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Accelerated junctional rhythm and non-alternans repolarization lability precede ventricular tachycardia in *Casq2*^{−/−} mice

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Abstract

Background—Calsequestrin-2 (CASQ2) is a Ca²⁺ buffering protein of myocardial sarcoplasmic reticulum. *CASQ2* mutations underlie a form of catecholaminergic polymorphic ventricular tachycardia (CPVT). The CPVT phenotype is recapitulated in *Casq2*^{−/−} mice. Repolarization lability (RL) - beat-to-beat variability in the T wave morphology - has been reported in long-QT syndrome, but has not been evaluated in CPVT.

Methods and Results—ECG from *Casq2*^{−/−} mice was evaluated with respect to heart rate (HR) and RL changes prior to onset of ventricular tachycardia (VT) to gain insight into arrhythmogenesis in CPVT. Telemetry from unrestrained mice (3-month-old males, 5 animals of each genotype) and ECG before and after isoproterenol administration in anesthetized mice was analyzed. Average HR in sinus rhythm (SR), occurrence of non-sinus rhythm and RL were quantified. HR was slower in *Casq2*^{−/−} animals. Accelerated junctional rhythm (JR) occurred more frequently in *Casq2*^{−/−} mice and often preceded VT. In *Casq2*^{−/−} mice, HR increased prior to VT onset, prior to onset of JR and on transition from JR to VT. RL increased during progression from SR to VT and after isoproterenol administration in *Casq2*^{−/−}, but not in *Casq2*^{+/+} animals. Isoproterenol did not increase repolarization alternans in either genotype.

Conclusions—Accelerated JR, likely caused by triggered activity in His/Purkinje system, occurs frequently in *Casq2*^{−/−} mice. Absence of CASQ2 results in increased RL. Increase in HR and in RL precede onset of arrhythmias in this CPVT model. Non-alternans RL precedes ventricular arrhythmia in wider range of conditions than previously appreciated.

Keywords

calsequestrin; catecholaminergic polymorphic ventricular tachycardia; junctional rhythm; repolarization lability; repolarization alternans; sudden death

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Introduction

Calsequestrin (CASQ) is a Ca^{2+} -binding protein present primarily in junctional sarcoplasmic reticulum of skeletal and cardiac muscle; the cardiac form (CASQ2) is encoded by a separate gene.^{1, 2} The primary role of CASQ2 is buffering of the sarcoplasmic reticulum Ca^{2+} ions, but another role for CASQ2 has emerged recently: CASQ2 regulates the open probability of ryanodine receptor 2 (RyR2).^{3, 4} Binding of Ca^{2+} -free CASQ2 to the luminal side of RyR2 augments RyR2 inactivation and contributes to normal termination of calcium-induced calcium release. In the setting of sarcoplasmic reticulum Ca^{2+} overload, lower availability of Ca^{2+} -free CASQ2 may result in spontaneous overload-induced calcium release, a potentially arrhythmogenic phenomenon.⁵

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a hereditary arrhythmic syndrome manifested by propensity to polymorphic or bidirectional ventricular tachycardia (VT) and risk of sudden cardiac death in the setting of β -adrenergic stimulation.⁶ The more common autosomal dominant form of the disease is usually caused by a mutation in *RYR2*.^{7, 8} The less common autosomal recessive form of CPVT is caused by *CASQ2* mutations.^{9, 10} An abnormal sarcoplasmic reticulum Ca^{2+} release occurs in both CPVT forms; during diastole, activation of the electrogenic cardiac $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) tends to depolarize cell membrane and may result in triggered ectopic activity known as delayed after depolarization (DAD).

Casq2 has been knocked-out in the mouse to create a CPVT model. *Casq2*^{-/-} animals exhibit the key features of the human disease: polymorphic or bidirectional VT during exercise or catecholamine administration, and structurally normal heart (though left ventricular hypertrophy is observed in aged animals).¹¹ Although CPVT-related arrhythmias are associated with high sympathetic tone and preceded by sinus tachycardia during treadmill exercise, sinus bradycardia has been described in *Casq2*^{-/-} mice.^{11, 12}

CPVT is important both clinically and as a model of arrhythmias related to impaired Ca^{2+} handling. In contrast to other hereditary arrhythmias, such as long-QT syndrome (LQTS) or Brugada syndrome, no characteristic abnormality is usually present on resting ECG. Repolarization lability (RL) in the form of T wave alternans,¹³⁻¹⁵ QT interval variability^{16, 17} and non-alternans T-wave lability¹⁸⁻²¹ has been associated with propensity to ventricular arrhythmias in a wide variety of settings, but RL in CPVT or its animal models has not been well studied.

A large body of literature links T-wave alternans to ventricular arrhythmias and sudden cardiac death.²² Abnormal handling of intracellular Ca^{2+} seems to underlie T-wave alternans,²³⁻²⁵ but T-wave alternans is actually more difficult to induce in Langendorff-perfused hearts of *Casq2*^{-/-} mice than in the wild-type animals.³

Non-alternans RL – i.e., beat-to-beat change in T wave morphology that does not follow an alternans pattern – has been described in patients with congenital LQTS¹⁸ and in a canine LQTS model.²¹ On a cellular level, non-alternans changes in action potential morphology occur in both canine and rabbit models of LQTS.^{21, 26} In another rabbit model of LQTS,¹⁹ non-alternans RL appears to be mechanistically related to secondary oscillations of calcium transient. These oscillations of calcium transient can eventually cause early after depolarizations, but precede the onset of ventricular ectopy. Although abnormal Ca^{2+} release from sarcoplasmic reticulum underlies arrhythmogenesis in CPVT, it is not known whether non-alternans RL occurs in CPVT models.

In this paper, we report data from a detailed evaluation of ECG recordings in *Casq2*^{-/-} mice with respect to heart rate (HR), RL and rhythm changes prior to VT onset.

Methods

Studies were carried out according to NIH guidelines and approved by the Animal Care and Use Committees of Vanderbilt University. *Casq2*^{-/-} mice were generated as described before.¹¹ Ten male animals (5 *Casq2*^{-/-}, 5 *Casq2*^{+/+}) underwent ECG recording at 3 months of age. Surface ECG in anesthetized mice was recorded before and after application of i.p. isoproterenol at a dose of 1.5 mg/kg. ECG monitoring in 10 unrestrained mice (age, gender and genotype same as above) was performed as reported previously.¹¹ The animals were allowed to recover for 72 hours after monitor implantation. Continuous ECG monitoring was performed during usual activity for 24 hours.

ECG analysis

ECG signal was sampled at 1 kHz (telemetry) or 2 kHz (isoproterenol experiments). The signals were analyzed off-line with software created in C++ Microsoft Visual Studio. After automatic detection of R wave peaks, the signal was low-pass filtered at 100 Hz using 3-pole Butterworth filter. Smooth cubic spline (passing through fiducial points preceding R wave by 10 ms) was subtracted to minimize baseline fluctuation. All recordings were manually reviewed; poor-quality data segments were excluded. During each 1 hour data segments, several ECG features were analyzed:

- a. presence of rhythm distinct from sinus rhythm (SR)
- b. average HR (expressed as average RR interval during SR) and total HR variability (standard deviation of normal RR intervals; SDNN)
- c. if present, an example of each of the following events was tagged in each 1 hour ECG segment:
 - “stable SR”: SR preceded and followed by at least 300 s of continuous SR
 - “VT or complex ventricular ectopy”: either VT of > 3 beats duration or frequent ventricular ectopy (bigeminy, trigeminy) of at least 2 s duration
 - “junctional rhythm”: at least 60 beat segment of continuous JR, terminating with SR
 - “pre-junctional SR”: 60 beat SR immediately preceding JR
 - “pre-VT SR”: 60 beat SR segment immediately preceding a segment of VT or complex ventricular ectopy (without intervening JR)
 - “pre-VT junctional rhythm”: 60 beat JR segment immediately preceding VT or complex ventricular ectopy

For the tagged rhythm episodes listed above, 60 consecutive beats were analyzed with respect to the following:

- average HR (average RR interval) and short-term HR variability (root-mean-square of successive RR interval differences; RMSSD)
- signal-averaged tracing and the corresponding signal lability were evaluated in a similar way as described before.^{18, 19} Briefly, for each time-point in the cardiac cycle, ECG signal lability was defined as root-mean-square (RMS) of beat-to-beat amplitude differences at a given time with respect to R wave peak. This was only calculated when the 60 consecutive beats were of SR or JR, without intervening ventricular ectopy.
- average value of signal lability (defined above) was calculated during early repolarization window (30 to 35 ms after R wave peak) and

normalized for amplitude (maximal value minus minimal value) of the signal-averaged QRS complex. After logarithmic transformation, this value was called “repolarization lability”, or RL. See Fig. 1 and its legend for more details.

- alternans component of RL was quantified as RMS of signal-averaged difference between the odd and even beats in the early repolarization window. After normalization for QRS amplitude and logarithmic transformation, this value was called “repolarization alternans”
- duration of the QRSJ complex, defined as the time interval from the R wave peak to the moment when the signal-averaged ECG signal drops under 5% of the QRS complex amplitude. In mice, ventricular repolarization starts before completion of ventricular depolarization. The terminal portion of the high-frequency deflection, which would be classified as end of QRS in humans, actually corresponds to beginning of repolarization. The closest human equivalent is the J wave. For this reason, we use the term “QRSJ complex duration” for this variable.

ECG signal quality in each of the 60-beat data segment was quantified using the following parameters:

- high-frequency noise: high-frequency signal was defined as the difference between the raw and low-pass filtered signal. The RMS of the high-frequency signal was calculated from the 60 consecutive beats at each point in the window ranging from 35 to 80 ms after R wave peak and normalized for QRS complex amplitude. The maximal value in this interval was used as a measure of high-frequency noise, such as skeletal myopotentials.
- baseline fluctuation: this was calculated as RMS of differences between amplitudes of subsequent fiducial points (after low-pass filtering, before baseline subtraction), normalized for QRS amplitude. Since spline subtraction is an imperfect way to remove baseline fluctuations, high values signify residual signal contamination with low-frequency noise
- QRS lability: defined as RMS of beat-to-beat values of the signal at the time of maximal value of signal-averaged QRS, normalized for QRS amplitude. High values are associated with beat-to-beat changes in QRS morphology, expected during changes in animal posture and resulting changes of cardiac axis with respect to the vector of ECG lead

Elevation of any of these parameters indicates decreased signal quality.

Statistical analysis

Frequency of categorical variables was compared using Fisher exact test (VassarStats; faculty.vassar.edu/lowry/VassarStats.html). The rest of the statistical analysis was performed in Microsoft Excel 2007. Variables obtained from *Casq2*^{+/+} and *Casq2*^{-/-} animals were compared using unpaired t-test. Variables from the same animals during different rhythm episodes were compared using paired t-test; only those 1 hour segments which contained both rhythms of interest were evaluated. All reported p-values are two-tailed. P-values <0.05 were considered significant.

Results

Heart rhythm

VT episodes, frequently of bidirectional morphology, occurred in all *Casq2*^{-/-} (133±118 episodes over 24 hours), but in none of the *Casq2*^{+/+} mice. Episodes of accelerated JR occurred in both *Casq2*^{-/-} and *Casq2*^{+/+} animals, but were markedly more frequent in the former: accelerated JR was present in 93 out of 120 one-hour long data segments in the *Casq2*^{-/-}, and in 26 out of 120 segments in the *Casq2*^{+/+} animals ($p<0.0001$; Fig. 2A,B). VT was preceded by JR rather than sinus rhythm (Fig. 2C) in 436 out of 664 (65.7%) episodes; most JR episodes did not escalate to VT.

Heart rate

Average RR interval during 24 hour monitoring period during SR was longer in the *Casq2*^{-/-} than in the *Casq2*^{+/+} mice (130 ± 10 vs. 111 ± 5 ms; $p<0.005$), indicating slower HR in *Casq2*^{-/-} mice.^a Total HR variability (SDNN) did not differ between genotypes (11 ± 4 vs. 12 ± 2 ms; NS).

In the *Casq2*^{-/-} animals, RR interval was longer during stable SR than during SR preceding onset of accelerated JR (140 ± 14 vs. 131 ± 15 ms; $p<0.0001$) and during stable SR compared to SR preceding VT or complex ventricular ectopy onset (128 ± 8 vs. 115 ± 8 ms; $p<0.0001$). RR interval during JR was shorter than during the preceding SR (115 ± 8 ms vs. 131 ± 15 ; $p<0.0001$) and further decreased prior to VT or complex ventricular ectopy onset (115 ± 7 vs. 110 ± 6 ms; $p<0.0001$). Short-term HR variability (RMSSD) did not change during any of these rhythm transitions.

Repolarization lability in *Casq2*^{-/-} mice

During SR, RL increased before the onset of accelerated JR, as compared to stable SR (-3.73 ± 0.62 vs. -4.24 ± 0.78 ; $p<0.0005$).^b Compared to stable SR, RL was also elevated during SR prior to onset of VT or complex ventricular ectopy (-3.09 ± 0.37 vs. -4.38 ± 0.37 ; $p<0.0001$). During JR, RL was higher than during the preceding SR (-3.08 ± 0.42 vs. -3.761 ± 0.60 ; $p<0.0001$) and increased again prior to VT or complex ventricular ectopy onset (-3.18 ± 0.39 vs. -2.98 ± 0.39 ; $p<0.005$; Fig. 3). These transitions were accompanied by an increase in at least one ECG noise index (Table I).

Repolarization morphology

In addition to accelerated junctional rhythm, another unexpected phenomenon was noted prior to VT or complex ventricular ectopy onset in *Casq2*^{-/-} mice, namely a gradual change in the morphology of late high-frequency deflection (Fig. 3). The high-frequency deflection reflects both depolarization and early repolarization in mice. As detailed in Methods, we used duration of the QRSJ complex to quantify this change in morphology of early repolarization. QRSJ complex duration did not differ between stable SR and SR preceding JR (19 ± 4 vs. 20 ± 5 ms; NS), but it did increase during SR preceding VT or complex ventricular ectopy (18 ± 3 vs. 24 ± 4 ms; $p<0.0001$). QRSJ complex duration during JR was longer than during the preceding SR (23 ± 5 vs. 20 ± 4 ms; $p<0.0001$) and additional prolongation occurred prior to VT or complex ventricular ectopy onset (23 ± 5 vs. 27 ± 5 ms; $p<0.0001$).

^aHR in beats per minute and RR interval in ms are related by the formula $RR = 60,000/HR$. Longer RR interval is synonymous with slower HR.

^bAll RL calculated in this paper are negative, because signal lability is always substantially smaller than QRS amplitude. RL value of, for example, -3.5 , indicates higher repolarization lability than -4.5 .

Isoproterenol effect

Before isoproterenol administration, RR interval was longer (i.e., HR was slower) in *Casq2*^{-/-} than in *Casq2*^{+/+} animals (147±12 vs. 122±5 ms; $p < 0.01$). After isoproterenol, RR interval was similar in both genotypes (108±4 vs. 108±7 ms; NS). RR interval decrease following isoproterenol was significant in both genotypes (Fig. 4A).

Isoproterenol increased RL in *Casq2*^{-/-} (-3.41±0.33 vs. -3.05±0.52; $p < 0.05$), but not in *Casq2*^{+/+} animals (-4.23±0.53 vs. -4.12±0.38; NS; Fig. 4B, Fig. 5). RL in SR was higher in *Casq2*^{-/-} than in *Casq2*^{+/+} mice before (-3.41±0.33 vs. -4.12±0.38; $p < 0.02$) and after isoproterenol (-3.05±0.52 vs. -4.23±0.53; $p < 0.01$; Fig. 4B). RL change after isoproterenol was significant in *Casq2*^{-/-} ($p < 0.05$), but not in *Casq2*^{+/+} animals. Interestingly, repolarization alternans did not change significantly after isoproterenol in either genotype (*Casq2*^{-/-}: -5.87±0.72 vs. -5.72±0.88; *Casq2*^{+/+}: -5.26±0.95 vs. -6.80±0.85) and did not differ between the two genotypes.

QRSJ complex duration in SR was similar in *Casq2*^{-/-} and *Casq2*^{+/+} mice at baseline (16±1 vs. 15±1 ms; NS), but became significantly longer in *Casq2*^{-/-} after isoproterenol (24±3 vs. 16±2 ms; $p < 0.005$; Fig. 4C). There was no significant change in QRSJ complex duration after isoproterenol administration in *Casq2*^{+/+}, although a significant increase ($p < 0.01$) occurred in *Casq2*^{-/-} mice.

None of the 3 indices of ECG signal noise increased in SR after isoproterenol administration compared to baseline in animals of either genotype (Table I).

Discussion

We describe novel ECG phenomena preceding VT onset in the *Casq2*^{-/-} animals: frequent episodes of accelerated JR, changes in QRSJ complex morphology, and increase in HR and RL, though not of repolarization alternans. These dynamic ECG changes occur both during spontaneous activity in unrestrained mice and after isoproterenol administration. They link the underlying molecular abnormality to mechanism of arrhythmogenesis on the animal level. Presence of sinus bradycardia reported previously in unrestrained *Casq2*^{-/-} animals was confirmed.

Sinus bradycardia

Abnormally slow sinus rate has been reported in mice lacking CASQ2 before.^{11, 12} Sinus bradycardia also occurs in CPVT.^{6, 9} It remains uncertain how the sinus node dysfunction relates to the Ca²⁺ handling abnormality. Nevertheless, spontaneous Ca²⁺ release from SR stores is important for normal sinus node automaticity. According to the “calcium clock” theory,²⁷ gradual Ca²⁺ accumulation in sarcoplasmic reticulum of SAN cells eventually results in spontaneous Ca²⁺ release and contributes to diastolic depolarization through NCX activation. Absence of CASQ2 is known to increase frequency of Ca²⁺ sparks in isolated ventricular myocytes.^{28, 29} Therefore, acceleration rather than delay of the “calcium clock” might be expected. However, the *Casq2*^{-/-} mice have lower sarcoplasmic reticulum Ca²⁺ content than the wild-type animals;¹¹ it is possible that in the absence of β-adrenergic stimulation, the amplitude of Ca²⁺ release in SAN cells in *Casq2*^{-/-} animals is too small to promote automaticity. Moreover, augmentation of CaMKII activity by oxidation has been shown to result in apoptosis of SAN cells and SAN dysfunction.³⁰ Conceivably, chronic Ca²⁺ leakage in *Casq2*^{-/-} mice may have a similar effect on CaMKII activity. Regardless of the mechanism, sinus bradycardia caused by CASQ2 absence attests to the importance of this protein in normal SAN automaticity.

Accelerated junctional rhythm

Accelerated JR in *Casq2*^{-/-} mice has not been reported before to the best of our knowledge. A report on mice with *Casq2* deletion by another group does mention a presence of JR in an anesthetized animal, but this was interpreted as a junctional escape rhythm.¹² The data reported here establish that JR is markedly more common in *Casq2*^{-/-} animals. This is best understood as accelerated JR rather than escape rhythm during especially profound episodes of sinus bradycardia: HR during SR actually increases before JR onset, and increases again during transition from SR to JR. Average RR interval during JR is shorter than during SR, and comparable to average RR interval during SR in *Casq2*^{+/+} animals. It is likely that accelerated JR in *Casq2*^{-/-} mice is a manifestation of triggered activity in the His/Purkinje tissue. Interestingly, Purkinje fibers appear to be more prone to triggered activity than working ventricular myocardium in another CPVT model involving *Ryr2* mutation.³¹ If the level of β -adrenergic stimulation required to elicit triggered activity is lower in His/Purkinje tissue than in working ventricular myocardium of *Casq2*^{-/-} mice, the observed pattern of JR episodes, which frequently precede VT episodes, but also occur without VT, would not be surprising. We are not aware of any reports describing accelerated JR in CPVT patients. However, JR occurs in digitalis toxicity, a condition which, like CPVT, is characterized by DADs.

Repolarization lability and ECG morphology changes

RL in the form of T-wave alternans, non-alternans T wave lability and QT interval variability has been associated with clinical arrhythmias in humans and animal models. However, arrhythmias in CPVT are believed to arise as a consequence of DADs, which occur after repolarization is completed. Nevertheless, repolarization abnormality has been reported in CPVT patients: Viitisalo et al.³² described dynamic T wave splitting in CPVT subjects, and Nof et al.³³ found a marked QT prolongation and T wave changes after a post-pacing pause in a CPVT kindred with *RYR2* mutation. Thus, the Ca^{2+} handling abnormality underlying CPVT can affect electrical systole.

We had some concern regarding the interpretation of increased RL prior to JR and VT in the telemetry data: if the episodes of spontaneous JR and VT are triggered by increased physical activity, the analysis could be biased by increased ECG noise due to skeletal myopotentials and changes of the cardiac axis as the animal changes position. The increase in noise level does indeed occur prior to JR and VT (Table I). However, we believe that the increase in RL is not solely attributable to increased noise, for 2 reasons:

1/ in several cases (e.g. Fig. 3B), we observed a distinct peak of ECG lability as the ECG signal returns to baseline at the end of QRSJ complex; this would be difficult to explain by the nonspecific effects discussed above

2/ more importantly, no increase in noise prior to VT onset was observed in the experiments involving isoproterenol administration to anesthetized animals. Nevertheless, RL increase was still present under these conditions.

Moreover, increased RL was accompanied by a characteristic change in QRSJ complex morphology (Fig. 3, Fig. 5A). The QRSJ complex duration assessed in the present paper is similar to the Qb interval described by Danik et al.³⁴ In that report, this interval corresponded approximately to 50% repolarization (APD50) on monophasic action potential recording. Thus, the QRSJ complex changes we observed likely reflect increased voltage gradients in ventricular tissue during early repolarization phase.

The cellular mechanism underlying increased RL and altered early repolarization morphology remains uncertain, but likely involves altered Ca^{2+} handling. It is possible that

in the setting of β -adrenergic stimulation and increased Ca^{2+} loading of sarcoplasmic reticulum, spontaneous (i.e., not triggered by L-type Ca channel opening) Ca^{2+} release occurs relatively early after AP upstroke – before AP termination – in *Casq2*^{-/-} animals. This would activate NCX and prolong phase 3 of AP in spatially heterogeneous manner, leading to steeper voltage gradients and prolonged QRSJ complex duration. If there is a stochastic component to the spontaneous Ca^{2+} release on the cellular level, it could manifest as beat-to-beat variability of action potential course and, on the animal level, as RL. This mechanism does appear to operate in LQTS.¹⁹

Of note, tachycardia-induced repolarization alternans is actually more difficult to induce in *Casq2*^{-/-} hearts than in *Casq2*^{+/+} hearts,³ presumably because of shorter time constant of junctional sarcoplasmic reticulum filling. Consistent with these findings, we have not observed any increase in repolarization alternans after isoproterenol administration in *Casq2*^{-/-} animals.

In summary, we report several new ECG findings in *Casq2*^{-/-} animals that are relevant to arrhythmogenesis in this CPVT model. They may provide new insights into arrhythmogenesis in CPVT and related conditions. Specifically, non-alternans RL has been associated with arrhythmias in the setting of impaired repolarization, but has not been previously reported in CPVT. Non-alternans RL has also been reported in human subjects prior to spontaneous VT onset²⁰ and in the setting of canine myocardial ischemia.³⁵ It is therefore possible that it might be a more general marker of arrhythmic propensity, and merits further study.

Limitations

Although this model provides important insights into human CPVT, murine cardiac physiology is sufficiently different from that of large mammals that some of the findings may not be relevant clinically. Increased RL has been linked to arrhythmias in other settings, but at this time, there is no firm evidence that the phenomena described in this paper are causally related to arrhythmogenesis in *Casq2*^{-/-} mice.

Conclusion

Accelerated JR, likely caused by triggered activity in His/Purkinje system, occurs frequently in *Casq2*^{-/-} mice. HR acceleration precedes onset of JR and VT or complex ventricular ectopy in these animals. Absence of CASQ2 increases RL. Further increase of RL occurs prior to VT or complex ventricular ectopy onset both in unrestrained mice and after isoproterenol administration, but the alternans component of RL remains unchanged. The association between non-alternans RL and ventricular arrhythmias may be more general than previously appreciated. The possible mechanistic link between RL and arrhythmogenesis merits further study.

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Fig. 1.

The algorithm for calculation of repolarization lability (**RL**) is illustrated. **A** Sixty consecutive QRST complexes are aligned along the R wave peak. For clarity, only 5 color-coded complexes are shown and signal lability is exaggerated. At each time (**t**) in a portion of the cardiac cycle, the sequence of 60 signal amplitudes is used to calculate signal lability. **B** Signal lability is calculated from differences between amplitudes of consecutive QRST complexes ($\Delta_1, \Delta_2, \dots, \Delta_{59}$) at time **t**. Only Δ_1 and Δ_{59} are shown for clarity. Color-coding is consistent with panel **A**. **C** Lability of the ECG signal at time **t** is calculated as root-mean-square of $\Delta_1, \dots, \Delta_{59}$, i.e. as the square root of the average of the squared differences at time **t**. The signal lability values are plotted as the green curves in Fig. 3 and Fig. 5. Average signal lability in the early repolarization window (i.e. **t** ranging from 30 ms to 35 ms after R wave peak) is normalized for amplitude of QRS complex (**QRS_{max}** – **QRS_{min}**) and **RL** is calculated as the natural logarithm of the value. It is a measure of short-term beat-to-beat repolarization signal variability which includes, but is not limited to, repolarization alternans. It is closely related to the measures of T-wave lability reported in references 18 and 19.

Fig. 2. Examples of ECG signal from unrestrained mice. Each panel shows a continuous recording. Duration of RR intervals in ms is indicated in red. Time from the beginning of the file is displayed at the beginning of each rhythm strip in **hh:mm:ss** format. **A** Sinus rhythm in a *Casq2*^{+/+} animal. **B** Transition from SR to JR in a *Casq2*^{-/-} animal. **C** JR precedes onset of bidirectional ventricular tachycardia in a *Casq2*^{-/-} animal. Note the slower SR in *Casq2*^{-/-} animal in panel **B** compared to *Casq2*^{+/+} in panel **A**, and the slight increase in HR during transition from SR to JR in **B**.

Fig. 3.

Signal-averaged ECG (**red**) and signal lability (**green**) calculated from 60 consecutive beats from a *Casq2*^{-/-} animal during stable SR (**A**) and SR preceding VT onset (**B**). The Y-axis of the lability signal is magnified 10-fold with respect to the signal-averaged ECG. The blue horizontal bars indicate 25 ms time interval. The parallel vertical bars mark the window used for RL calculation. The horizontal arrows denote QRSJ complex duration. RL and QRSJ complex duration both increase before VT onset.

Fig. 4.

Comparisons of mean RR interval (A), RL (B) and QRSJ complex duration (C) in *Casq2*^{-/-} animals (KO) before and after isoproterenol, and between *Casq2*^{+/+} (WT) and *Casq2*^{-/-} after isoproterenol. The black symbols (‡ < 0.01; § < 0.05) indicate difference between values measured before and after isoproterenol. White symbols (* < 0.01; # < 0.05) signify differences between values obtained from *Casq2*^{+/+} and *Casq2*^{-/-} animals. RL and QRSJ complex duration all increase after isoproterenol in *Casq2*^{-/-} animals, and are higher in *Casq2*^{-/-} than *Casq2*^{+/+} animals after isoproterenol.

Fig. 5.

Examples of signal-averaged ECG and signal lability in a *Casq2*^{-/-} (**A**) and *Casq2*^{+/+} (**B**) animal before (**left**) and after (**right**) isoproterenol. The meaning of the symbols is same as in Fig. 3. After isoproterenol, RL and QRSJ complex duration increase in *Casq2*^{-/-} animal, but change minimally in *Casq2*^{+/+}.

Table I

Values of ECG noise indices in *Casq2*^{-/-} mice during telemetry and isoproterenol experiments. In the telemetry data, at least one of the indices increases when the rhythm progresses from stable SR to pre-VT rhythm. No decline in ECG quality is observed after isoproterenol administration.

	QRS lability	Baseline lability	HF noise
stable SR vs. pre-junctional SR	0.038±0.017 vs. 0.065±0.037 [†]	0.012±0.005 vs. 0.021±0.012 [†]	0.007±0.005 vs. 0.014±0.007 [†]
stable SR vs. pre-VT SR	0.037±0.016 vs. 0.084±0.028 [†]	0.010±0.004 vs. 0.029±0.010 [†]	0.005±0.008 vs. 0.019±0.017 [†]
Pre-junctional SR vs. junctional rhythm	0.066±0.037 vs. 0.084±0.029 [†]	0.020±0.012 vs. 0.034±0.014 [†]	0.014±0.007 vs. 0.018±0.009 [*]
Junctional rhythm vs. junctional rhythm pre-VT	0.077±0.025 vs. 0.088±0.031 [*]	0.031±0.013 vs. 0.033±0.012 NS	0.018±0.009 vs. 0.021±0.010 NS
SR before isoproterenol vs. SR after isoproterenol	0.048±0.020 vs. 0.068±0.025 NS	0.029±0.012 vs. 0.038±0.011 NS	0.024±0.009 vs. 0.024±0.011 NS

^{*}
p<0.05

[†]
p<0.0001