Precision medicine in catecholaminergic polymorphic ventricular tachycardia: Recent advances toward personalized care

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ABSTRACT

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a rare inherited cardiac ion channelopathy where the initial disease presentation is during childhood or adolescent stages, leading to increased risks of sudden cardiac death. Despite advances in medical science and technology, several gaps remain in the understanding of the molecular mechanisms, risk prediction, and therapeutic management of patients with CPVT. Recent studies have identified and validated seven sets of genes responsible for various CPVT phenotypes, including RyR2, CASQ-2, TRDN, CALM1, 2, and 3, and TECRL, providing novel insights into the molecular mechanisms. However, more data on atypical CPVT genotypes are required to investigate the underlying mechanisms further. The complexities of the underlying genetics contribute to challenges in risk stratification as well as the uncertainty surrounding nongenetic modifiers. Therapeutically, although medical management involving beta-blockers and flecainide, or insertion of an implantable cardioverter defibrillator remains the mainstay of treatment, animal and stem cell studies on gene therapy for CPVT have shown promising results. However, its clinical applicability remains unclear. Current gene therapy studies have primarily focused on the RyR2 and CASQ-2 variants, which constitute 75% of all CPVT cases. Alternative approaches that target a broader population, such as CaMKII inhibition, could be more feasible for clinical implementation. Together, this review provides an update on recent research on CPVT, highlighting the need for further investigation of the molecular mechanisms, risk stratification, and therapeutic management of this potentially lethal condition.

Keywords: Catecholaminergic polymorphic ventricular tachycardia, gene therapy, risk stratification, stress-induced arrhythmias

INTRODUCTION

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a rare, potentially lethal inherited cardiac ion channelopathy characterized by physical or emotional stress-induced bidirectional or polymorphic ventricular tachycardia (VT). Since its characterization by Coumel *et al.* in the 1970-1980s,[1] multiple genotypes and their genetic substrate have been discovered and contributed to the phenotypic variants we now observe. CPVT mainly affects children and adolescents, resulting in syncope and sudden cardiac death at a very early

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age, with high mortality of up to 31%, and 4- and 8-year cardiovascular event rates being up to 33% and 58%, respectively.[2] There is a high need for efficient intervention and management of CPVT.

Despite rapid development in medical sciences and technology, there is a lack of a comprehensive review of the recent advances in molecular mechanisms, risk prediction, and therapeutic management among CPVT patients.[3] In clinical practice, about 40% of patients carry unidentified disease-specific mutations.[4] With the establishment of new genetic and molecular techniques, new information has been elucidated with regard to the cellular mechanisms underlying CPVT. The central role of the dysregulation of intracellular calcium concentration in the pathophysiological mechanism of such conditions has been better understood through a series of expression studies and murine models. The complex interaction between genetic variants is slowly being unmasked. The in-depth understanding of the underlying mechanisms along with the rise of precision medicine approaches, such as gene therapy, has reshaped the landscape of CPVT treatment and provides an optimistic future for CPVT patients and their families. This review article aims to explore new molecular mechanisms revealed within recent literature before showing how it has informed new epidemiological and risk stratification insights and summarizing advances with recent CPVT treatment options.

MOLECULAR MECHANISMS OF CATECHOLAMINERGIC POLYMORPHIC VENTRICULAR TACHYCARDIA

Implicated genes in catecholaminergic polymorphic ventricular tachycardia

Since the discovery of CPVT, various mutations have been identified that result in a range of pathological subtypes. The core mechanism underlying each subtype is a pathological release of Ca^{2+} spontaneously in the sarcoplasmic reticulum (SR) of cardiomyocytes during diastole upon beta-adrenergic stimulation, which induces arrhythmogenic action potentials [Figure 1].[5,6] Mutations in genes that encode for the calcium-releasing unit (CRU) play a significant role in the disease pathogenesis.[6] The primary type of CPVT mutation, RyR2, was identified through a family pedigree analysis accompanied by DNA analysis to identify a pathogenic missense mutation (Arg4497Cys).[7] Additional information through genetic linkage studies helped to provide particular information regarding the chromosomal locations of these mutations to be mapped on 1q42–q43, which coincided with the locations of RyR2.[8] Since then, many other mutations have been identified, but only seven mutations (RyR2, CASQ2, CALM1,2,3, TRDN, and TECRL) have been validated based on a recent reappraisal.[9] According to the Association for European Paediatric and Congenital Cardiology, only the identification of pathogenic RyR2 and CASQ2 gene variants is regarded as diagnostic. Mutations found within other rarer variants must be considered on a case-to-case basis.[10] Thus, the first section aims to discuss our current understanding of these genes and their relationship to CPVT as well as the associated overlapping syndromes [Figure 1].

RyR2

The most typical form of CPVT, also known as CPVT-1, is an autosomal dominant form associated with a mutation in the human cardiac ryanodine receptor gene located on chromosome 1q42-43.[11] These mutations are found in 60% of patients with CPVT and affect a cluster of proteins.[12,13] Mutations with codon 2200–2500 are associated with structural changes in the binding site responsible for the binding of FKBP12.6, while mutations starting within codon 3700 correlate with transmembrane segments.[13] The cardiac ryanodine receptor is located on the SR, playing an important role in regulating intracellular Ca^{2+} release to coordinate with cardiac muscle excitation-contraction coupling.[14] The initial excitation process begins with the action potential depolarization of voltage-gated L-type Ca^{2+} channels (LTCC); a small Ca^{2+} influx current results in a 1000-fold greater Ca^{2+} through RyR2 through SR Ca^{2+} storage, leading to subsequent contraction of cardiomyocytes.[15] Such spatial and temporal patterns of LTCC and RyR2 activation lead to a coordinated Ca^{2+} influx, causing synchronous Ca^{2+} release events known as sparks.[14] Normal RyR2 activity is closely regulated by FKBP12.6 (Calstabin2) through protein kinase A (PKA) phosphorylation, with the RyR2 macromolecular complex containing 4 RyR2 subunits binding one molecule of calstabin2.[11,15] Increased levels of phosphorylation result in increased dissociation of Calstabin2, leading to increased contractility and cardiac output.[11]

In CPVT, the release of catecholamine results in an increase in cAMP, increased PKA activation, and increased phosphorylation cascade.[11] This prevents Calstabin2 from being able to perform its normal function of preventing aberrant activity. Activation of beta-adrenergic receptor signaling results in a higher amount of Ca2+ available within the SR. Within these settings, leaky RyR2 channels would increase diastolic calcium release, which may then increase the incidence of delayed after depolarization (DADs) and life-threatening ventricular arrhythmia (VT). In addition, the excess in Ca^{2+} stores would also result in the phenomenon of overload-induced Ca^{2+} release (SOICR), causing an increase in Ca^{2+} concentrations.[11] The N-terminal and central domains of the RyR2 channels are thought to perform an

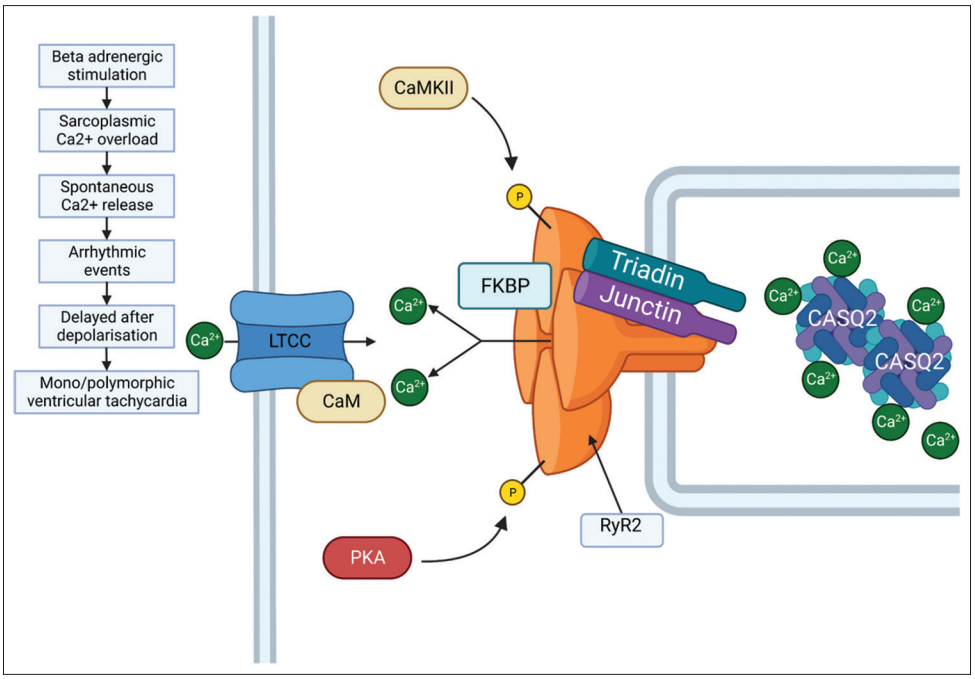

Figure 1: General Mechanisms of Arrhythmia induction in catecholaminergic polymorphic ventricular tachycardia. Ca2+: Calcium ion, CaM: Calmodulin, CaMKII: Calcium-calmodulin (CaM)-dependent protein kinase II, CASQ2: Calsequestrin 2, FKBP: FK506 binding proteins, LTCC: L-Type Calcium Channel, PKA: Protein kinase A, RyR2: Ryanodine receptor 2 Reproduced from[6] with permission

important function in acting as a regulatory component for channel gating and stabilizing the RyR2 channel.[16] This correlates with another pathological mechanism hypothesized, which postulates that the mutation on the RyR2 domains results in a weakened N-terminal to central domain interaction, causing an unzipping of the RyR2 and a resulting increase in spontaneous interaction.[11] However, genotype-phenotype correlations are not extensive. However, mutations in certain regions may yield more pathogenic phenotypes. For example, interactions between the central and N-terminal domains of a RyR2 subunit perform a crucial stabilizing function in normal RyR2 subunit behavior. The RyR2 channel becomes improperly controlled as a result of mutations in the N-terminal, which then impacts the structural stability overall.[16]

Since the identification of RyR2 mutation with CPVT, close to 170 genes have been identified with the condition, with 108 of these mutations being mapped by Silvia *et al.* on the RyRy2 cryo-electron microscopy diagram.[17] Interestingly, these mutations are seen throughout the coding region of the gene, and using such information together with GWAS would be crucial in providing better management of individual patients, with an emphasis on precision medicine in the future.

Calsequestrin

Calsequestrin (CASQ2) gene is the second-most common gene mutation in CPVT, leading to the autosomal recessive CPVT (CPVT2).[11] These mutations mainly reside on the short arm of chromosome 1 and affect around 3%–5% of diagnosed CPVT patients. The primary mutation types within CASQ2 are missense, deletion, and nonsense mutations. In all cases, an observable decrease or complete loss of CASQ2 protein is observed, explaining a rather homogeneous characteristic of presentations shared among the patients. However, a recent multicenter international study has suggested a new potential form of dominant CPVT caused by missense CASQ2 mutations.[18] Although the exact genetic mechanism is still unclear, the proposed hypothesis is that such CASQ2 prevents the polymerization of CASQ2 normal structure, exerting a dominant negative effect.

Normally, CASQ2 functions as a Ca^{2+}-binding glycoprotein with high capacity and low affinity.[19-21] Found exclusively within the junction of the SR, CASQ2 monomers primarily aggregate from the monomeric forms into tetrameric forms to further increase their Ca^{2+} capacity and can hold up to 40 Ca^{2+} ions.[21] CASQ2 thus plays the important role of regulating Ca^{2+} levels within the SR and promoting the reuptake of Ca^{2+}. During excitation-contraction coupling, the normal molecule of CAS2 would be polymerized upon the junction of the SR with two other important Ca^{2+} regulatory proteins, junctin and triadin,[21] which operate together with the RyR2 release channel from the CRU. The CRU would then respond to changes in cytosolic Ca^{2+} concentration to release Ca^{2+} for cardiac muscle contraction.

The exact pathophysiological mechanism of the impacts of CASQ2 mutation has not been fully elucidated. Studies

on mouse models suggest that loss of the CASQ2 gene results in a much faster increase in intraluminal free Ca^{2+}, leading to higher levels of available Ca^{2+} that would cause the spontaneous activation of RyR2.[21] Simultaneously, the loss of CASQ2 removes its inhibitory role upon the calcium release mechanism, causing a larger store of Ca^{2+} in SR. This is supported in mice models with overexpression of CASQ2, which have a significantly reduced level of Ca^{2+} induced and spontaneous release of Ca^{2+}.[21] For mice with the CASQ2 gene removed, they displayed a significantly slower heart rate, but no effects on cardiac contractility were reported.[22] When such a population of mice underwent a catecholamine challenge with β-adrenergic agonist isoproterenol, they displayed a higher level of premature ventricular complexes with reporting of VTs in a small population, reflecting the typical phenotype seen within humans. The events of VT were also increased within such mice populations during an exercise stress test measurement, which coincides with the much higher level of spontaneous diastolic Ca^{2+} release events in CASQ2-absent mice.[22] This provides a direct pathophysiological mechanism explaining why the absence or loss of CASQ2 causes premature spontaneous SR Ca^{2+} release, triggering additional heartbeats and ultimately resulting in arrhythmic events with a high phenotypic resemblance to those observed during CPVT.

Further research is needed to study the ultrastructural changes resulting from CASQ2 mutations, which have been associated therapeutically with the correction of electrophysiological abnormalities, suppression of triggered activities, and reduction in the events of VT.[11] Human cardiac cells harboring the D307H mutation exhibit immature morphology with disorganized myofibrils, enlarged SR, and a reduced number of caveolae, suggesting that ultrastructural changes may play a role in the pathological mechanisms underlying CPVT.[23]

Calmodulin

Calmodulin gene mutation (CALM1/CALM2/CALM3) is a rare mutation that is suspected of causing an autosomal dominant form of CPVT in <1% of patients, giving rise to CPVT-4 and CPVT-6.[11] These mutations were identified in 30 cases spread over nine different families. The two main variants seen among the largest registry on CALM mutation were p. Asn54Ile on CALM1 and p. Ala103Val on CALM3,[24] with rarer mutations found within CALM2 on 2p21.[25] The cardiomyocytes of these patients showed an observable increase in RyR2 opening probability and an increase in the development of DADs and triggered beats associated with the CALM3 mutation. Calmodulin is a ubiquitous protein in the human body, playing an important Ca^{2+}-dependent regulatory function.[26] In cardiac muscle, calmodulin provides another important regulatory role within cardiac muscle contraction through the control of phosphorylation of SR Ca^{2}+ pump activator, phospholamban, which exerts an inhibitory effect on SERCA to control the contractility of the heart.[27]

The current understanding of the exact pathophysiological mechanism and how it links to the structural components of calmodulin structure is not clear, however, it is likely related to the large sarcoplasmic Ca^{2} + release channel RyR2 sharing the same phenotypic expression.[22] Proof of concept studies using knock-in animal models and human-induced pluripotent stem cells are missing and are another area of research.[11] A recent study making use of nuclear magnetic resonance was able to provide insight into the particular structural effects of CALM1 p. Asn53Ile allele on its interaction between calmodulin and RyR2.[28] Such mutation resulted in a new hydrophobic site within the structure of calmodulin and disrupted its normal interaction with the RyR2 channel, leading to reduced opening probability of RyR2, and ultimately resulting in observable arrhythmic events seen in CPVT patients.[28] Further investigations within this direction combining animal, genetics, and molecular studies, will be crucial in further understanding the complexity of the mechanism in which these rarer mutations result in arrhythmia and VT.

Triadin

Mutations within the triadin (TRDN) gene also result in another rare form of CPVT (CPVT-5) with an autosomal recessive inheritance manner, otherwise known as triadin knockout syndrome (TRDN-KO).[29] Under normal physiological circumstances, triadin works together with its counterpart, junctin, as specialized proteins aiding with the binding of Ca^{2+} buffering protein calsequestrin upon the RyR2 receptor.[30] Therefore, the loss of the triadin molecule prevents this direct interaction between calsequestrin and the RyR2 channel. The normal location of the triadin gene is located on chromosome 6, made up of at least 41 exons and 420 kb.[30] The first genetic mutation associated with the triadin protein was first reported in 2012 in a 2-year-old boy experiencing syncope, with molecular analysis revealing a c.del53_56ACAG homozygous deletion within exon 2 of TRDN. Another mutation reported is a c.176C>G missense mutation in exon 2.[31] In both forms, no expression or expression of a nonfunctional form of triadin protein is observed.

A likely pathophysiological explanation for TRDN mutations is an increase within spontaneous SR Ca^{2+} release seen with triadin-null mice in animal model studies.[32] Due to the intimate relationship between triadin and other components of the CRU, it is a probable mechanism that disruption of any of these protein subunits could prime the heart for VT. One of the first mechanisms is the reduced co-expression of

CASQ2 within junctional SR, an already established cause leading to arrhythmias, as explained above. Another likely mechanism is the loss of L-type calcium channel inactivation, causing a significant increase in Ca^{2+} influx, thus leading to spontaneous Ca^{2+} release and delayed after depolarization during catecholamine surges.[30]

TECRL

Trans-2,3-enoyl-CoA reductase-like protein is another rare form of mutation that has been reported, categorized as atypical CPVT-3. The normal TECRL gene can be mapped on chromosome 4q13 and contains 12 exons.[11] The normal function of TECRL protein aids within lipid metabolism and aids within the underlying metabolic process present within mitochondria.[33] Two main genetic mutations associated with the TECRL gene have been reported within the literature – a substitution mutation of Arg196Gln reported within two French Canadians, and a splice site mutation c.331 + 1G>A in a Sudanese family was identified.[34]

In order to better understand the pathophysiological effects of TECRL mutations that lead to arrhythmic events in CPVT, a functional study was conducted by Devalla *et al.* through the use of human induced pluripotent stem cells (hiPSC) from one of the affected patients.[34] The induced cells within the homozygous splice site mutation are seen to have elevated diastolic Ca^{2+}, slower decay of cytosolic Ca^{2+} transients, and a prolongation within action potential. When these hiPSC lines underwent a noradrenaline challenge, they also exhibited an increase in propensity for delayed after depolarizations. They also demonstrated mutations within TECRL 331 + 1G>A mutation affected canonical calcium handling proteins with a 52% reduction in RyR2 protein and an 85% reduction in CASQ2 proteins.[34] Nevertheless, additional studies through the formation of hiPSC lines from patients or targeting mutation within healthy control lines would be important in providing insight into the underlying pathophysiological mechanism of TECRL mutation that causes CPVT. Clarification on the prevalence of TECRL mutation would also be needed to prevent underdiagnosis, as currently there is no clear consensus on the prevalence of TECRL mutations, with values ranging from <1% up to 5%.[35]

Genetic overlap

Rarer forms of CPVT have also been found with mutations associated with CALM, TRDN and TECRL genes [Table 1]. Notably, although genes such as KCNJ2, ANK2, and SCN5A have been disputed as pathogenic genes of CPVT, they could cause overlapping phenotypes of CPVT and other congenital arrhythmias, such as Brugada Syndrome and Long-QT syndrome (LQTS).[9,36] Moreover, rare cases of arrhythmogenic right ventricular cardiomyopathy (ARVC) and familial polymorphic ventricular tachycardia (FPVT) have also been reported. 6 rare autosomal dominant mutations within the RyR2 gene were associated with ARVC, while a recent disease locus mapping to 1q42-q43 has also pinpointed mutations within the cardiac calcium release channel of RyR2 to be associated as a cause of FPVT.[37,38] A rare RyR2 novel loss of function mutation I4855M has also been described to cause a deadly combination of left ventricular noncompaction and CPVT.[39]

For atypical CPVT, calmodulin mutations have been associated with a wide variety of arrhythmic conditions, with the two most prevalent pathological phenotypes being CPVT and LQTS respectively, along with idiopathic ventricular fibrillation and CPVT-LQTS overlapping phenotypes.[24] A recessive null triadin gene mutation could also lead to a unique pathology that combines the presentation of both CPVT and LQTS-known as triadin knockout syndrome (TKOS). Patients would present with extensive T-wave inversion, QT elongation, and further exacerbation of the condition upon exercise stress.[40] The presentation of TKOS has been characterized as actually a rare overlap syndrome of both LQTS and CPVT; therefore, early genetic diagnosis is crucial in informing subsequent treatment and in ensuring that patient families are adequately informed. Finally, TECRL mutations have also been implicated in other arrhythmic events, most notably LQTS, since both pathologies are closely related to an increase in diastolic calcium concentration and prolongation of action potential.[34]

RISK STRATIFICATION IN CATECHOLAMINERGIC POLYMORPHIC VENTRICULAR TACHYCARDIA

Due to the high risk of sudden cardiac death from VTs in CPVT, risk stratification is essential to identify subgroups of patients that require more aggressive monitoring and treatment.[1] The development of a comprehensive risk stratification tool would aid in the individualized tailoring of CPVT management based on risk status, thus optimizing the outcomes of medical interventions. Hayashi *et al.* showed that compliance with beta-blocker treatment reduced cardiac events amongst 16 relatives with positive stress test results ($P = 0054$).[40] Kallas *et al.* further reported that dual therapy, where patients are prescribed beta blocker in addition to flecainide and/or left cardiac sympathetic denervation, significantly decreased the risk of cardiac events from 48% to 10% when compared to beta-blocker or flecainide use alone ($P < 0.001$).[41] These results highlight the importance of initiating CPVT patients on optimal medical management to reduce their risks of developing sudden cardiac death. In addition, accurate risk stratification would reduce the likelihood of overtreatment. Considering the side effects and costs

implicated with each medical procedure, there is a high need for reliable guidelines on offering treatments based on the likelihood of a patient with CPVT developing adverse cardiac events.

Inadequate screening

Unfortunately, current guidelines and methods for CPVT screening are not very reliable in identifying patients at risk of arrhythmias and its downstream effects, making it difficult for specialists to identify patients who require medical therapy. At present, the exercise stress test is considered the first line when screening for relatives of CPVT probands, whereby the test results are deemed positive with the induction of VTs.[42] This screening method on 67 relatives of 17 genetically positive probands has reported a sensitivity and specificity for a positive CVPT-related genotype of 50% and 97%, respectively ($P < 0.001$).[40] Furthermore, this same study observed how results of the exercise stress test might change in certain individuals during follow-up– when the exercise-stress tests were repeated in 14 genotypically positive relatives with initially negative stress-test results, three displayed a positive result during retesting. This shows that more screening methods are required in conjunction with the exercise-stress test to accurately and safely stratify patients' risks of developing CPVT and its associated cardiac events. However, creating a thorough screening and risk stratification tool for CPVT patients has been met with multiple barriers [Table 2]. The complex pathophysiology of CPVT has led to the genetic and environmental factors underlying it being poorly understood – this makes it challenging for experts to agree on a comprehensive risk stratification tool. This review will discuss a few of these factors below.

Incomplete penetrance and variable expressivity

Multiple studies have found that the variants in CPVT-associated genes display incomplete penetrance. The recent rise of genome-wide association studies (GWAS) has complicated our confidence in variant significance and disease penetrance; variants that were once thought to be rare and strongly linked to a particular disease are now brought into question as they are found to be present in healthy populations. This reduces the variant's signal-to-noise ratio (SNR), where the noise is defined as the background rate at which the variant is found in healthy individuals.[43] In the case of CPVT, an exome sequencing study done on noncardiac pediatric patients reported that CPVT variants of likely pathogenic/pathogenic (LP/P) significance were found in this population at a frequency of 20 times, the baseline disease prevalence.[44] This finding was replicated by Ezekian *et al.* which stated that the CPVT-associated LP/P variants and variants of uncertain significance (VUS) are found incidentally in healthy individuals at a rate of 0.2% and 9%, respectively, equivalent to 2 and 90 times the disease prevalence in the population.[43] The high prevalence of LP/P variants and VUS in the population adds a layer of complexity to creating a risk stratification tool, as this indicates that the vast majority of variants are not highly penetrant. The emergence of AI and machine learning could be a solution, as a recent study demonstrated the possibility of using machine learning models to determine the pathogenicity of a RyR2 VUS.[45] Another issue is that the GWAS responsible for these findings are conducted in populations with a majority of European descent, with very few studies analyzing incidental findings of pathogenic variants in other ethnic groups generally as well as specifically for CPVT genotypes.[44,46-48] This meant that we could not confidently state whether an epidemiological predilection exists for some genetic variants or not. For instance, recent studies in China revealed RyR2, CASQ2, and TECRL mutations identified in CPVT patients, which is consistent with the literature. However, it also noted that there were no patients identified with mutations in CALM1, CALM2, CALM3, and TRDN mutations.[47,49] Although these variants are inherently rarer, it nonetheless suggest the importance to encourage collaboration globally to identify potential epidemiological patterns. In addition, numerous novel variants were also discovered in these studies, such as the c. 14861C > G RyR2 variant reported in Hong Kong.[49] Their pathogenicity is yet to be established; however, this highlights the need to encourage genetic testing among different ethnic groups to gather more comprehensive data regarding the genetic variants of CPVT to prompt further investigations.

Possible presentations in CPVT are also influenced by the compound effect of having multiple variants, yet the effects of such a phenomenon are not well researched. A study conducted on participants from the PACES CPVT Registry found that only fifteen out of 193 patients reported having ≥2 variants, representing 8% of the CPVT cohort.[50] This challenges the statistical analysis of phenotypic differences between CPVT patients with one versus multiple CPVT-related variants. Therefore, more studies must be done to establish the prognostic value of having ≥2 variants present. By observing how multiple variants coexist to produce phenotypic differences in other cardiac channelopathies, Coll *et al.* hypothesized that the presence of a second variant in CPVT could act as a risk modifier.[51] These "second hits," which may carry a relatively small physiologic effect in isolation, can alter the phenotype produced when acting in conjunction with the primary CPVT-associated variant. Examples of such genetic modifiers include the RyR2-p. G1886S, which could potentially increase the risk of VTs in patients with heart failure.[50] It is also important to note how various variant positions may affect their pathogenicity – variants of the RyR2 gene were linked to

greater risks of arrhythmia development when found on the C-terminus relative to the N-terminus.[52] This could be due to the variants in the C-terminus interrupting the process of proper channel formation, thus increasing the likelihood of improper calcium release.[41] It has also been hypothesized that the presence of two RyR2 variants in the trans phase may lead to worse disease prognosis, due to the number and orientation of RyR2 subunits affected.[50] Therefore, identification of these genetic modifiers and their physiological functions is necessary for the creation of a comprehensive polygenic risk score and risk stratification tool.

The complexities of multiple genetic variants in CPVT are further complicated by practical limitations observed in clinical practice. Inconsistent screening in the family is problematic; whilst cis phase variants would likely not contribute to a change in CPVT phenotype, trans phase variants would theoretically lead to increased severity.[50] It is unfortunate, therefore, that parental screening is often incomplete due to clinician uncertainties and technological inadequacies in the past, leading to insufficient data currently to further decipher the impact of multiple variants. Hence, it would be ideal if genetic counseling and family screening were to be offered in a specialized multidisciplinary clinic, with both types of variants evaluated.[50,53]

Nongenetic modifiers

In addition to the presence of genetic factors, the natural history of CPVT and its disease progression are hypothesized to be affected by various nongenetic factors. Early studies suggested that having the male sex and an earlier age of symptom onset are significant predictors of adverse cardiac events.[54,55] Hayashi *et al.* also found in their analysis of 101 CPVT patients that there were no significant differences in the rate of cardiac events (fatal or near-fatal) that occur between probands, referring to the first individual in a family to receive the diagnosis of CPVT, and their relatives.[55] Interestingly, a recent multicenter study with a total of 133 patients refuted these previous findings; their conclusions suggested that only proband status, not sex nor age at first symptom onset, plays a significant and independent role in risk prediction of time to earliest cardiac event ($P = 0.008$; hazard ratio = 4.4). In the same study, probands were also found to be more susceptible to adverse cardiac events in comparison to relatives, with 1-and 10-year event-free survival rates of 94% and 56% vs 100% and 91%, respectively.[41] This may be linked to the finding that probands have more RyR2 de novo variants and/or variants located on the C-terminus. Previous studies have suggested male sex as a predictor for arrhythmic risk,[56,57] but Kallas *et al.* found that this previous conclusion may have been a result of the younger age at which males get diagnosed with CPVT, resulting in them being more likely to be probands than females ($P = 0.031$).[41] These inconclusive results across multiple studies suggest that more analysis on each of these nongenetic modifiers is required.

ADVANCES IN CATECHOLAMINERGIC POLYMORPHIC VENTRICULAR TACHYCARDIA THERAPY

The current state-of-the-art approaches: Advantages and weaknesses

As an arrhythmia-generating condition with an adrenergic nature, CPVT is typically treated with beta-blockers, which have long been established as the first-line option. According to the expert consensus in 2013, avoidance of high-intensity exercise and beta-blockers are the only class 1 recommendations for all patients with a diagnosis of CPVT (as per the guidelines from the Heart Rhythm Society and the European Heart Rhythm Association).[42] They are also recommended to relatives with a pathogenic mutation but a negative exercise stress test, as severe cardiac events, could still be observed within that population.[40,58,59] Beta-blockers are effective and relatively safe when used as the sole treatment; however, their use can be complicated by non-adherence, improper titration leading to underdosing, undesired effects (which may be caused by the preference for non-selective beta-blockers), as well as breakthrough arrhythmias.[57] Among the numerous beta blockers available, nadolol is often the drug of choice due to its association with fewer cardiac events and a narrower "arrhythmic window."[57]

Another pharmacotherapeutic option is flecainide, a sodium channel blocker. Since its first report of anti-arrhythmic effects in CPVT patients in 2011, flecainide is now recommended in guidelines as a suitable addition to beta-blocker therapy.[42,60] In their 2015 study, Roston *et al.* reported that among the almost 50 CPVT patients in the PACES CPVT registry, those adherent to optimal doses had not experienced a single episode of breakthrough syncope or cardiac arrest.[57] Its therapeutic mechanism, however, is still unclear, and could be attributed to several mechanisms involving sodium channels and RyR2 blockade.[61] Moreover, a recent study has also suggested that flecainide's interaction with certain genetic variants could lead to arrhythmogenesis, highlighting the necessity of further research on its pharmacological mechanism.[62]

In terms of invasive treatment, two main options currently exist: implantable cardioverter-defibrillator (ICD) or left cardiac sympathetic denervation (LCSD). Although the therapeutic effect of LCSD has been reported since 2008 and repeated on a larger scale,[63,64] it is underutilized globally without clear reasons.[65] This procedure involves

ablating the lower part of the stellate ganglion along with the second and third thoracic ganglia, and the fourth ganglion is cauterized, disrupting sympathetic signaling to the heart. LCSD has been shown to not only reduce the occurrence of life-threatening events by over 80% but also improve the quality of life among patients.[63] Thus, in addition to the expert consensus recommendation, LCSD as a one-off procedure could also be considered in patients with poor compliance with medical treatment.

On the other hand, the use of ICD is more popular in CPVT. However, a systematic review by Roston *et al.* in 2018 showed that ICDs have a high chance of complications; patients have a 20% chance of suffering an electrical storm or inappropriate shock, and a 33% chance of experiencing device-related complications.[66] An inappropriate or even appropriate shock could precipitate more, and potentially fatal, VTs and myocardial changes.[66,67] However, this does not necessarily mean that ICDs should not be recommended. In the same study, it was found that nearly half of the ICD patients had one appropriate shock. If they are simultaneously the patients that receive most of the inappropriate shocks, it could still result in a net mortality benefit for them.

Despite the various weaknesses among the current therapeutic measures displayed by the literature, further investigation of current approaches is warranted to achieve optimal recommendations [Table 3]. However, novel approaches, such as precision medicine and gene therapy, are rapidly gaining attention and should also be explored. In particular, novel therapies are urgently needed for atypical CPVT mutations. One example is CPVT associated with TRDN variations, also known as TKOS. Clemens *et al.* described that conventional therapies such as beta-blockers, flecainide, and LCSD are largely unsuccessful in preventing arrhythmic events in this variant.[29] Over 70 percent of patients with TKOS experience cardiac events with beta-blocker treatment and all patients who received LCSD experienced at least one breakthrough event.[29] The latest development in precision medicine could therefore contribute majorly to the future management of atypical CPVT [Figure 2].

Gene therapy: New pathways towards precision medicine

According to the Precision Medicine Initiative, precision medicine refers to the "emerging approach for disease prevention and treatment that takes into account people's individual variations in genes, environment, and lifestyle."[72] The concept is of particular importance in the context of CPVT due to the multiple genetic variants and phenotypic differences across each genotypic mutations, yet the conventional treatment is still largely taking an "average patient" approach.

Gene therapy is an important aspect of precision medicine. Human gene therapy seeks to modify or manipulate the expression of a gene or to alter the biological properties of living cells, which can be achieved in different mechanisms: replacing a disease-causing gene with a healthy copy of the gene, inactivating a disease-relevant gene that is not functioning properly, or introducing a new or modified gene into the body to help treat disease.[73]

To have a panoramic view of the development of gene therapy for CPVT, we systematically searched the studies in three mainstream electronic databases (PubMed, Cochrane, and Embase) using the following rules: (CPVT) AND (gene OR genetic) AND (therapy OR treatment). The retrieval time was from the establishment of the database to December 31, 2022. The result showed that there were fewer than 5 papers in total before 2000 (since 1993), followed by a progressive rise [Figure 3]. Recent studies using mice models and human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have shown reassuring results on multiple occasions [Figure 4]. To understand the different approaches trialed for each class of CPVT, it is necessary to first briefly discuss the benefits and limitations of recombinant adeno-associated virus (rAAV), the most commonly used delivery modality in genetic therapy for cardiac diseases. Compared to other modalities such as oligonucleotides and modified mRNA, the most significant advantage rAAV offers is its ability to target specific cell types. For cardiac tissue, the most commonly used ones are AAV6, AAV8, and AAV9.[74] However, its limitations are also relevant – the potential development of adaptive immunity by the host means that it is best to have a precise prediction of dosing in the initial administration to ensure the durability and therapeutic effect.[75] Jeune *et al.* have shown that neutralizing antibodies in AAV6, 8, and 9 have a prevalence ranging from 19 to 37%.[75] However, methods such as hemapheresis and antibodies cleavage protein are currently being developed to combat this potential barrier to the wider application.[76] Another limitation of AAV is the limited cargo capacity (~5 kb), which meant that the most prevalent type of mutations among CPVT patients (around 60%)-mutations in the RyR2 gene, with a cDNA length of 14901 nucleotides, would require methods other than gene replacement, such as allele silencing and the use of CRISPR Cas 9.[11]

CASQ-2 + RyR2 catecholaminergic polymorphic ventricular tachycardia gene therapy

Current studies and trials on gene therapy largely focused on the correction of mutations in CASQ-2 variants or RyR2 variants [Figure 5]. While rAAV gene therapy for RyR2 mutations would need to take into account the cargo capacity, the coding sequence of the CASQ-2 gene only consists of around 1.2 kb, and thus could be fully

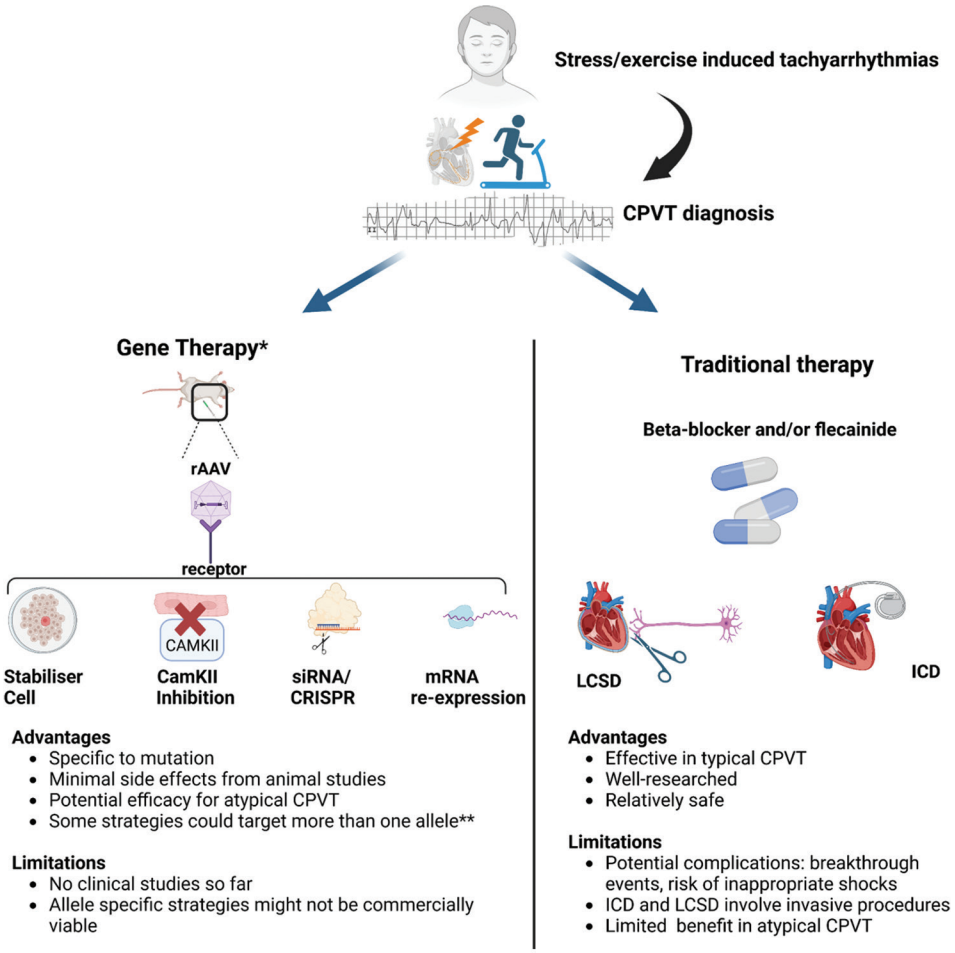

Figure 2: Overview of traditional versus gene therapy for the treatment of catecholaminergic polymorphic ventricular tachycardia. CPVT: Catecholaminergic polymorphic ventricular tachycardia CRISPR: Clustered regularly interspaced short palindromic repeats, mRNA: Messenger ribonucleic acid, rAAV: Recombinant adeno-associated virus, siRNA: Small interfering ribonucleic acid, LCSD: Left cardiac sympathetic denervation, ICD: Implantable cardioverter-defibrillator

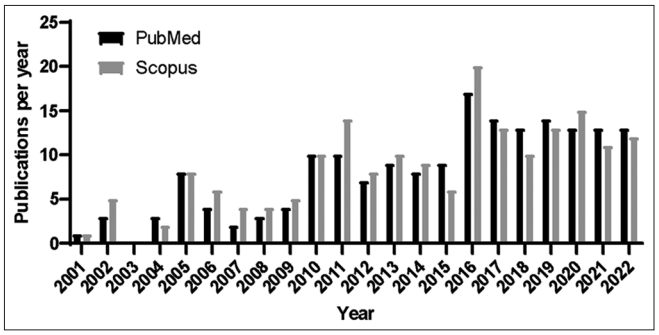

Figure 3: The number of publications on genetic therapy of catecholaminergic polymorphic ventricular tachycardia by year

inserted. The first successful CASQ-2 proof-of-concept trial was published in 2012 when Denegri *et al.* managed to induce CASQ2 expression in newborn knockout (KO) mice using AAV serotype 9.[77] The results were obtained 20 weeks postinjection of viral vectors and showed a substantial reduction in the development of arrhythmias *in vivo* using ECG telemetry on adrenaline stimulation (15/15 in uninfected KO mice compared to 1/10 in AAV-infected KO mice).[77] Further studies replicating the same method on knock-in mouse models also show a reduction or termination of life-threatening arrhythmias.[78,79] Interestingly, both studies have shown that partial transduction is sufficient to mount a therapeutic response. In Denegri *et al.*'s study, only 40% of cardiomyocytes are infected, and Kurtzwals-Josefson showed that a 33% or above expression of normal CASQ-2 level could significantly decrease VTs, while levels below it are still protective. This finding could implicate a potential marker for therapeutic quality in the future. Aside from targeting calsequestrin mutation directly, modified calmodulin could also be effective in treating CASQ-2-associated CPVT.[80] Liu *et al.* has shown that along with calsequestrin, calmodulin is also a modulator on the refractory duration of RyR2, and therefore could be an alternate target in Cas-Q 2 mediated CPVT. The introduction of an engineered calmodulin through an AAV vector into mouse models led to reduced diastolic Ca waves upon catecholamine

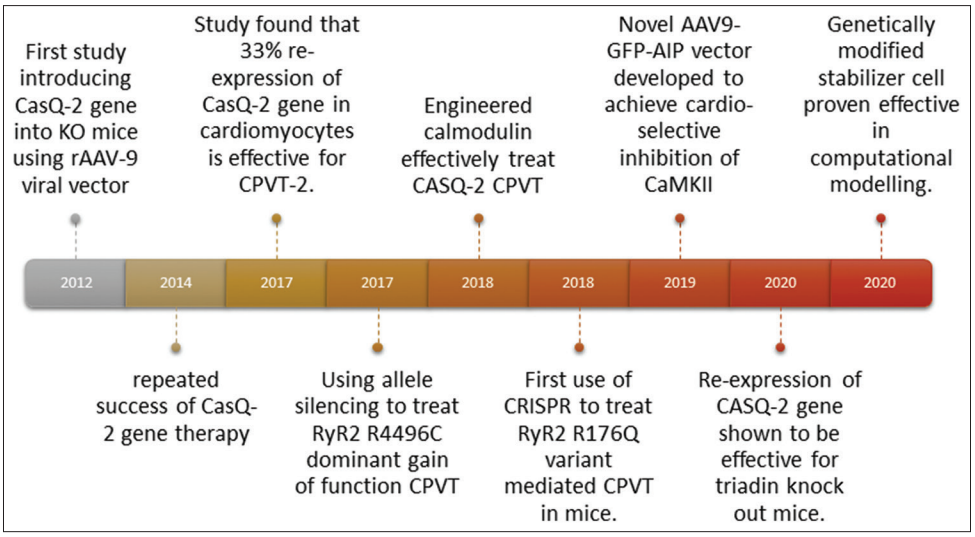

Figure 4: The development of gene therapy strategies each year from 2012 to 2020. AAV9: Adeno-associated virus 9, GFP: Green fluorescent protein, AIP: Autocamtide-2-related inhibitory peptide

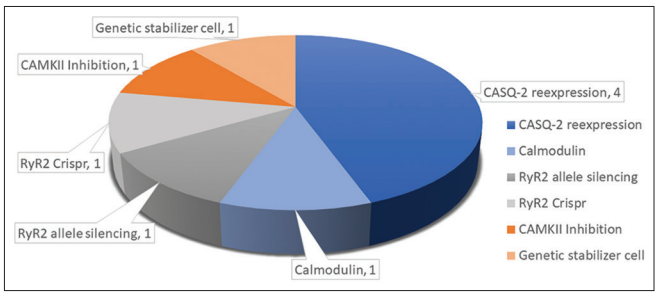

Figure 5: Distribution of gene therapy strategies. The number on each slice represents the number of studies using each strategy

stimulation in isolated cardiomyocytes. When tested at 8 weeks postinfection *in vivo*, uninfected CASQ2 R33Q mice had an 88% (7 out of 8) mortality rate upon arrhythmia induction, while infected mice had an 88% (7 out of 8) survival rate, showing its potential in preventing arrhythmias. Finally, it should also be noted that the CASQ-2 targeted therapy could also potentially mediate TRDN-induced CPVT.[81] Cacheux *et al.* showed that calsequestrin and triadin proteins are intimately linked, and triadin KO mice models demonstrated an 80% decrease in Calsequestrin protein level. Re-expression of the calsequestrin gene, however, seems to be able to reverse the effect, as the CASQ protein expression level has elevated to an average of 63%. More importantly, this partial reversal was enough to reverse the phenotypic effect of Ca^{2+} homeostasis disruption associated with TRDN deletion as ECG recording showed a 52% decrease in CPVT presentations between untreated and treated KO mice models.[81]

To overcome the limitation of gene replacement strategies posed by the colossal size of the RyR2 gene, other methods, such as allele silencing and genome editing, were conducted. Allele-specific silencing refers to a method where the mutant allele is selectively silenced, such that its functional impact could be alleviated. This method relies on two prerequisites – that the mutation causes a dominant-negative or gain of function; and that the normal allele could maintain cellular function once the mutant allele is silenced.[74] Researchers in the Priori lab showed that allele silencing is an effective strategy to treat CPVT caused by dominant, gain-of-function RyR2 mutations.[82] The approach was tested on a knock-in murine model with heterozygous mutations of R4496C in RyR2 that has been thoroughly characterized by the same team over the last decade.[83] They successfully identified a selective siRNA (siRyR2-U10) that was then packaged into an AAV serotype 9 vector and introduced into the murine model through intraperitoneal injection. Results from transcriptional analysis after 8 and 30 days and phenotypic studies after 8 weeks show an increase in the WT and mutant RYR2 mRNA ratio, and catecholamine-induced DADs and VTs are reduced. This again reinforces the idea that a partial reduction of mutated RyR2 protein would be sufficient to achieve a therapeutic response.[78,84] One of the concerns over the use of allele silencing is the overall reduction of protein production. While the total RyR2 was reduced by 15% in the infected mice model, this has not caused any change in cardiac function assessed by echocardiography.[82]

The introduction of CRISPR-Cas9 has opened up a wide range of possibilities for genetic study and therapy.[85] While there are some studies utilizing this novel technology *in vitro* to investigate gene function and potential therapeutic role in inherited cardiac conditions.[86-88] Its therapeutic potential *in vivo* in terminally differentiated cells, such as cardiomyocytes is currently limited since it relies on a homologous-directed repair pathway to produce precise editing results. This relies on the S and G2 phases of a cell cycle.[89]

Table 1: Summary of genetic variants of catecholaminergic polymorphic ventricular tachycardia

Gene variant	Normal function	Pathogenic mechanism	Inheritance mode
RYR2	Regulation of Ca2+ release in SR	Gain of function (more leaky) RYR2 protein	AD
CASQ-2	Regulate Ca2+ level as a binding glycoprotein in SR	Large decrease or complete loss of CASQ2 protein Abnormal polymerisation of CASQ-2 protein*	AR or AD
CALM 1, 2, 3	Control phosphorylation of phospholamban, inhibiting SERCA	Loss of function increases opening probability of RyR2*	AD
TRDN	Aids binding of CASQ-2 protein with RyR2 in SR to increase Ca2+ buffering capacity	Reduced expression of CASQ-2* Loss of L-type calcium channel inactivation*	AR
TECRL	Fatty acid and lipid metabolism in ER of heart and skeletal muscle	Impaired calcium homeostasis* Reduced expression of RyR2 and CASQ-2 proteins*	AR

*Potential mechanisms. SR: Sarcoplasmic reticulum, Ca2+: Calcium ion, SERCA: Sarco/endoplasmic reticulum Ca2+-ATPase pump, ER: Endoplasmic reticulum, AD: Autosomal dominant, AR: Autosomal recessive, TRDN: Triadin, CASQ-2: Calsequestrin-2

Table 2: Summary of the challenges encountered in risk stratification for catecholaminergic polymorphic ventricular tachycardia patients

Challenges	Explanation	Sources
Inadequacy of current screening guidelines	Currently, exercise-stress testing is considered as the first line screening tool for relatives of CPVT proband. However, its sensitivity for a positive CVPT-related genotype was low - 50%, (P<0.001). Results of the exercise stress test could also change on retesting	[40,42]
Uncertainty in variant significance	High frequency of incidental VUS/LP/pathogenic variant findings in an ostensibly healthy population, reducing the variant's SNR. This indicates that the vast majority of CPVT variants demonstrate incomplete penetrance Variant positions may affect their pathogenicity, for example, trans versus cis positioning, worse prognosis for patients with variants found in the C-terminus instead of the N-terminus	[41,43,44,50,52] [50]
Influence of compound effects and multiple variants	Not well-studied as multi-variant CPVT is extremely rare (~8% of CPVT cohort) Hypothesized that presence of a second variant may act as a risk modifier	[50] [51]
Epidemiological variance in CPVT variants	Current CPVT variant's significance are decided upon GWAS findings in populations with a majority of European descent It has been found, however, that there are epidemiological variance in CPVT-associated variants (e.g., variants specifically reported in China)	[45] [47-49]
Limited CPVT patient population data	CPVT is rare Clinical uncertainties and technological inadequacies in the past leads to insufficient CPVT patient data collection	[50,53]
Inconclusive results on nongenetic modifiers	More analysis on the suggested nongenetic modifiers required, for example, sex, earlier age of symptom onset, proband status	[42,54-56]

SNR: Signal-to-noise ratio, CPVT: Catecholaminergic polymorphic ventricular tachycardia, VUS: Variants of uncertain significance, LP: Likely pathogenic, GWAS: Genome-wide association studies

Table 3: Summary of the traditional therapies for catecholaminergic polymorphic ventricular tachycardia patients

Therapies	Category	Studies	Advantages	Limitations
Beta-blockers	Pharmacological	[54,55,68]	Safe, effective Widely available	Incomplete protection Nadolol (preferred drug choice) has variable availability internationally
Flecainide	Pharmacological	[62,69]	Superior efficacy add-on to beta-blocker monotherapy Suitable for pediatric patient	Mechanism unclear Could be arrhythmogenic in certain variants
ICD	Invasive	[66,70]	Appropriate shocks could be life-saving by terminating ventricular arrhythmias	Risk of complications from inappropriate shocks, including lethal arrhythmias
LCSD	Invasive	[63,64,71]	Permanent effects Avoids incompliance	Temporary Horner syndrome Limited efficacy predictors

ICD: Implantable cardioverter-defibrillator, LCSD: Left cardiac sympathetic denervation

Nonetheless, Pan *et al*. obtained remarkable results using an AAV9-mediated Crispr/Cas9 approach that targets the RyR2 R176Q variant in heterozygous murine models.[90] The technique resulted in a 30% decrease in total RyR2 mRNA in infected mice with the heterozygous R176Q mutation and effectively eliminated arrhythmic events in the intervention group. What's perhaps more impressive is the allele specificity demonstrated. While there was a significant decrease of mRNA and RyR2 protein in heterozygous mice with the R176Q variant, wild-type mice that were also injected with the viral vector showed no change in RyR2 mRNA or protein levels. This advantage could make it a more attractive gene therapy option compared to allele silencing, which generally has lower specificity and requires repeated dosing.

Challenges in translational implementation and alternative approaches

Despite the remarkable results achieved from the above

trials on gene therapy, we must not overlook the challenge they face in translating their findings to day-to-day clinical practice. While the therapeutic aspect seems promising, potential areas of concern such as proarrhythmic risk, ethical consideration, and commercial viability should be further investigated.[74,91] One potential challenge is the incomplete coverage of the genetic spectrum of CPVT with gene-specific targeted therapy.[91] Therefore, an allele-independent strategy might be more feasible as it could target a wider range of patients with CPVT. A hallmark feature of CPVT is a normal resting ECG, since its pathophysiology relies on a double hit mechanism-an alteration in SR Ca^{2+} release due to mutation, as well as raised SR Ca^{2+} concentrations, often caused by adrenergic stimulation.[11] Hence, a target for an allele-independent strategy is the inhibition of Ca^{2+}/calmodulin-dependent PKA II, a key signaling molecule that facilitates RyR2 Ca^{2+} release upon beta-adrenergic receptor stimulation. A crucial challenge to overcome is to develop a cardioselective vector and peptide to induce silencing only in the heart, and not in other systems, such as the CNS, where CaMKII is indispensable.[92] The vector AAV9-GFP-AIP developed by Bezzerides *et al.* has successfully achieved cardioselective inhibition of CaMKII, both in murine models and iPSC-HCMs.[91] When assessing for arrhythmia vulnerability using programmed ventricular stimulation, the infected mice show a 60% decrease in the frequency of induced arrhythmias and a reduced duration of arrhythmias. Moreover, they also show that CaMKII inhibition does not increase proarrhythmic risk. Another interesting strategy that could also be applied more generally is stabilizer cell gene therapy.[93] Researchers from the Priori lab manipulated the source-sink relationships confirmed by the aforementioned strategies, where partial transduction of ventricular myocytes is sufficient to prevent the generation of VTs, and simulated the effect of genetically modified stabilizer cells distributed in 1D, 2D, and 3D tissues using computational modeling. Overall, they showed that 20%–50% of stabilizer cells could suppress the generation of delayed after depolarizations (DADs), a core mechanism underlying arrhythmia generation in CPVT patients[93] [Figure 5].

CONCLUSIONS AND RECOMMENDATIONS

In summary, the current management for CPVT involves identification and workup of the probands, with exercise restriction and beta-blockers being the mainstay therapy. Flecainide may be added to patients who do not respond to beta-blockers. While whether LCSD is superior to flecainide is unclear, it can be considered for patients who suffer from recurrent syncope despite receiving optimal medical therapy.

With the rapid and recent advances in the understanding and management of CPVT, this topical review aims to provide the latest updates in molecular genetics, risk stratification, and gene therapy;[94,95] three areas that have developed substantially over the past few years. From discovering the RyR2 gene mutation initially, studies have now identified and validated seven sets of genes (RyR2, CASQ-2, TRDN, CALM1, 2 and 3, and TECRL) responsible for various CPVT phenotypes with novel insights into each gene's molecular mechanisms. However, the scarcity of atypical CPVT meant that major gaps still exist in our understanding of their pathogenetic mechanisms. More data on the atypical CPVT genotypes are needed for further research to decipher their underlying mechanisms. The complexities and opacity of the underlying genetics also translate to clinical practice, particularly in terms of risk stratification and personalized treatment.[96] Further research to determine the significance of genetic variants, as well as nongenetic modifiers, would improve the current screening recommendations. Finally, animal and stem cell studies on gene therapy for CPVT presented promising results over the last decade. However, the clinical applicability of many of these strategies remains dubious. These studies also extensively focus on RyR2 and CASQ-2 variants, which constitute 75% of all CPVT cases. More strategies looking at alternative approaches that could target a wider population, such as Ca^{2}+/calmodulin-dependent protein kinase II (CaMKII) inhibition, could be more feasible for implementation in clinical practice. Ruxolitinib, which is approved for treating myelofibrosis, has been identified as a CaMKII inhibitor from drug repurposing studies, may be used as anti-arrhythmic agent.[97]

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Conflicts of interest

There are no conflicts of interest.

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