Purkinje fibers, and atria [10]. In sinoatrial nodal cells [50], $Ca_V1.3$ can contribute up to ≈50–70% of $I_{Ca,L}$ [51,52]. The N-terminus of LRRC10 in the GeeC construct (see above), being guided to LTCCs by a $Ca_V\beta$-targeting nanobody, has been reported to augment $I_{Ca,L}$ through $Ca_V1.3$ in HEK-293 cells [38]. It will be important to test whether the native full-length LRRC10 is also capable of associating with and modulating $Ca_V1.3$.

Conclusion

LRRC10 is now recognized as a cardiac LTCC accessory protein, yet the molecular mechanism of its action and its physiological significance are only starting to be defined. Many remaining questions, some highlighted above, await further investigation. Novel experimental models such as conditional knockout mice and human induced-pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) may provide additional insights into the roles of LTCC regulation by LRRC10 in cardiac biology and diseases. The cardiac-specific expression of LRRC10 gives cardiac LTCCs a unique signature and regulation compared to LTCCs present in other tissues in the body. A better understanding of how LRRC10 impacts the trans-sarcolemmal Ca^{2+} influx and intracellular Ca^{2+} homeostasis may enable new preventive and/or therapeutic strategies for cardiomyopathy, heart failure, and arrhythmias.

Disclosure statement

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Author contributions

NS and TJK conceptualized the review, reviewed and interpreted the literature, drafted and revised the manuscript, and approved the version of manuscript to be published. All authors agree to be accountable for all aspects of the work.

Data availability statement

Data availability is not applicable as no new data were generated for this review article.

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