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Evaluation of Differences in Automated QT/QTc Measurements between Fukuda Denshi and Nihon Koden Systems

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Abstract

Background: Automatic measurement becomes a preference, and indeed a necessity, when analyzing 1000 s of ECGs in the setting of either drug-inducing QT prolongation screening or genome-wide association studies of QT interval. The problem is that individual manufacturers apply different computerized algorithms to measure QT interval. We conducted a comparative study to assess the outcomes with different automated measurements of QT interval between ECG machine manufacturers and validated the related heart rate correction methods.

Methods and Results: Herein, we directly compared these different commercial systems using 10,529 Fukuda Denshi ECGs and 72,754 Nihon Kohden ECGs taken in healthy Japanese volunteers. Log-transformed data revealed an equal optimal heart rate correction formula of QT interval for Fukuda Denshi and Nihon Kohden, in the form of $QTc = QT/RR^{-0.347}$. However, with the raw data, the optimal heart rate correction formula of QT interval was in the form of $QTc = QT+0.156\times(1-RR)$ for Fukuda Denshi and $QTc = QT+0.152\times(1-RR)$ for Nihon Kohden. After optimization of heart rate correction of QT interval by the linear regression model using either log-transformed data or raw data, QTc interval was ~10 ms longer in Nihon Kohden ECGs than in those recorded on Fukuda Denshi machines. Indeed, regression analysis revealed that differences in the ECG machine used had up to a two-fold larger impact on QT variation than gender difference. Such an impact is likely to be of considerable importance when ECGs for a given individual are recorded on different machines in the setting of multi-institutional joint research.

Conclusions: We recommend that ECG machines of the same manufacturer should be used to measure QT and RR intervals in the setting of multi-institutional joint research. It is desirable to unify the computer algorithm for automatic QT and RR measurements from an ECG.

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Introduction

Prolongation of the QT interval is an intermediate phenotype associated with an individual's increased propensity to develop a ventricular tachyarrhythmia called Torsades de Pointes and increased risk of sudden cardiac death (SCD) [1,2,3,4].

All new drugs must undergo a 'thorough QT/QTc' (TQT) study to detect drug-induced QT prolongation. Indeed, in accordance with the GCP (good clinical practice) E14 guidance, development of several drugs that prolong QTc by >5 ms was abandoned by the relevant companies [5]. Therefore, accurate and consistent measurement of the QT/QTc interval is increasingly important not only for clinical benefit, but also from the pharmaceutical drug safety screening perspective.

It is well established that the rare Mendelian diseases of extreme QT duration, the long-QT syndromes [6] [7], are risk factors for SCD [8]. However, recent population-based cohort studies demonstrated that multiple common genetic variants that contribute to the repolarization affect the risk of SCD in the general population [9] [10]. Although individual common variants would be expected to induce only modest increments in QT interval (3–6 ms per locus) [10], these common variants could, individually and in aggregate, more markedly increase SCD through influencing susceptibility to arrhythmogenic triggers such

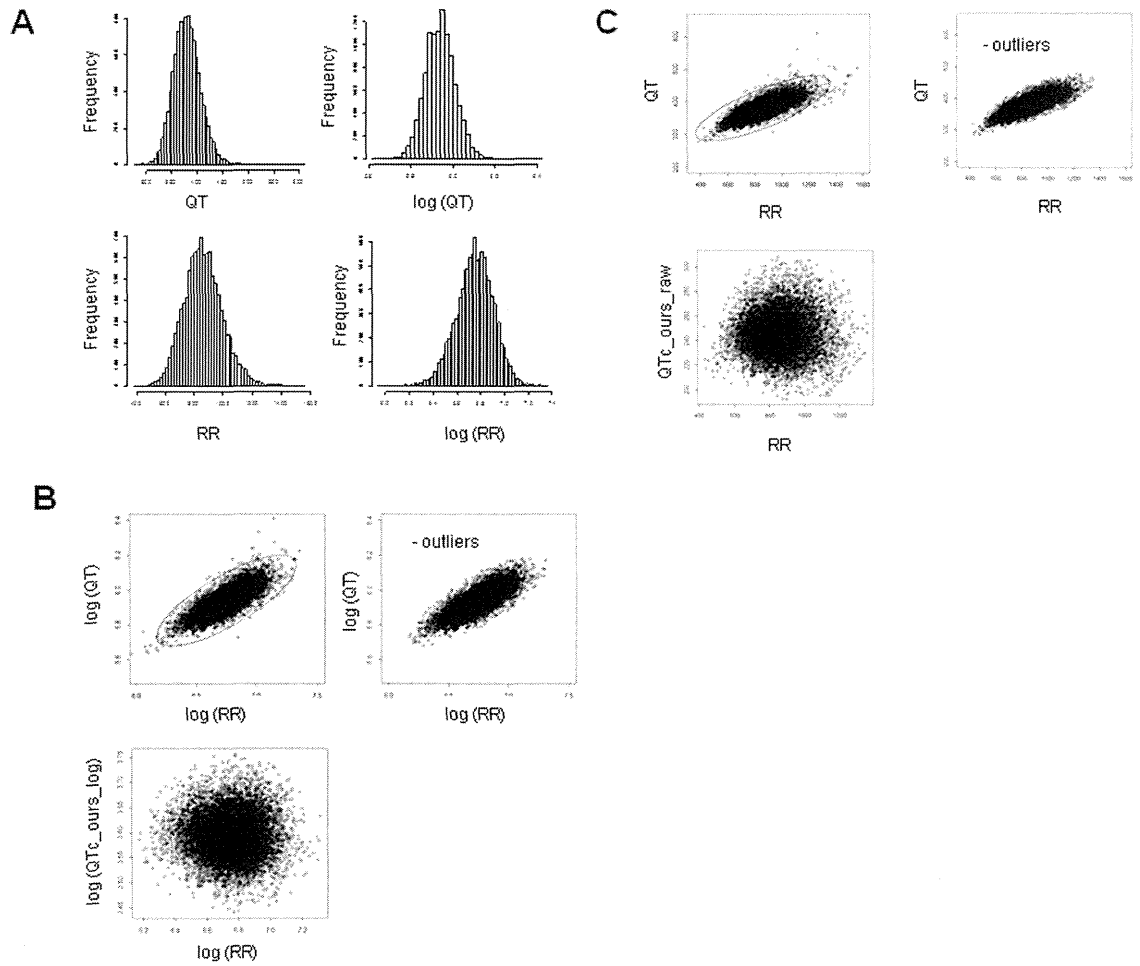

Figure 1. Analysis of resting Fukuda Denshi ECGs. (A) Histograms of QT, log-transformed QT, RR, and log-transformed RR intervals. (B) Scatter plots of log QT versus log RR and log QTc_ours log versus log RR. (C) QT versus RR and QTc_ours raw versus RR. Units of all variables are ms.
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as myocardial ischemia, electrolyte disturbance, or QT-prolonging medications [11]. In fact, a 10-ms increase in the observed QT interval was associated with an increased risk of SCD (hazard ratio, 1.19; 95% CI, 1.07 to 1.32; $P = 0.002$) [12].

QT interval is inversely correlated with heart rate. Generally, QT intervals are corrected for heart rate so that QTc is equal to QT if the heart rate is 60 beats per minute, i.e., RR interval of 1 s. Various formulae have been used to correct QT interval (QTc) with respect to heart rate, and such correction can be done by the linear regression model using either raw data or log-transformed data.

Measurement of QT interval by surface electrocardiogram (ECG) is performed either manually or automatically. Manual measurement, in which the end of T wave is determined as the intersection between the tangent to the steepest down-slope of the T wave and the isoelectric line, is made according to published guidelines, but is time-consuming and contains inter-reader variability. For automated measurements, difficulties in delineating the end of T wave are encountered when either the ECG is polluted with noise or the T wave is flat, bifid, biphasic, or overlapping on a U wave [13] [14]. However, automated QT measurement technologies are evolving rapidly and their precision has been increasingly demonstrated to be appropriate in various studies [15]. Automatic measurements also show greater consistency and are more suitable for processing a large volume of data.

In Japan, Nihon Kohden and Fukuda Denshi together provide most of the ECG machines used clinically. However, each of these manufacturers uses a different automatic computerized algorithm to measure QT interval and different methods for heart rate correction of QT interval, with Nihon Kohden adopting the ECAPS12 formula [QTc (ECAPs12) = QT+(1–RR)/7] and Fukuda Denshi the Bazett formula [QTc (Bazzet) = $QT/RR^{0.5}$]. Surprisingly, the impact of such system differences on the clinical results has been ignored. Thus, we conducted a comparative study to assess the outcomes with different automated measurements of QT interval between ECG machine manufacturers and validated the related heart rate correction methods.

Methods

Ethics statement

Since all the data used in this study were anonymous data, the ethical committee of Fukuda Denshi and Nihon Kohden both had a same judgment that there is no ethical problem to provide us with anonymous data. IRB committee at Keio University said that the submission of this study for the approval by IRB is not necessary.

Evaluation of automatic QT and RR measurements in adult resting ECGs by Fukuda Denshi and Nihon Kohden

The QT interval and RR interval in healthy Japanese were measured from 12-lead digital ECGs based on automated algorithms. Fukuda Denshi and Nihon Kohden provided anonymous data sets. Nihon Kohden ECGs were taken at 2010 by ECG-1450. Fukuda Denshi ECGs were taken at 1999 by FCP-4000 series (Algorithm to determine the T wave end is the same as current ECGs.).

We evaluated resting ECGs recorded on a Fukuda Denshi machine from 10,529 adult (≥20 years old) healthy subjects (8,631 males aged 46.1±9.2 years and 1,898 females aged 45.3±7.9 years) and resting ECGs recorded on a Nihon Kohden machine from 72,754 adult (≥20 years old) healthy subjects (42,673 males aged 49.9±15.3 years and 30,081 females aged 53.6±16.5 years). Histograms of QT, RR, log-transformed QT, and log-transformed RR intervals demonstrated normal (Gaussian) distributions for these variables (Figure 1A and Figure 2A), although the distribution of each log-transformed variable was closer than the raw variable to the normal distribution (Data S1).

Definition of outliers

Scatter plots of log QT versus log RR are shown in Figure 1B and Figure 2B. Outliers were defined as the plots outside the contour line that includes 99.9% of the integral of the probability density function of the bivariate normal distribution defined by the means, SDs, and the correlation coefficient of the observed values (log QT versus log RR or QT versus RR) (Figure 1B, 1C, 2B, 2C). The contour line of a bivariate normal distribution is expressed by an ellipse on a plane defined by two means, two SDs and a correlation coefficient. After the ourliers ourside of the ellipse were excluded, the remaining data were used for the analysis.

Correction of QT by heart rate

Regression analysis was performed separately for the data from two manufacturers, Nihon Koden and Fukuda Denshi for the calculation of optimal heart rate correction formula. In one analysis, we used the log-transformed RR value as an independent variable and the log-transformed QT value as the dependent variable, while in the other, we used raw RR interval as an independent variable and raw QT value as the dependent variable. After performing the linear regression analysis, we used the estimated coefficients for constructing the correction formula. When both independent and dependent variables are in the raw form, the formula used for the regression analysis was as follows.

$$QT = aRR + X + b,$$

where a and b are coefficients to be estimated, and X denotes a variable for the variation other than RR that affects QT. Units of QT and RR are sec. Using the estimated coefficients, the formula for the calculation of QTc (ours_raw) was constructed as follows,

$$QTc(ours\_raw) = QT + a(1 - RR)$$

where, a is the estimated coefficient.

Note that QTc (ours_raw) = X+b+a, and this adjustment for the intercept was made so that QTc (ours_raw) = QT when RR = 1.

When both independent and dependent variables are in the log form, the formula used for the regression analysis was as follows.

$$\log QT = c \log RR + Y + d,$$

where c and d are coefficients to be estimated, and Y denotes a variable for the variation other than log RR that affects QT. Using the estimated coefficients, the formula for the calculation of log QTc was constructed as follows,

$$\log QTc(ours\_\log) = \log QT - c \log RR,$$

or

$$QTc(ours\_\log) = \frac{QT}{RR^c}$$

Note that log QTc (ours_log) = Y+d, and this adjustment for the intercept was made so that QTc (ours_log) = QT when RR = 1.

We also validated other published heart correction formula as comparison.

The formulas for the correction of QT by Fredericia, Bazzet, Framingham and ECAPs12 methods were as follows.

$$QTc(Fredericia) = \frac{QT}{RR^{\frac{1}{3}}}$$

$$QTc(Bazzet) = \frac{QT}{RR^{0.5}}$$

$$QTc(Fra\ min\ gham) = QT + 0.154(1 - RR) \qquad (16)$$

$$QTc(ECAPs12) = QT + \frac{1 - RR}{7}$$

Note that, in all the correction methods, QTc = QT when RR = 1. We calculated those published QTc values using the data from the two manufacturers, and tested for differences between the manufacturers by Student t-test for each gender for each published method for QTc calculation.

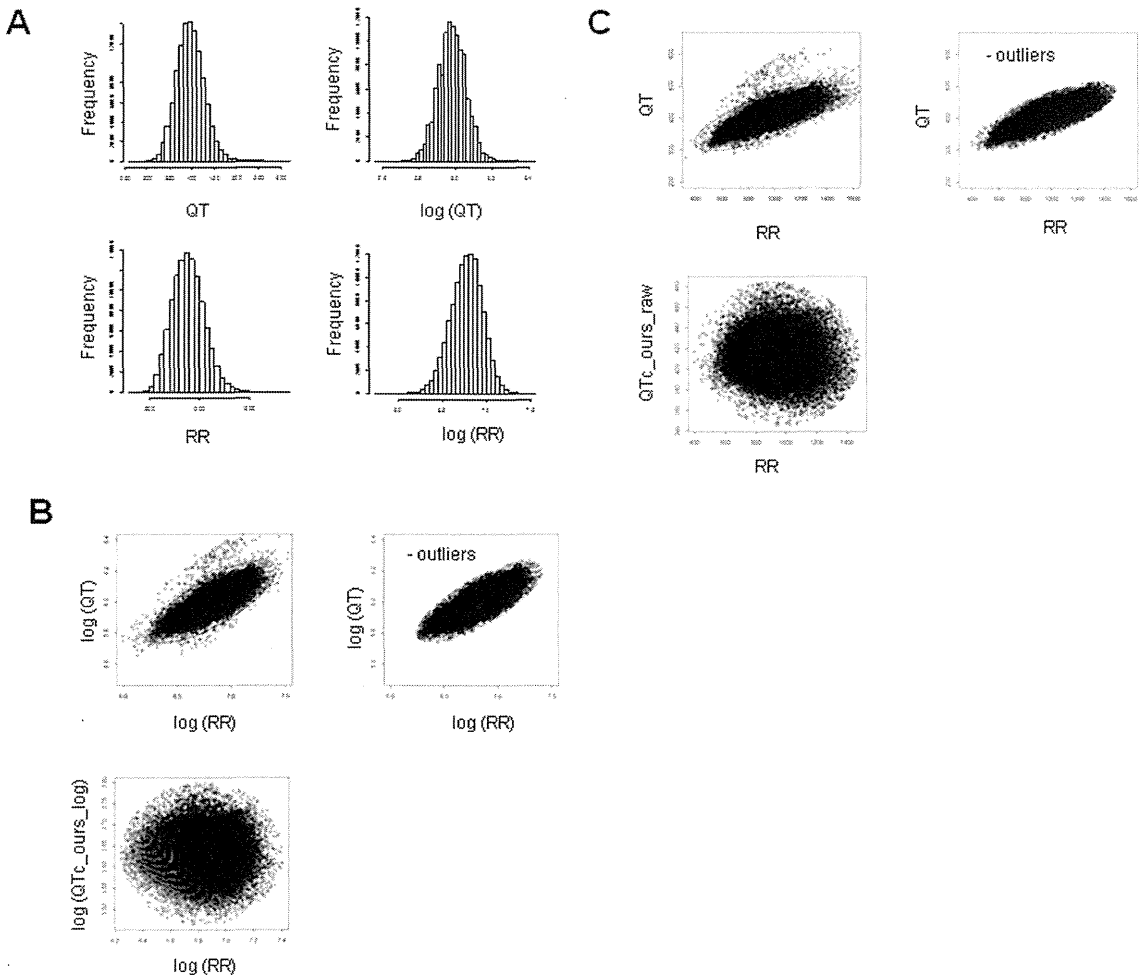

Figure 2. Analysis of resting Nihon Kohden ECGs. (A) Histograms of QT, log-transformed QT, RR, and log-transformed RR intervals. (B) Scatter plots of log QT versus log RR and log QTc_ours log versus log RR. (C) QT versus RR and QTc_ours raw versus RR. Units of all variables are ms.
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Manufacturer-related effects on QT variation

The values obtained by each correction method for QT were compared between the two manufacturers for each gender by Student t-test.

Next, to examine whether the manufacturer-related differences in automatic measurement of QT interval was independent of other factors such as gender and age, we combined the data sets from Fukuda Denshi and Nihon Kohden ECGs, and performed a multivariate regression analysis. Thus, the combined data were analyzed by the linear multivariate regression model shown by the following formula

$$QT = aX + bGender + cAge + Y,$$

where X denotes a manufacturer (0 or 1), Gender denotes a gender (coded as 1 or 2), Age denotes an age in year and Y denotes the residual variable. We tested whether a is equal to 0. Age was expressed by year because relation between QT and age was nearly linear in each gender as judged by the visual inspection.

Statistical analysis

All statistical analyses were performed using R environment (version 2.15.0). Data are presented as means ± SD. A value of $P<0.05$ was considered statistically significant.

Results

Optimal correction formula for QT interval

After the elimination of outliers, the remaining 8,595 male and 1,889 female adult resting Fukuda Denshi ECGs and the remaining 42,398 male and 29,936 female adult resting Nihon Kohden ECGs were subjected to QT interval analysis. In the following formulas for the correction of QT by RR, the units of QT and RR are sec.

Regression analysis using the log-transformed RR value as an independent variable and the log-transformed QT value as the dependent variable produced a regression coefficient of 0.347 (95% CI, 0.342 to 0.352, $P<2.0\times10^{-16}$, R-squared = 0.609) for Fukuda Denshi ECGs, whereas 0.347 (95%CI, 0.345 to 0.349; $P<2.0\times10^{-16}$, R-squared = 0.612) for Nihon Kohden ECGs. Therefore, an appropriate correction formula for both Fukuda Denshi and Nihon Kohden would be in the form of QTc (ours_log) = $QT\times RR^{-0.347}$. When this correction was made, as expected, the effect of heart rate on QTc was negligible as shown by scatter plots of log (QTc) (ours_log) versus log (RR) (Figure 1B and Figure 2B).

We next tested other published methods as to whether those methods efficiently remove the effects of RR. We performed the linear regression analysis by using log-transformed RR as an independent variable and log-transformed QTc calculated according to Fredericia or Bazzet method as the dependent variable. Note that both Fredericia and Bazzet methods assume the linearity between log-transformed QT and RR. The effect of RR on QTc (Fredericia) remained as shown by the linear regression model using log-transformed variables ($\beta = 0.0103$; 95% CI, 0.00513 to 0.0155; $P = 9.54\times10^{-5}$ for Fukuda Denshi ECGs, $\beta = 0.0144$; 95% CI, 0.0125 to 0.0164; $P = 2\times10^{-16}$ for Nihon Kohden ECGs). If the effect of RR is completely removed, the correlation coefficient β would be 0. The effect of RR on QTc (Bazett) was large as shown by the linear regression model using log-transformed variables ($\beta = -0.156$; 95% CI, −0.151 to −0.157; $P = 2\times10^{-16}$ for Fukuda Denshi ECGs, $\beta = -0.152$; 95% CI, −0.150 to −0.154; $P = 2\times10^{-16}$ for Nihon Kohden ECGs). The regression coefficient after correction by Bazett's formula indicated excessive correction of QT by RR in normal Japanese resting ECGs, as previously reported [17].

We also performed a regression analysis using raw RR interval as an independent variable and a raw QT value as the dependent variable. After excluding the outliers, the remaining 8,578 male and 1,885 female Fukuda Denshi ECGs and the remaining 42,309 male and 29,960 female Nihon Kohden ECGs were subjected to analysis, which produced a regression coefficient of 0.156 (95%CI 0.154–0.158, $P<2.0\times10^{-16}$, R-squared = 0.608) for Fukuda Denshi ECGs and 0.152 (95%CI, 0.151 to 0.153, $P<2.0\times10^{-16}$, R-squared = 0.607) for Nihon Kohden ECGs. Therefore, an appropriate correction formula is in the form of QTc (ours_raw) = QT+0.156×(1-RR) for Fukuda Denshi and QTc (ours_raw) = QT+0.152×(1-RR) for Nihon Kohden. When this correction was made, as expected, the effect of heart rate on QTc was negligible as shown by scatter plots of QTc (ours_raw) versus RR (Figure 1C and 2C). This formula is relatively close to the Framingham method, i.e., QTc = QT+0.154×(1-RR).

Comparison of automatic QT measurements in adult resting ECGs between Fukuda Denshi and Nihon Kohden

Table 1 summarizes our comparison data. The proportion of outliers under the assumption of bivariate normal distribution of log-transformed QT vs. log-transformed RR was larger in Nihon Kohden ECGs than in Fukuda Denshi ECGs, especially in male recordings (0.64% vs. 0.42% in males, $P = 0.014$, by Fisher's exact test; 0.48% vs. 0.47% in females, $P = 1.00$, by Fisher's exact test).

The average uncorrected automatic QT interval was 378.6±25.7 ms in males and 388.1±26.2 ms in females for Fukuda Denshi and 404.0±28.4 ms in males and 406.4±27.3 ms in females for Nihon Kohden. Therefore, the average uncorrected automatic QT interval was longer in Nihon Kohden ECGs than in Fukuda Denshi ECGs (difference of mean; 25.4 ms in males and 18.3 ms in females, $P<2.2\times10^{-16}$ for both males and females, Student t-test).

After optimizing the heart rate correction of QT interval by the linear regression model using the log-transformed data, QTc (ours_log) interval by Fukuda Denshi in males was 400.1±16.8 ms, and in females was 407.2±17.7 ms, while QTc (ours_log) interval by Nihon Kohden in males became 412.9±17.8 ms, and in females became 418.8±17.4 ms ($P<2.2\times10^{-16}$ for both males and females, Student t-test). After optimizing the heart rate correction of QT interval by the linear regression model using raw data, QTc (ours_raw) interval by Fukuda Denshi in males was 400.5±15.9 ms, and in females was 407.3±16.8 ms, while QTc (ours_raw) interval by Nihon Kohden in males was 412.3±17.4 ms, and in females was 418.4±17.1 ms (difference between two manufacturers, $P<2.2\times10^{-16}$ for both males and females, Student t-test). Thus, after optimizing for heart rate correction of QT interval by the linear regression model using either log-transformed data or raw data, the QTc (our method) interval was ~10 ms longer in Nihon Kohden than Fukuda Denshi ECGs.

The effect of ECG manufacturer on automatic measurement of QT interval

To examine whether the manufacturer-related differences in automatic measurement of QT interval was independent of other factors such as gender and age, we combined the data sets from Fukuda Denshi and Nihon Kohden ECGs, and performed a multivariate regression analysis using a log-transformed RR value, gender, age and ECG manufacturer as independent variables, and a log-transformed QT value as the dependent variable, as described in the Methods. The analysis produced the following regression coefficients: 0.348 for log-transformed RR (95%CI, 0.346 to 0.349; $P<2.0\times10^{-16}$), −0.0123 for gender (male) (95%CI, −0.0117 to −0.0129, $P<2.0\times10^{-16}$), 0.000686 for age (95%CI, 0.000667 to 0.000704, $P<2.0\times10^{-16}$), and, of note, 0.0275 for ECG manufacturer (Nihon Kohden) (95%CI, 0.0266 to 0.0283, $P<2.0\times10^{-16}$). Assuming that the average QT interval is 400 ms, the inter-manufacturer difference in QT interval was 11.1 ms.

Similarly, a multivariate regression analysis using raw RR, gender, age and ECG manufacturer as independent variables, and a raw QT value as the dependent variable revealed a regression coefficient for RR of 0.153 (95%CI, 0.152 to 0.153, $P<2.0\times10^{-16}$), −5.154 for gender (male) (95%CI, −4.917 to −5.391, $P<2.0\times10^{-16}$), 0.276 for age (95%CI, 0.268 to 0.284, $P<2.0\times10^{-16}$), and, of note, 10.610 for ECG manufacturers (Nihon Kohden) (95%CI, 9.808 to 10.962, $P<2.0\times10^{-16}$), resulting in an inter-manufacturer difference for QT of 10.6 ms.

Effect of the difference in ECG manufacturer on automatic measurement of RR interval

The automatic RR interval by Fukuda Denshi was 859.7±132.8 ms in males and 876.5±123.3 ms in females, while the automatic RR interval by Nihon Kohden was 947.6±151.2 ms in males and 922.6±133.4 ms in females. We verified the causes of inter-manufacturer differences in RR interval. A multivariate regression analysis using age and ECG manufacturer as independent variables and an RR value as the dependent variable revealed a regression coefficient for ECG manufacturer (Nihon Kohden) of 84.95 in males and 44.72 in females. Surprisingly, RR interval was longer in Nihon Kohden ECGs than in Fukuda Denshi ECGs, even after adjustment for age. Thus, RR interval was 9.43% longer in males and 4.97% longer in

Table 1. Comparison of automatic QT measurements in adult resting ECGs between Fukuda Denshi and Nihon Kohden.

gender number age	QT	RR	QTc (log)		QTc (raw)	
			study	statistic	study	statistic
Nihon Kohden						
			corrected coefficient: 0.347		corrected coefficient: 0.152	
male 42,673 49.9±15.3	404.0±28.4	947.6±151.2	Ours	412.9±17.8	Ours	412.3±17.4
			Fredericia	412.5±17.8	Framingham	412.2±17.4
			Bazett	417.2±20.8	ECAPs12	411.6±17.4
female 30,081 53.6±16.5	406.4±27.3	922.6±133.4	Ours	418.8±17.4	Ours	418.4±17.1
			Fredericia	418.3±17.5	Framingham	418.2±17.1
			Bazett	424.8±19.6	ECAPs12	417.4±17.2
Fukuda Denshi						
			corrected coefficient: 0.347		corrected coefficient: 0.156	
male 8,631 46.1±9.2	378.6±25.7	859.7±132.8	Ours	400.1±16.8	Ours	400.5±15.9
			Fredericia	399.2±17.7	Framingham	400.2±15.9
			Bazett	410.3±20.0	ECAPs12	398.6±15.9
female 1,898 45.3±7.9	388.1±26.2	876.5±123.3	Ours	407.2±17.7	Ours	407.3±16.8
			Fredericia	406.4±17.7	Framingham	407.0±16.9
			Bazett	416.1±19.7	ECAPs12	405.6±17.0

Units of RR, QT and all corrected forms of QT in this table are ms.
The results of the tests of differences in QT, RR, QTc(ours_log), Fredericia, Bazett, QTc(ours_raw), Framingham and ECAPs12 between Nihon Koden and Fukuda Denshi for each gender were all $P<2.2\times10^{-16}$ (Student t-test).
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females on Nihon Kohden ECGs than on Fukuda Denshi ECGs ($P<2.0\times10^{-16}$).

Discussion

What we expect from automatic measurements of QT/QTc interval varies with the times. The automatic approach has been widely used to rule out high-risk patients for SCD in clinical practice. However, as automatic QT measurement technologies have evolved with concomitant increases in precision, automatic measurement becomes a preference, and indeed a necessity, when analyzing 1000 s of ECGs in the setting of either large, multicenter, genome-wide association studies (GWAS) of QT interval identifying common genetic variants among ethnic groups or drug-inducing QT prolongation screening [15]. Automatic QT measurement has advantages over manual measurements from tracings in such settings in terms of reproducibility and time reduction. According to our experience, errors often occur in automatic measurements because the identification of the end of the T wave is difficult in some cases. They were excluded as outliers outside the contour line that includes 99.9% of the integral of the probability density function of the bivariate normal distribution (log QT vs. log RR or raw QT vs. raw RR).

We initiated the present study because the difference in QTc between different manufacturers was big enough to disturb our GWAS for ECG parameters even if it is too little to be noticed in clinical situations. ECG machines from Fukuda Denshi and Nihon Kohden dominate the market of ECG in Japan; however, these companies independently developed automated computer algorithms for QT measurement. In addition, they apply different methods for heart rate correction of QT interval, with Fukuda Denshi machines using Bazett's formula and Nihon Kohden using the ECAPs12 formula. Despite such key differences, the validity of QT correction formulae and the differences in absolute values of QTc between Fukuda Denshi and Nihon Kohden recordings have never been considered.

Using automatic measurements of QT and RR intervals from 10,529 Fukuda Denshi ECGs and 72,754 Nihon Kohden ECGs recorded for healthy individuals, we found that corrected QTc values calculated by either Bazett's or ECAPs12 formulae had residual heart rate dependence after correction. When we used log-transformed data, the optimal heart rate correction formula of QT interval was equivalent between Fukuda Denshi and Nihon Kohden systems, and would be in the form of QTc (our method) = $QT\times RR^{-0.347}$. However, when we used raw data, the optimal heart rate correction formula of QT interval was in the form of QTc (our method) = $QT+0.156\times(1-RR)$ for Fukuda Denshi and QTc (our method) = $QT+0.152\times(1-RR)$ for Nihon Kohden. Since our study included the largest number of subjects from the Japanese ancestry, we proposed our own correction formulas for QT to adjust for RR.

In addition, after optimization of heart rate (HR) correction of QT interval by the linear regression model using either log-transformed data or raw data, QTc (our method) interval was ~10 ms longer in Nihon Kohden ECGs than in those recorded on Fukuda Denshi machines. Indeed, regression analysis revealed that differences in the ECG machine used had up to a two-fold larger impact on QT variation than gender difference. Such an impact is likely to be of considerable importance when ECGs for a given individual are recorded on different machines in the setting of multi-institutional joint research. For example, the effect of various factors (gene, gender, age, drug etc) on QT interval can never be evaluated without the information about the ECG manufacturer.

The explanation for this difference remains speculative but one would emphasize that Fukuda Denshi and Nihon Kohden adopt different definitions for measurement of QT interval. Fukuda Denshi adopts the average duration of QT interval, in which QT interval is defined as the mean of 12-lead measurements. By contrast, Nihon Kohden adopts the global duration of QT interval, in which QT interval is defined by the earliest QRS onset in one lead and latest offset of T-end in any other lead (wave onset and offset do not necessarily appear at the same time in all leads because the activation of wave fronts propagates differently). Such difference should yield longer QT interval by Nihon Kohden ECG machine than Fukuda Denshi ECG machine.

Surprisingly, even after adjustment for age, the automatic measurements of RR interval are 9.43% longer in males and 4.97% longer in females on Nihon Kohden ECGS than on those recorded by Fukuda Denshi systems. So far, we have no explanation for such a big difference in RR interval, which Fukuda Denshi calculates as the mean RR interval during recording time, whereas Nihon Kohden calculates RR interval by length of time between the first beat and the last beat during recording time divided by number of beats −1. Neither manufacturer excludes premature beats from automatic measurements of RR interval.

In conclusion, inter-manufacturer difference of QT interval was ~10 ms even after optimizing the heart rate correction of QT interval. Based on these study results, we recommend that ECG machines of the same manufacturer are used to measure QT and RR intervals in the setting of multi-institutional joint research. It is desirable to unify the computer algorithm for automatic QT and RR measurements from an ECG and optimize the heart rate correction formula of QT interval. Otherwise, interchangeable formula should be established.

Limitations

A motive of the present study was that we found clear differences in average QTc's between different sampling sites for 3,000 healthy individuals. Although they were clustered into two different groups, we were able to identify neither the manufacturers nor the correction formula for QTc in each sampling site. In contrast, the manufacturers and correction formula but not phenotypes are clear in the present data set with about 80,000 subjects. Therefore, a limitation of this study is that the two different data sets are quite different in nature since the former is from healthy individuals while phenotypes of the latter are unknown, the manufacturer data are obtained from the latter but not from the former and the correction formula are obtained from the latter but not from the former.

There are, in general, two different approaches in medicine, i.e. one by the analysis of a small number of individuals in detail and the other by analyzing big data from multiple individuals using statistics. Both of the approaches are important because each of them has drawbacks and both of the approaches compensate for the drawbacks of each other. Our present study employed the latter approach.

This is a retrospective study. Since we were not able to collect all covariates including BMI, some factors not included in our analysis may have been important confounding factors. So far, we have no explanation for a difference in RR interval between ECG machine manufacturers. We could not exclude the confounding variables that influence autonomic nervous system activity.

To the best of our knowledge, this is the first work to assess the outcomes with different automated measurements of QT interval between ECG machine manufacturers. The next step is to obtain ECG data from many individuals for each of whom the machines from the two manufacturers are used at the same time.

Supporting Information

Data S1 The log-transformed QT and RR assumed a closer to normal distribution than the raw variables.
(DOCX)

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Author Contributions

Conceived and designed the experiments: MS YA YK NN ST KF. Performed the experiments: MS SK NK. Analyzed the data: MS SK NK. Contributed reagents/materials/analysis tools: MS SK NK. Contributed to the writing of the manuscript: MS NK.

References

1. Tomaselli GF, Beuckelmann DJ, Calkins HG, Berger RD, Kessler PD, et al. (1994) Sudden cardiac death in heart failure. The role of abnormal repolarization. Circulation 90: 2534–2539.
2. Straus SM, Kors JA, De Bruin ML, van der Hooft CS, Hofman A, et al. (2006) Prolonged QTc interval and risk of sudden cardiac death in a population of older adults. J Am Coll Cardiol 47: 362–367.
3. Vrtovec B, Delgado R, Zewail A, Thomas CD, Richartz BM, et al. (2003) Prolonged QTc interval and high B-type natriuretic peptide levels together predict mortality in patients with advanced heart failure. Circulation 107: 1764–1769.
4. Schouten EG, Dekker JM, Meppelink P, Kok FJ, Vandenbroucke JP, et al. (1991) QT interval prolongation predicts cardiovascular mortality in an apparently healthy population. Circulation 84: 1516–1523.
5. Salvi V, Karnad DR, Panicker GK, Kothari S (2010) Update on the evaluation of a new drug for effects on cardiac repolarization in humans: issues in early drug development. Br J Pharmacol 159: 34–48.
6. Splawski I, Shen J, Timothy KW, Lehmann MH, Priori S, et al. (2000) Spectrum of mutations in long-QT syndrome genes. KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. Circulation 102: 1178–1185.
7. Priori SG, Schwartz PJ, Napolitano C, Bloise R, Ronchetti E, et al. (2003) Risk stratification in the long-QT syndrome. N Engl J Med 348: 1866–1874.
8. Moss AJ, Schwartz PJ, Crampton RS, Tzivoni D, Locati EH, et al. (1991) The long QT syndrome. Prospective longitudinal study of 328 families. Circulation 84: 1136–1144.
9. Newton-Cheh C, Eijgelsheim M, Rice KM, de Bakker PI, Yin X, et al. (2009) Common variants at ten loci influence QT interval duration in the QTGEN Study. Nat Genet 41: 399–406.
10. Pfeufer A, Sanna S, Arking DE, Muller M, Gateva V, et al. (2009) Common variants at ten loci modulate the QT interval duration in the QTSCD Study. Nat Genet 41: 407–414.
11. Roden DM (2006) Long QT syndrome: reduced repolarization reserve and the genetic link. J Intern Med 259: 59–69.

12. Noseworthy PA, Havulinna AS, Porthan K, Lahtinen AM, Jula A, et al. (2011) Common genetic variants, QT interval, and sudden cardiac death in a Finnish population-based study. Circ Cardiovasc Genet 4: 305–311.
13. Darpo B, Agin M, Kazierad DJ, Layton G, Muirhead G, et al. (2006) Man versus machine: is there an optimal method for QT measurements in thorough QT studies? J Clin Pharmacol 46: 598–612.
14. Hnatkova K, Gang Y, Batchvarov VN, Malik M (2006) Precision of QT interval measurement by advanced electrocardiographic equipment. Pacing Clin Electrophysiol 29: 1277–1284.
15. Couderc JP, Garnett C, Li M, Handzel R, McNitt S, et al. (2011) Highly automated QT measurement techniques in 7 thorough QT studies implemented under ICH E14 guidelines. Ann Noninvasive Electrocardiol 16: 13–24.
16. Sagie A, Larson MG, Goldberg RJ, Bengtson JR, Levy D (1992) An improved method for adjusting the QT interval for heart rate (the Framingham Heart Study). Am J Cardiol 70: 797–801.
17. Funck-Brentano C, Jaillon P (1993) Rate-corrected QT interval: techniques and limitations. Am J Cardiol 72: 17B–22B.

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CLINICAL RESEARCH

Exon 3 deletion of *RYR2* encoding cardiac ryanodine receptor is associated with left ventricular non-compaction

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Aims Ryanodine receptor gene (*RYR2*) mutations are well known to cause catecholaminergic polymorphic ventricular tachycardia (CPVT). Recently, *RYR2* exon 3 deletion has been identified in patients with dilated cardiomyopathy (DCM) and/or CPVT. This study aimed to screen for the *RYR2* exon 3 deletion in CPVT probands, characterize its clinical pathology, and confirm the genomic rearrangement.

Methods and results Our cohort consisted of 24 CPVT probands. Polymerase chain reaction (PCR)-based conventional genetic analysis did not identify any mutations in coding exons of *RYR2* in these probands. They were screened using multiplex ligation-dependent probe amplification (MLPA). In probands identified with *RYR2* exon 3 deletion, the precise location of the deletion was identified by quantitative PCR and direct sequencing methods. We identified two CPVT probands from unrelated families who harboured a large deletion including exon 3. The probands were 9- and 17-year-old girls. Both probands had a history of syncope related to emotional stress or exercise, exhibited bradycardia, and were diagnosed with left ventricular non-compaction (LVNC). We examined 10 family members and identified six more *RYR2* exon 3 deletion carriers. In total, there were eight carriers, of which seven were diagnosed with LVNC (87.5%). Two carriers under the age of 4 years remained asymptomatic, although they were diagnosed with LVNC. Using quantitative PCR and direct sequencing, we confirmed that the deletions were 1.1 and 37.7 kb in length.

Conclusion *RYR2* exon 3 deletion is frequently associated with LVNC. Therefore, detection of the deletion offers a new modality for predicting the prognosis of patients with LVNC with ventricular/atrial arrhythmias, particularly in children.

Keywords *RYR2* exon 3 deletion • Catecholaminergic polymorphic ventricular tachycardia • Left ventricular non-compaction • Bradycardia • Genetic screening • Multiplex ligation-dependent probe amplification

Introduction

Cardiac ryanodine receptor (RyR2) is an essential Ca^{2+} release channel of the sarcoplasmic reticulum (SR) and plays a central role in excitation–contraction coupling in cardiomyocytes.[1] Abnormal Ca^{2+} leak from SR due to RyR2 dysfunction generates delayed afterdepolarization (DAD) and causes catecholaminergic polymorphic ventricular tachycardia (CPVT).[2] Catecholaminergic polymorphic ventricular tachycardia is characterized by exercise-induced ventricular tachyarrhythmias and sudden cardiac death in structurally normal hearts.[3]

In 2007, Bhuiyan *et al.*[4] reported two families who carried a 1.1 kb deletion of ryanodine receptor gene (*RYR2*) including exon 3. The carriers displayed sinoatrial and atrioventricular node dysfunction, atrial fibrillation, and atrial standstill, in addition to exercise-related ventricular arrhythmias. Some affected members also displayed left ventricular dysfunction and were diagnosed with dilated cardiomyopathy (DCM). Other CPVT families were subsequently identified

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What's new?

- Ryanodine receptor gene (*RYR2*) exon 3 deletion was suspected as a genetic cause of left ventricular non-compaction.
- Left ventricular non-compaction associated with *RYR2* exon 3 deletion was related with catecholaminergic polymorphic ventricular tachycardia and/or bradycardia.
- The deletion range varied among each family. To confirm the deletion and to characterize the precise location of the genomic deletion, we performed quantitative polymerase chain reaction (PCR), long-range PCR, and direct sequencing.

as carrying a 1.1 or 3.6 kb deletion including exon 3.[5,6] In one patient with 1.1 kb deletion, increased trabeculation of the left ventricle was observed, suggesting the presence of non-compaction cardiomyopathy [left ventricular non-compaction (LVNC)].[5] Left ventricular non-compaction is a form of cardiomyopathy characterized by a pattern of prominent trabecular meshwork and is thought to be caused by an arrest of normal endomyocardial morphogenesis.[7] Although LVNC is primarily classified as a cardiomyopathy, its genetic background is not yet fully elucidated.[8]

In the present study, we screened for *RYR2* exon 3 deletion in 24 probands diagnosed with CPVT and identified two carriers with the deletion. In addition to exercise-induced ventricular tachycardia and bradycardia, echocardiography identified the presence of LVNC in both probands. Here, we performed detailed clinical and genetic analyses on the probands and their family members who carried *RYR2* exon 3 deletion.

Methods

Study population

The study cohort consisted of 24 CPVT probands (13 females), who were referred to two institutes in Japan, Shiga University of Medical Science and Kyoto University Graduate School of Medicine, for genetic evaluation. Their mean age at registration was 15.0 ± 2.2 years. In addition to CPVT probands, we included five probands with LVNC identified by echocardiography—four of them were diagnosed with DCM (one female) and one female was diagnosed with sick sinus syndrome (SSS). The mean age of the five probands with DCM and SSS was 47.4 ± 10.5 years. All subjects submitted written informed consent in accordance with the guidelines approved by each institutional review board. They were evaluated according to medical history, physical examination, standard 12-lead electrocardiography (ECG), exercise stress test, ambulatory 24 h ECG (Holter) monitoring, and echocardiography. Left ventricular non-compaction was diagnosed according to previously proposed echocardiographic criteria:[9] (i) the absence of coexisting cardiac abnormalities, (ii) the presence of a two-layer structure, including a compacted thin epicardial band and a much thicker non-compacted endocardial layer of trabecular meshwork with deep endomyocardial spaces, a maximal end systolic ratio of non-compacted to compacted layers of >2, and (iii) colour Doppler evidence of deep perfused intertrabecular recesses.

Genetic analysis

Catecholaminergic polymorphic ventricular tachycardia probands were found to be negative for mutations in all coding exons of *RYR2*, *CASQ2*, *KCNJ2*, *KCNQ1*, and *SCN5A*, as verified by polymerase chain reaction (PCR)-based direct sequencing. cDNA sequence of *RYR2* was identified on the basis of GenBank reference sequence NM 001035.2. To identify *RYR2* exon 3 deletion, we performed multiplex ligation-dependent probe amplification (MLPA) analysis. The probes for MLPA analysis of *RYR2* exons 3 and 97 (SALSA MLPA Kit P168) were purchased from MRC Holland. Because the MLPA Kit P168 consists of ARVC/D-related genes, only *RYR2* exons 3 and 97 were included. Multiplex ligation-dependent probe amplification was performed according to the manufacturer's instructions. To confirm the precise location of the deletion, long-range PCR was performed using Tks G flex polymerase (Takara Bio) for reactions using previously reported primer pairs[4] or KOD FX neo (TOYOBO) for newly designed primer pairs (*Table 1*). Conventional direct sequencing was performed using ABI PRISM-3130 sequencer (Applied Biosystems).

Quantitative polymerase chain reaction analysis for detecting deletion range

Quantitative PCR (qPCR) primers (*Table 1*) for Universal Probe Library system (Roche Diagnostic GmbH) were designed with the Universal

Table 1 Primer design for long-range PCR and qPCR

Primer set	Probe number	Primer	
		Forward	Reverse
Long PCR1	–	5′-agctgctgagtagcagaaccag-3′	5′-gctactcaccaccacagact-3′
intron 1	10	5′-ttcactggaaatcagtgagca-3′	5′-caggcaaagcccaatattttt-3′
intron 2-1	24	5′-cgaggtatcatttcacagattgg-3′	5′-gccaaagtgcttataccgtca-3′
intron 2-2	20	5′-tcttgctggcctcatgaata-3′	5′-ttgggtctgcactgctttta-3′
exon 3	67	5′-tgatgctgctgactgctctt-3′	5′-ggtgttagccagcatctcct-3′
intron 3	58	5′-gcccacagaagctttcagat-3′	5′-ttctccttctcgttgcctgt-3′
exon 4	–	5′-tgtggtgcaaggaccaaat-3′	5′-attagaggcgagagcaagca-3′
intron 4	86	5′-caaaaaccttgactgtttaatctcaa-3′	5′-caacatagtgagactccatctatacca-3′
KCNE1	78	5′-gcatcatgctgagctacatcc-3′	5′-agacgttgaatgggtcgttc-3′
Long PCR2	–	5′-agctgctgagtagcagaaccag-3′	5′-tagaggcgagagcaagcatgcac-3′

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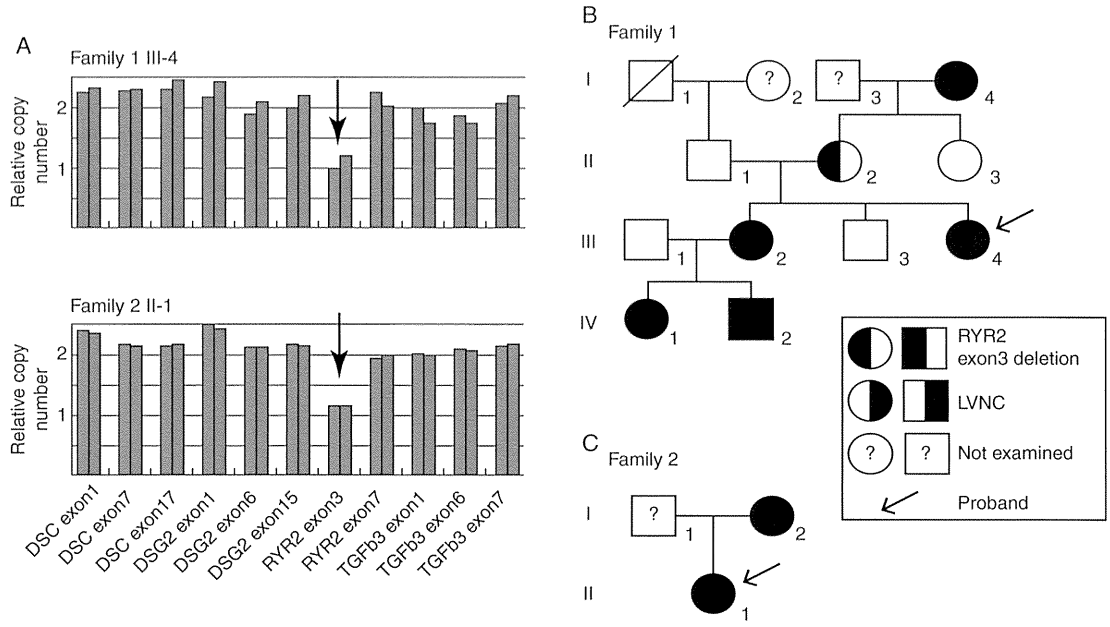

Figure 1 Multiplex ligation-dependent probe amplification analysis and family trees. (*A*) Multiplex ligation-dependent probe amplification analysis of the probands. Both graphs show the decrease in ryanodine receptor gene (*RYR2*) exon 3 (arrows). (*B*, *C*) Family trees of the *RYR2* exon 3 deletion carriers.

Table 2 Clinical summary of carriers with *RYR2* exon 3 deletion

Family	Patient		Age	Syncope	HR	LVNC	CPVT	Other	Medication	Device
1	I	4	80	−	70 (PM)	+	−	SSS	−	PM
	II	2	52	−	75 (AF)	−	−	AF	Verapamil	−
	III	2	25	+	44	+	+	SSS	BB	ICD
		4	17	+	54	+	+	SSS	BB	ICD
	IV	1	3	−	83	+	−	−	−	−
		2	1	−	78	+	−	−	−	−
2	I	2	38	+	52	+	+	−	−	−
	II	1	9	+	54	+	+	CAVB	BB	PM

AF, atrial fibrillation; Age, age at clinical evaluation; BB, β-blocker; CAVB, complete atrioventricular block; CPVT, catecholaminergic polymorphic ventricular tachycardia; ICD, implantable cardioverter-defibrillator; LVNC, left ventricular non-compaction; Other, other clinical diagnosis; PM, pacemaker; SSS, sick sinus syndrome; +, positive; −, negative or none.

Probe Library Assay Design Center (https://www.roche-applied-science.com/). We performed relative quantitation using the Light Cycler probe master mix (Roche). With the exception of the exon 3 primer setting, we introduced the human G6PD gene assay (Roche) labelled with LC yellow 555 as the reference and performed dual-colour analysis. For quantification of exon 3, we used the reference probe–primer set designed for *KCNE1* located on chromosome 21 and performed monocolour analysis. For exon 4, we used the SYBR green method because we could not design the probe set for the exon (*Table 1*). All qPCRs were performed using LightCycler 480 instrument (Roche Diagnostic GmbH), and the data were analysed using $\Delta\Delta C_T$ method.

Results

Multiplex ligation-dependent probe amplification analysis

No mutations were detected in any exons of *RYR2* of 24 CPVT probands using conventional PCR methods. We next performed MLPA analysis to identify the *RYR2* exon 3 deletion and identified two probands with the deletion. *Figure 1A* (indicated as arrows) shows the results of MLPA analysis in the two probands. The ratio of copy

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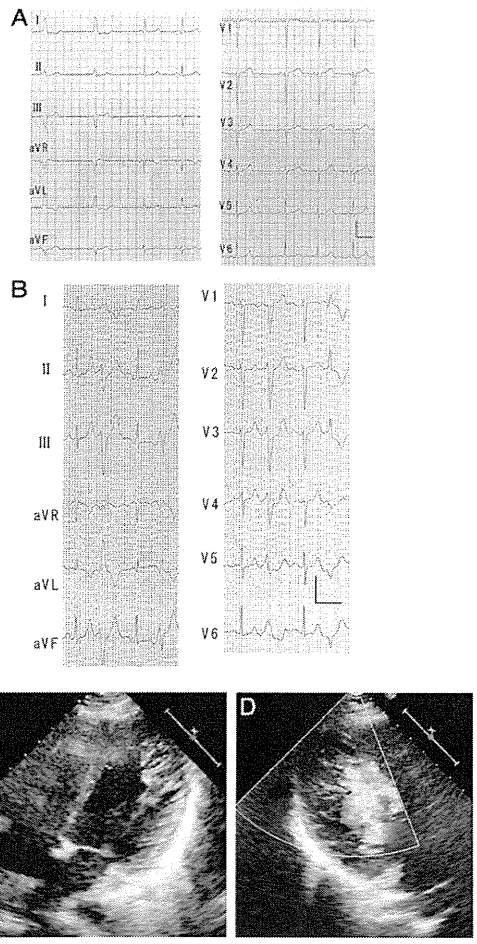

Figure 2 Clinical characteristics of Family 1. Twelve-lead ECG at rest (*A*) and multifocal PVCs recorded in the exercise stress test of proband III-4 (*B*). Atrioventricular dissociation was intermittently detected in ECG at rest. (*C*) Apical four chamber view showed a thick non-compaction layer and an increased trabeculation in the proband (III-4). (*D*) Colour Doppler echocardiogram of III-2 showing the intertrabecular recesses filled with blood from the left ventricular cavity. Scale bars indicate 1 mV and 400 ms.

number for *RYR2* exon 3 was half compared with that of controls, suggesting that these probands carried a deletion in exon 3. We further performed MLPA analysis in 10 family members from the two families and identified 6 family members carrying the exon 3 deletion (*Figure 1B* and *C*). Although we screened for *RYR2* exon 3 deletion in five other patients with LVNC, no one carried the deletion.

Clinical characteristics

Table 2 summarizes the clinical characteristics of the eight carriers with *RYR2* exon 3 deletion.

Family 1

The proband (III-4) was a 17-year-old girl diagnosed with CPVT, with a history of repeated syncope related to exercise (*Figure 1B*). Her ECG at rest showed sinus bradycardia (54 b.p.m.) and intermittent atrioventricular dissociation (*Figure 2A*). In addition, bidirectional ventricular tachycardia was detected by Holter recording (data not shown). Frequent premature ventricular contractions (PVCs) were observed with increasing heart rate during the treadmill exercise test (TMT) (*Figure 2B*), and echocardiography revealed the presence of LVNC (*Figure 2C*). β-Blocker therapy failed to suppress the syncope and worsened the bradycardia. Therefore, she was administered flecainide after implantable cardioverter-defibrillator (ICD) implantation. Her sister (III-2) had also experienced syncope while swimming in her teens and was diagnosed with LVNC on echocardiography (*Figure 2D*). Her heart rate at rest was 44 b.p.m., and PVCs were also observed in TMT with increasing heart rate. She was also diagnosed with CPVT and was administered flecainide after ICD implantation. Her 3-year-old daughter (IV-1) and 1-year-old son (IV-2) were asymptomatic, although their ECGs showed mild bradycardia at age, 83 and 78 b.p.m., respectively. Furthermore, they were diagnosed with LVNC on echocardiography. The proband's mother (II-2) was asymptomatic but had persistent atrial fibrillation, and verapamil was prescribed to control her heart rate since the age of 47. Her echocardiography did not show LVNC. The proband's maternal grandmother (I-4) underwent pacemaker implantation due to severe bradycardia. Moreover, she was diagnosed with LVNC on echocardiography. Other family members in the family tree did not complain of any cardiac symptoms and showed no apparent ECG abnormality.

Family 2

The proband was a 9-year-old girl (II-1) who lost consciousness while playing a game to beat a drum (*Figure 1C*). When she was newborn, premature atrial contractions were observed and was diagnosed with peripheral pulmonary stenosis. She was followed up until the age of 2 years, during which she did not exhibit any morphological or neurological abnormalities. She experienced syncope at the age of 1 and 4 but had recovered immediately both times. She suffered a third episode of syncope at the age of 9 and was admitted to the emergency hospital by an ambulance. Her ECG showed bradycardia (54 b.p.m.), Mobitz type 1 atrioventricular block, and T-wave inversions in lead V1–3 (*Figure 3A*). In an exercise stress test, PVCs appeared immediately and changed to polymorphic ventricular tachycardia (VT) with increasing heart rate (*Figure 3B*), and she was diagnosed with CPVT. Left ventricular non-compaction diagnosis was confirmed on echocardiography (*Figure 3C*) and cardiac magnetic resonance imaging (MRI). In both examinations, her left ventricular compaction layer was thin with a prominent trabeculation. In contrast MRI image, non-compaction area was clearly visualized. For the evaluation of bradycardia, she was performed electrophysiological study and was diagnosed with complete atrioventricular block. A pacemaker was implanted, and she was treated with carvedilol (0.1 mg/kg), which successfully suppressed her syncope thereafter. Her mother (I-2) also experienced syncope while exercising at the age of 12 and 35. Her mother's ECG (*Figure 3D*) was similar to the proband's, showing bradycardia (52 b.p.m.), T-wave inversions from V1 through V5 leads, and atrioventricular dissociation was suspected. Polymorphic VT was recorded in the exercise stress test

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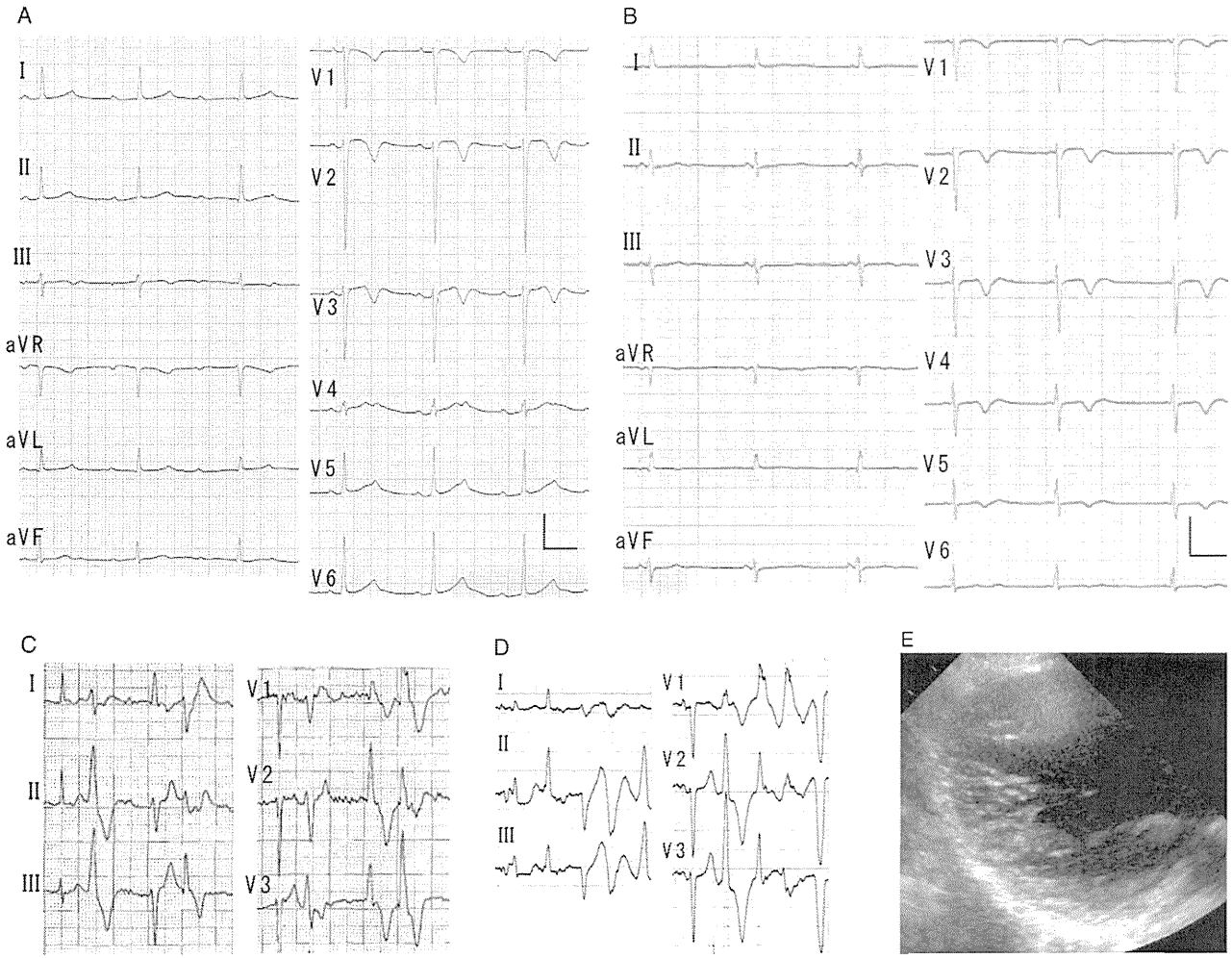

Figure 3 Clinical characteristics of Family 2. Twelve-lead electrocardiography (ECG) at rest of the proband (II-1) (*A*) and her mother (I-2) (B) showed Morbitz type 1 AV block (*A*) and sinus bradycardia (*B*). Polymorphic ventricular tachycardia was recorded in an exercise stress test of the proband (*C*) and RYR2 exon-3 deletion in LVNC her mother (*D*). (*E*) Apical four chamber view of the proband, which showed a noncompaction layer and numerous trabeculations. Scale bars indicate 1 mV and 400 ms.

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Table 3 Clinical summary of reported families with *RYR2* exon 3 deletion

Family	Deletion (kb)	Carrier (*n*)	Atrial arrhythmia (*n*)	Sinoatrial dysfunction (*n*)	AV block (*n*)	Ventricular arrhythmia (*n*)	LV dysfunction (*n*)	LVNC (*n*)	Screened CPVT families (*n*)	Reference
1	1.1	11	10	7	3	11	4	0	{155}	{4,6}
2	1.1	2	2	1	1	2	1	0		
3	3.6	1	–	–	–	–	–	–		{6}
4	1.1	4	2	2	1	4	0	0	{33}	{5}
5	1.1	2	1	1	0	2	0	1		
6	1.1	1	0	1	0	1	0	1	{1}	{11}
7	37.7	6	1	3	1	2	0	5	{24}	{This study}
8	1.1	2	0	1	1	1	0	2		
Total (%)	–	29	16 (55.2)	16 (55.2)	7 (24.1)	23 (79.3)	5 (17.2)	9 (31.0)	213	

AV, atrioventricular; LV, left ventricular; CPVT, catecholaminergic polymorphic ventricular tachycardia; ICD, implantable cardioverter-defibrillator; LVNC, left ventricular non-compaction.

(*Figure 3E*) and Holter recordings. Therefore, her mother was diagnosed with CPVT. In addition to the diagnosis of CPVT, her echocardiography revealed LVNC as well. We could not obtain further clinical information of maternal side because they did not give the consent to our study.

Of the eight carriers of *RYR2* exon 3 deletion, seven had LVNC (87.5%), whereas four were diagnosed with CPVT (50%). These results are summarized in *Table 2*. No carriers showed left ventricular dysfunction (*Table 3*).

In remaining 22 CPVT probands without *RYR2* exon 3 deletion, we did not identify any other cases of LVNC.

Analysis of detailed genomic rearrangement

Bhuiyan *et al.* were the first to report a large genomic rearrangement in *RYR2*. Here, we performed long-range PCR using primer pairs (long PCR1) located in introns 2 and 3, as previously designed.[4] We confirmed the presence of PCR products in the two probands (*Figure 4A*). In one proband (F2-II-1), we detected two clear bands similar to the previous report.[4] The sizes of the PCR products were 3.9 and 2.8 kb. In contrast, only one band was detected in the other proband (F1-III-4), the intensity of which was faint compared with the control. To confirm the deletion range for proband F2-II-1, we performed direct sequencing and confirmed the same deletion (*Figure 4B* and *C*) reported in 2007.[4] The deletion was c.169-198_c.273+823 del1126, and the deletion caused an in-frame deletion of 35 amino acids p.Asn57_Gly91. To identify the specific range of the deletion in F1-III-4, we designed primer pairs (*Table 1*) and performed multiple qPCR spanning from intron 1 to exon 4. *Figure 4D* summarizes the results of qPCR; the deletion started 35 kb after exon 2 and ended before exon 4. To confirm the exact deletion range, we designed new primer pairs (*Table 1*, long PCR2), the location of forward and reverse primers were shown in arrows in *Figure 4D*, and performed a long-range PCR reaction. As a result, we could confirm a PCR product in the proband, which was longer than 10 kb (*Figure 4E*, arrow), the product was not visible in the control sample. In the sequence analysis, we confirmed that the deletion was c.169-22924_c.273+14653 del37682 insCAT, corresponding to a 35 amino acid deletion (p.Asn57_Gly91-del35), which was consistent with another proband (*Figure 4F* and *G*).

Discussion

Clinical phenotype of *RYR2* exon 3 deletion carriers

Since the first report from Bhuiyan *et al.*[4] in 2007, *RYR2* exon 3 deletion has been identified in six families with an atypical CPVT phenotype.[4–6] *Table 3* summarizes the clinical manifestations of 29 deletion carriers from eight different families, including the two families examined in this study. Of the 29 carriers, 23 (79.3%) patients suffered from ventricular arrhythmias, and they were highly complicated with atrial arrhythmias ($n = 16$, 55.2%) or sinoatrial dysfunction ($n = 16$, 55.2%). The frequency of supraventricular arrhythmias was higher than that reported for CPVT probands harbouring *RYR2* mutations.[10] Although the first report emphasized left ventricular dysfunction,[4] cardiac function was reduced in only five patients

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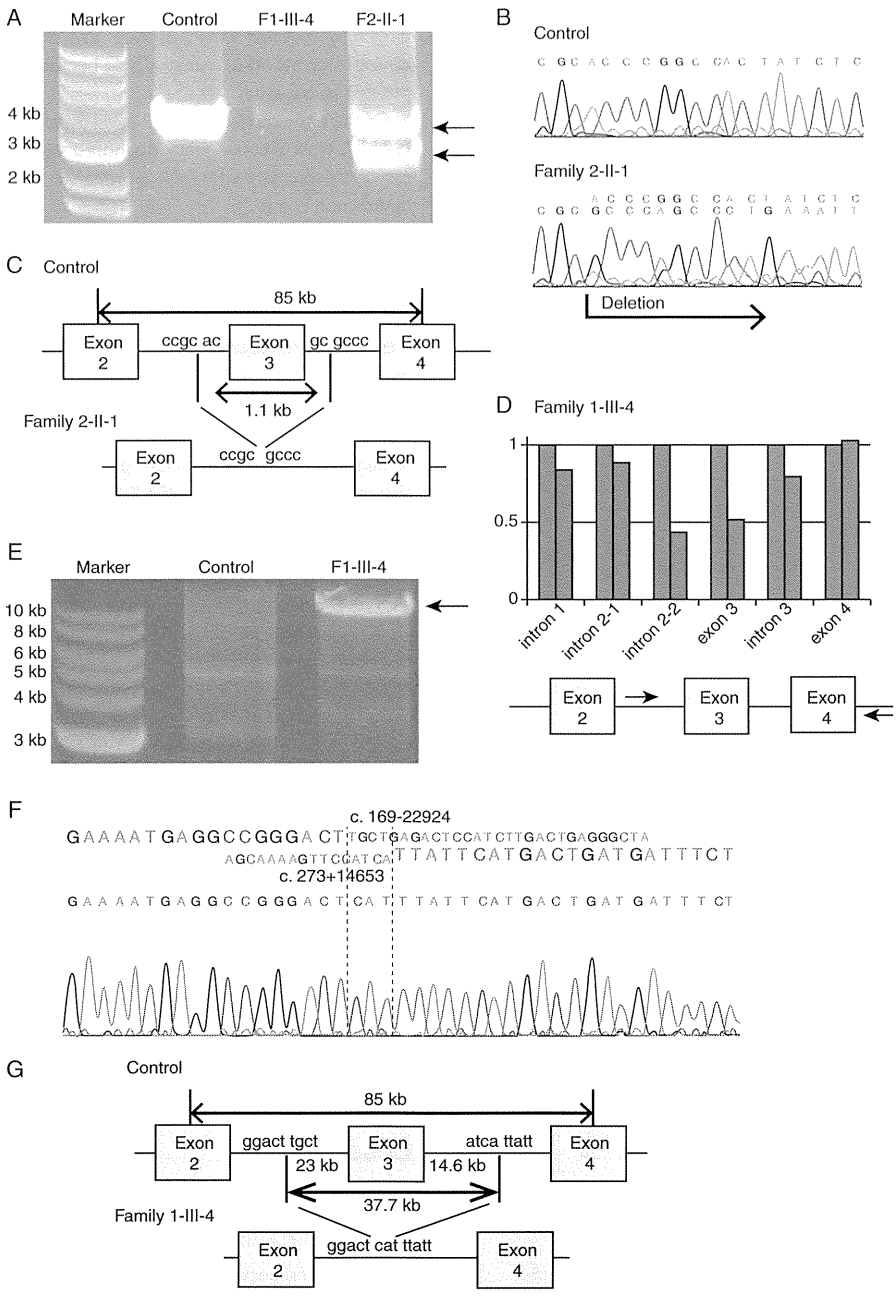

Figure 4 Detailed analysis of genomic rearrangement. (*A*) Long-range PCR amplification with the primers designed in introns 2 and 3. Polymerase chain reaction products of 3.9 and 2.8 kb (arrows) were obtained with DNA from F2-II-1. The PCR product with DNA from F1-III-4 was faint compared with that of the control. (*B*) DNA sequencing electropherogram of F2-II-1 showed the starting point of the large deletion. (*C*) The scheme simplified the genomic rearrangement in the proband F2-II-1. (*D*) The upper bar graphs display the relative ratio of the qPCR from DNA from family1-III-4. The ratios of intron 2-2 to exon 3 decreased. The lower scheme denotes the primer locations (arrows) in introns 2 and 4. (*E*) Long-range PCR amplification with the primers designed in introns 2 and 4. Polymerase chain reaction product longer than 10 kb (arrow) was obtained with DNA from F1-III-4 but was not obtained from control. (*F*) Upper sequence shows the 3′ end of the break point of the deletion and lower sequence denotes the 5′ end. Electropherogram displays three nucleotides (CAT) insertion between the break point. (*G*) Schema of the deletion in the proband of Family 1.

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(17.2%). In contrast, LVNC was diagnosed in seven of the eight carriers in our cohort, whereas only two patients were diagnosed with LVNC in the previous studies.[5,11] Potential reasons for the low detection rate of LVNC in previous studies may include the fact that the diagnostic criteria for LVNC are still debatable[8,12] and the fact that carriers in previous studies may not have been evaluated for LVNC. Therefore, a clear definition of LVNC and detailed echocardiography studies will be indispensable to demonstrate the relationship between LVNC and *RYR2* exon 3 deletion.

RYR2 exon 3 deletion as a cause of left ventricular non-compaction

As the cause of LVNC, many genes have been reported. Although an X-linked recessive inheritance has been reported in children,[13] LVNC has most commonly been reported to be transmitted as an autosomal dominant trait in adult familial cases.[7] Among the reported genetic cases, sarcomere gene mutations have been mostly identified in LVNC patients.[14] And among calcium handling genes, one calsequestrin2 (*CASQ2*) mutation, p.H244R was reported.[14] However, their penetrance was variable depending on the mutations and families, and a phenotype compatible with DCM along with LVNC was frequently observed. Relative to the variance in these previous reports, the penetrance of *RYR2* exon 3 deletion in our study was as high as the phenotype of LVNC (87.5%), suggesting that it may be a cause of LVNC.

In 2006, chromosome rearrangement, 46,XX,del(1)(q43q43), was identified in an infant with LVNC, facial dysmorphism, and psychomotor retardation.[15] Although other candidate genes for LVNC were examined, no mutations were identified. Because *RYR2* is located on chromosome 1q42–43, this case report supports our hypothesis that *RYR2* exon 3 deletion causes LVNC. More recently, a female patient with CPVT and LVNC was reported in the Netherlands.[11] The patient had no family history but carried the same *RYR2* exon 3 deletion that we identified in Family 2 (c.169-198_c.273+823 del1126).

Regarding *RYR2*, mutations other than deletion of exon 3 have not been reported to be associated with LVNC. In this connection, we identified 26 CPVT probands harbouring *RYR2* mutations, although none of them exhibited signs of LVNC in echocardiography.[16]

Functional change by the *RyR2* exon 3 deletion

In RyR2 mutations associated with CPVT, the threshold of store overload-induced Ca^{2+} release (SOICR) was reduced, which in turn increased the propensity for DADs and triggered arrhythmias.[17,18] The functional change induced by RyR2 exon 3 deletion was analysed using HL-1 or stable inducible HEK293 cell lines.[19] In addition to the change in SOICR, RyR2 exon 3 deletion reduced the threshold for termination of Ca^{2+} release and increased fractional Ca^{2+} release from SR, resulting in a further Ca^{2+} overload that was suspected to be associated with cardiomyopathy.[19] More studies are needed to elucidate the association between Ca^{2+} overload and the disturbance of the normal process of intrauterine endomyocardial morphogenesis.

Conclusions

Here, we reported for the first time that *RYR2* exon 3 deletion co-segregated with LVNC in two families, with both families exhibiting phenotypes that included sinus bradycardia, atrioventricular conduction disturbances, or atrial arrhythmias in addition to CPVT. Although we could not identify any *RYR2* exon 3 deletion in probands without CPVT phenotype, further studies are required to elucidate the association between LVNC and *RYR2* exon 3 deletion.

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Conflict of interest: none declared.

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References

1. Bers DM. Cardiac excitation–contraction coupling. *Nature* 2002;**415**:198–205.
2. George CH, Jundi H, Walters N, Thomas NL, West RR, Lai FA. Arrhythmogenic mutation-linked defects in ryanodine receptor autoregulation reveal a novel mechanism of Ca^{2+} release channel dysfunction. *Circ Res* 2006;**98**:88–97.
3. Leenhardt A, Lucet V, Denjoy I, Grau F, Ngoc DD, Coumel P. Catecholaminergic polymorphic ventricular tachycardia in children. A 7-year follow-up of 21 patients. *Circulation* 1995;**91**:1512–9.
4. Bhuiyan ZA, van den Berg MP, van Tintelen JP, Bink-Boelkens MT, Wiesfeld AC, Alders M *et al.* Expanding spectrum of human *RYR2*-related disease: new electrocardiographic, structural, and genetic features. *Circulation* 2007;**116**:1569–576.
5. Marjamaa A, Laitinen-Forsblom P, Lahtinen AM, Viitasalo M, Toivonen L, Kontula K *et al.* Search for cardiac calcium cycling gene mutations in familial ventricular arrhythmias resembling catecholaminergic polymorphic ventricular tachycardia. *BMC Med Genet* 2009;**10**:12.
6. Medeiros-Domingo A, Bhuiyan ZA, Tester DJ, Hofman N, Bikker H, van Tintelen JP *et al.* The RYR2-encoded ryanodine receptor/calcium release channel in patients diagnosed previously with either catecholaminergic polymorphic ventricular tachycardia or genotype negative, exercise-induced long QT syndrome: a comprehensive open reading frame mutational analysis. *J Am Coll Cardiol* 2009;**54**:2065–74.
7. Ichida F. Left ventricular noncompaction. *Circ J* 2009;**73**:19–26.
8. Oechslin E, Jenni R. Left ventricular non-compaction revisited: a distinct phenotype with genetic heterogeneity? *Eur Heart J* 2011;**32**:1446–56.
9. Jenni R, Oechslin E, Schneider J, Attenhofer Jost C, Kaufmann PA. Echocardiographic and pathoanatomical characteristics of isolated left ventricular non-compaction: a step towards classification as a distinct cardiomyopathy. *Heart* 2001;**86**:666–71.
10. van der Werf C, Nederend I, Hofman N, van Geloven N, Ebink C, Frohn-Mulder IM *et al.* Familial evaluation in catecholaminergic polymorphic ventricular tachycardia: disease penetrance and expression in cardiac ryanodine receptor mutation-carrying relatives. *Circ Arrhythm Electrophysiol* 2012;**5**:748–56.
11. Szentpali Z, Szili-Torok T, Caliskan K. Primary electrical disorder or primary cardiomyopathy? A case with a unique association of noncompaction cardiomyopathy and cathecolaminergic polymorphic ventricular tachycardia caused by ryanodine receptor mutation. *Circulation* 2013;**127**:1165–6.
12. Paterick TE, Tajik AJ. Left ventricular noncompaction: a diagnostically challenging cardiomyopathy. *Circ J* 2012;**76**:1556–62.
13. Ichida F, Tsubata S, Bowles KR, Haneda N, Uese K, Miyawaki T *et al.* Novel gene mutations in patients with left ventricular noncompaction or Barth syndrome. *Circulation* 2001;**103**:1256–63.
14. Hoedemaekers YM, Caliskan K, Michels M, Frohn-Mulder I, van der Smagt JJ, Phefferkorn JE *et al.* The importance of genetic counselling, DNA diagnostics, and cardiologic family screening in left ventricular noncompaction cardiomyopathy. *Circ Cardiovasc Genet* 2010;**3**:232–9.

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15. Kanemoto N, Horigome H, Nakayama J, Ichida F, Xing Y, Buonadonna AL *et al.* Interstitial 1q43-q43 deletion with left ventricular noncompaction myocardium. *Eur J Med Genet* 2006;**49**:247–53.
16. Kawamura M, Ohno S, Naiki N, Nagaoka I, Dochi K, Wang Q *et al.* Genetic background of catecholaminergic polymorphic ventricular tachycardia in Japan. *Circ J* 2013;**77**:1705–13.
17. Jiang D, Wang R, Xiao B, Kong H, Hunt DJ, Choi P *et al.* Enhanced store overload-induced Ca^{2+} release and channel sensitivity to luminal Ca^{2+} activation are common defects of RyR2 mutations linked to ventricular tachycardia and sudden death. *Circ Res* 2005;**97**:1173–81.
18. Jiang D, Xiao B, Yang D, Wang R, Choi P, Zhang L *et al.* RyR2 mutations linked overload-induced Ca^{2+} release (SOICR). *Proc Natl Acad Sci USA* 2004;**101**: 13062–67.
19. Tang Y, Tian X, Wang R, Fill M, Chen SR. Abnormal termination of Ca^{2+} release is a common defect of RyR2 mutations associated with cardiomyopathies. *Circ Res* 2012; **110**:968–77.

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ORIGINAL ARTICLE
Pediatric Cardiology and Adult Congenital Heart Disease

Fetal Bradyarrhythmia Associated With Congenital Heart Defects

– Nationwide Survey in Japan –

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Background: Because there is limited information on fetal bradyarrhythmia associated with congenital heart defects (CHD), we investigated its prognosis and risk factors.

Methods and Results: In our previous nationwide survey of fetal bradyarrhythmia from 2002 to 2008, 38 fetuses had associated CHD. Detailed clinical data were collected from secondary questionnaires on 29 fetuses from 18 institutions, and were analyzed. The 29 fetuses included 22 with isomerism, 4 with corrected transposition of the great arteries (TGA) and 3 with critical pulmonary stenosis; 14 had complete atrioventricular block (AVB), 8 had second-degree AVB, and 16 had sick sinus syndrome; 5 died before birth, and 10 died after birth (5 in the neonatal period). Neonatal and overall survival rates for fetal bradyarrhythmia with CHD were 66% and 48%, respectively. Pacemaker implantation was needed in 17 cases (89%). Beta-sympathomimetics were administered in utero in 13 cases and were effective in 6, but were not associated with prognosis. All cases of corrected TGA or ventricular rate ≥70 beats/min survived. A ventricular rate <55 beats/min had significant effects on fetal myocardial dysfunction (P=0.02) and fetal hydrops (P=0.04), resulting in high mortality.

Conclusions: The prognosis of fetal bradyarrhythmia with CHD is still poor. The type of CHD, fetal myocardial dysfunction, and fetal hydrops were associated with a poor prognosis, depending on the ventricular rate. (*Circ J* 2015; **79:** 854–861)

Key Words: Atrioventricular block; Bradyarrhythmia; Congenital heart defects; Fetus; Left atrial isomerism

Fetal bradyarrhythmia is an uncommon but life-threatening disease, especially in cases of complete atrioventricular block (AVB), which has a poor prognosis even in the postnatal period because of fetal hydrops and severe heart failure.[1–4] Fetal bradyarrhythmia may occur with and without associated congenital heart defects (CHD). In fetuses without CHD, the association of AVB with maternal anti-Ro/Sjögren's syndrome A antibodies is well established.[5,6] These antibodies damage the conduction system and the myocardium, and lead to AVB and myocarditis, respectively, resulting in a poor prognosis. We previously reported that fetal hydrops is associated with a poor prognosis in fetuses without CHD[7] and we suggested that myocardial dysfunction, rather than the severity of bradyarrhythmia, was associated with fetal hydrops. In contrast, in fetuses with CHD, congenital bradyarrhythmia is thought to result from the structural defect itself.[8,9] An embryological insult in the early gestational phase has been proposed to be the pathogenic mechanism of conduction system defects. Het-

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Table 1. Baseline Patient Characteristics

Characteristic	With CHD (n=29)	Without CHD (n=90)[7]	P value
At diagnosis			
GA (weeks)	25±5	26±5	0.15†
Atrial rate (beats/min)	113±22	135±20	<0.01
Ventricular rate (beats/min)	70±17	64±13	0.06†
Minimum ventricular rate (beats/min)‡	64±18	59±14	0.02†
Bradyarrhythmia			
Complete AVB	14 (48)	61 (68)	0.08
Second-degree AVB (2:1 AVB)	8 (28)	16 (18)	0.29
SSS	16 (55)	8 (9)	<0.01
Fetal hydrops	11 (38)	27 (30)	0.43
Fetal myocardial dysfunction	13 (45)	25 (28)	0.09
Fetal treatment	13 (45)	53 (59)	<0.01
FD	5 (17)	6 (7)	0.13
At birth			
GA (weeks)	35±5	35±4	0.87†
Body weight (g)	2,449±710	2,286±661	0.26†
Neonatal survival (>28 days)	19 (66)	73 (81)	0.08
Permanent pacemaker after neonatal period	17 (89)	29 (40)	<0.01

Data given as mean±SD or n (%). P<0.05, significant difference (†Student's t-test; other data analyzed using chi-squared test and Fisher's exact test). ‡Recorded in utero. AVB, atrioventricular block; CHD, congenital heart defects; FD, fetal death; GA, gestational age; SSS, sick sinus syndrome.

erotaxy and corrected transposition of the great arteries (TGA) often lead to discontinuity between the atrioventricular node and the ventricular conduction tissues, resulting in bradyarrhythmia,[10] and sick sinus syndrome (SSS) often occurs with heterotaxy and worsens the bradyarrhythmia.

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In the fetuses with bradyarrhythmia, an association with CHD was reported as a significant risk for the prognosis.[11–17] However, there is limited information on the risks related to outcomes of fetal bradyarrhythmia associated with CHD. Although Jaeggi et al reported that in all survived cases there was a ventricular rate >60 beats/min, the data were not based on multivariate analysis because of the limited number of cases, especially survivors.[18] Therefore, in this study we investigated the prognosis and risk factors for fetuses with bradyarrhythmia associated with CHD based on a large cohort in a Japanese database in recent years.

Methods

Study Design

Our primary nationwide questionnaire survey has been described in detail.[7] In brief, data were collected using questionnaires sent to Departments of Perinatology and Pediatric Cardiology at 750 institutions in Japan over 7 years (2002–2008). The response rate was 61% (455 institutions). Among 128 fetuses previously identified from 52 institutions, 38 (30%) had CHD, of which additional data were collected in 32 cases in a secondary survey.

All CHD cases were diagnosed prenatally using fetal echocardiography. Left or right atrial isomerism was confirmed by neonatal echocardiography, morphology of the auricle of the atrium during an operation such as pacemaker implantation (PMI), or postmortem examination. Fetal bradyarrhythmia was defined as a ventricular heart rate ≤100 beats/min. Fetal bradyarrhythmia was categorized into 3 subtypes: complete AVB, second-degree AVB (2:1 AVB), and SSS. Fetal SSS was defined as atrial heart rate ≤100 beats/min. These conditions were diagnosed prenatally using echocardiography or magnetocardiography, and confirmed in survivors by electrocardiography. Fetal myocardial dysfunction was subjectively evaluated by a pediatric cardiologist at each institution, based on contraction of the ventricles and the degree of cardiomegaly or atrioventricular valve regurgitation. In our secondary survey, the following additional perinatal data were collected: detailed anatomical features, fetal heart rate, fetal treatment, mode of pacemaker, operation, and outcomes during the follow-up period. Ventricular rate was defined as the minimum ventricular heart rate recorded in utero.

Statistical Analysis

Statistical analysis was performed using STATA 11.1 (StataCorp LP, College Station, TX, USA) and JMP 10 (SAS Institute, Cary, NC, USA). Data are presented as the mean±SD or number of patients and were evaluated with Student's t-test, or by Wilcoxon rank sum test when the data were not normally distributed. Categorical variables were evaluated using chi-squared test and Fisher's exact test. Time to death was analyzed using the Kaplan-Meier method with log-rank test. We also conducted univariate and multivariate Cox proportional hazard model analyses. The best prediction model was selected by backward elimination with P=0.10 as a criterion for exclusion. Stepwise analysis was used to adjust for baseline variables. P<0.05 was considered significant in all analyses.

Results

Baseline Characteristics

Of the 32 fetuses, 3 were excluded because of large atrial septal defect with maternal anti-Ro/Sjögren's syndrome A anti-

Table 2. Fetal Bradyarrhythmia With CHD (n=29)

Case no.	At diagnosis			Minimum V rate (beats/min)	Type of arrhythmia	Subtype of CHD	Hydrops
	GA (weeks)	A rate (beats/min)	V rate (beats/min)				
1	28	100	100	100	SSS	LAI	–
2	20	90	90	75	SSS	LAI	–
3	22	80	80	70	SSS	LAI	–
4	24	100	100	90	SSS	LAI	–
5	26	141	74	74	IIAVB	LAI	–
6	24	100	50	50	CAVB SSS	LAI	–
7	20	130	60	57	CAVB	LAI	–
8	20	90	90	90	SSS	LAI	–
9	29	98	89	89	IIAVB SSS	LAI	–
10	22	130	58	58	CAVB	LAI	–
11	21	120	60	59	IIAVB SSS	LAI	–
12	26	114	63	63	CAVB SSS	LAI	+
13	21	100	100	68	SSS	LAI	+
14	20	132	60	58	CAVB	LAI	–
15	25	120	70	60	IIAVB SSS	LAI	–
16	22	120	60	60	IIAVB	LAI	+
17	25	90	45	30	CAVB SSS	LAI	+
18	32	136	70	45	CAVB	LAI	+
19	20	53	53	36	SSS	LAI	+
20	17	90	45	40	CAVB SSS	LAI	+
21	37	136	67	67	IIAVB	RAI	–
22	22	110	55	45	CAVB SSS	RAI	+
23	28	140	80	75	CAVB	cTGA	–
24	36	100	100	99	IIAVB SSS	cTGA	–
25	29	120	60	55	IIAVB	cTGA	–
26	23	141	70	70	CAVB	cTGA	–
27	20	142	66	60	CAVB	cPS	+
28	28	135	56	56	CAVB	cPS	–
29	31	130	55	50	CAVB	cPS	+

A rate, atrial rate; AAI, atrium paced, atrium sensed, and pacemaker inhibited in response to sensed beat; IIAVB, second-degree AVB; AVVR, atrioventricular valve regurgitation; Beta, β-sympathomimetics; CAVB, complete AVB; CAVVR, common AVVR; cPS, critical pulmonary stenosis; cTGA, corrected transposition of the great arteries; DDD, dual-chamber inhibits and triggers; DDI, dual-chamber inhibits; LAI, left atrial isomerism; LD, late death (>28 days after birth); MR, mitral regurgitation; NND, neonatal death (≤28 days after birth); PMI, pacemaker implantation; RAI, right atrial isomerism; TR, tricuspid regurgitation; V rate, ventricular rate (minimum ventricular rate=minimum ventricular heart rate recorded in utero); VVI, ventricle paced, ventricle sensed, and pacemaker inhibited in response to sensed beat. Other abbreviation as in Table 1.

(Table 2 continued the next page.)

bodies, cardiac tumor and termination of pregnancy at 21 weeks of gestational age, respectively. Thus, a total of 29 fetuses were analyzed in the study. The baseline characteristics of the cases in this study compared with our previous study[7] are shown in Table 1. There were 5 deaths before birth and 10 after birth (5 in the neonatal period and 5 after the neonatal period). All except 1 died from heart failure within 6 months of birth despite multidisciplinary treatment. The overall survival rate was 48% in a mean follow-up period of 36±42 months. The ventricular rate at diagnosis in fetuses with CHD did not differ significantly from that in fetuses without CHD, but the atrial rate was lower in fetuses with CHD because of a higher incidence of SSS. Fetal myocardial dysfunction was frequently associated in fetuses with CHD than in those without CHD (45% vs. 28%, P<0.01). The incidence of fetal hydrops, fetal death and neonatal survival did not differ between fetuses with or without CHD. Of 19 neonatal survival cases, a permanent pacemaker was needed in 17 (89%), which was more frequent than in fetuses without CHD (40%).

Subtypes of CHD

The 29 fetuses with CHD were morphologically categorized into 3 subtypes: 22 with isomerism (20 left atrial isomerism, 2 right atrial isomerism), 4 with corrected TGA, and 3 with critical pulmonary stenosis (PS; Table 2; Figure 1). There were no cases of abnormal karyotype. Of the 22 fetuses with isomerism, 15 (68%) had SSS, 9 had complete AVB, and 6 had second-degree AVB. Of the 15 cases of SSS, 5 were associated with complete AVB, and 3 were associated with second-degree AVB. There were major extracardiac anomalies that required an operation after birth in 2 cases of left atrial isomerism (1 case of biliary atresia and 1 of jejunal atresia), which were not associated with a poor prognosis. In 2 cases of left atrial isomerism, there was a single umbilical artery.

All 5 fetal deaths were those with isomerism (4 left atrial isomerism, 1 right atrial isomerism) and associated fetal hydrops. In these cases, the ventricular rate ranged from 30 to 45 beats/min (median, 40) and all fetuses died in utero at 21–35 weeks of gestation (median, 32). Among the 8 neonatal and late deaths in cases of isomerism, 7 fetuses had fetal myocardial dysfunc-