Conclusions

The emerging field of neurocardiology is predicated on the dynamic interactions between the substrate of the heart and the neurohumoral control systems that regulate it. As detailed herein, there are inherent and acquired adaptions in both the heart and the nervous system that affect the progression of cardiac disease. With each year new insights are gained into these adaptations at the molecular, cellular, organ, and whole body level. Such information is critical to (1) identifying patients at high risk for future adverse outcome and (2) providing novel targets to pre-emptively manage such patients. Neuromodulation strategies show promise of sustaining cardiac function while maintaining electric stability.

Sources of Funding

Dr Shivkumar is supported by the National Heart, Lung, and Blood Institute (NHLBI; R01HL084261) and Dr Ardell was supported by NHLBI (R01 HL071830). Dr Ardell has grant funding from the St. Jude Medical, Glaxo Smith Klein, and Cyberonics Inc.

Disclosures

Dr Ardell serves as a consultant to Cyberonics Inc. The other authors report no conflicts.

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Circulation Research
JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Heart Association®

Cardiac Innervation and Sudden Cardiac Death

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Circ Res. 2015;116:2005-2019
doi: 10.1161/CIRCRESAHA.116.304679
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7330. Online ISSN: 1524-4571

Circulation Journal
Official Journal of the Japanese Circulation Society
http://www.j-circ.or.jp

ORIGINAL ARTICLE
Arrhythmia/Electrophysiology

Clinical and Pathological Impact of Tissue Fibrosis on Lethal Arrhythmic Events in Hypertrophic Cardiomyopathy Patients With Impaired Systolic Function

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Background: The natural history of hypertrophic cardiomyopathy (HCM) varies from an asymptomatic benign course to a poor prognosis. Myocardial fibrosis may play a critical role in ventricular tachyarrhythmias (VT/VF); however, the clinical significance of tissue fibrosis by right ventricular (RV) biopsy in the long-term prognosis of HCM patients remains unclear.

Methods and Results: We enrolled 185 HCM patients (mean age, 57±14 years). The amount of fibrosis (%area) was quantified using a digital microscope. Hemodynamic, echocardiographic, and electrophysiologic parameters were also evaluated. Patients with severe fibrosis had longer QRS duration and positive late potential (LP) on signal-averaged ECG, resulting in a higher incidence of VT/VF. At the 5±4 year follow-up, VT/VF occurred in 31 (17%) patients. Multivariate Cox regression analysis revealed that tissue fibrosis (hazard ratio (HR): 1.65; P=0.003 per 10% increase), lower left ventricular ejection fraction (HR: 0.64; P=0.001 per 10% increase), and positive SAECG (HR: 3.14; P=0.04) led to a greater risk of VT/VF. The combination of tissue fibrosis severity and lower left ventricular ejection fraction could be used to stratify the risk of lethal arrhythmic events in HCM patients.

Conclusions: Myocardial fibrosis in RV biopsy samples may contribute to abnormal conduction delay and spontaneous VT/VF, leading to a poor prognosis in HCM patients. (*Circ J* 2015; **79:** 1733–1741)

Key Words: Arrhythmias; Fibrosis; Histopathology; Hypertrophic cardiomyopathy; Prognosis

Hypertrophic cardiomyopathy (HCM) is usually recognized by left ventricular (LV) hypertrophy on echocardiography or a family history of HCM.[1] Histopathological changes, including myocardial hypertrophy, tissue fibrosis, or myocardial disarray,[2,3] may cause a distorted impulse propagation and inhomogeneous refractoriness, a substrate of electrical instability during tachycardia, which can lead to ventricular tachycardia (VT) or ventricular fibrillation (VF) and sudden cardiac death (SCD).

The natural history of HCM patients varies from an asymptomatic benign course to a poor prognosis because of heart failure (HF), lethal ventricular arrhythmias, or SCD.[4] Therefore, risk stratification in HCM patients has been a major issue. A positive late potential (LP) detected by signal-averaged electrocardiography (SAECG) has been used as a marker of electrical instability,[5] although myocardial scarring visualized by cardiac magnetic resonance (CMR) imaging can better predict long-term clinical outcome compared with other risk factors such as syncope and family history of SCD.[6–8] Myocardial fibrosis, as measured by late gadolinium enhancement (LGE) on CMR, was recently found to be an independent predictor of adverse outcome in HCM patients.[9,10] However, there are only a few case reports of the relationship between CMR-LGE and direct fibrotic changes.[11,12] It remains unclear

Received January 27, 2015; revised manuscript received March 29, 2015; accepted April 8, 2015; released online May 27, 2015 Time for primary review: 12 days

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ISSN-1346-9843 doi:10.1253/circj.CJ-15-0104

whether or not histopathological changes are associated with the risk of VT/VF and SCD in HCM patients.

In this study, we hypothesized that advanced myocardial fibrosis in HCM plays a critical role in lethal arrhythmic events, including VT/VF, implantable cardioverter-defibrillator (ICD) appropriate discharge, and SCD. We therefore quantified the fibrotic change in tissue samples from right ventricular (RV) biopsy and assessed its relevance to the long-term prognosis of HCM patients. This study examined the novel quantitative significance of tissue fibrosis in HCM patients associated with electrophysiological conduction abnormalities that lead to VT/VF and poor prognosis.

Methods

Diagnosis of Patients

We retrospectively surveyed 494 consecutive patients who had undergone RV endomyocardial biopsy at the National Cerebral and Cardiovascular Center between 1996 and 2011. The diagnosis of HCM was made on the basis of typical clinical, echocardiographic, and hemodynamic features according to established criteria,[1] used for a number of years, in the presence of LV wall thickness ≥15 mm without dilated ventricular chambers or any other cardiac or systemic disorders, including aortic stenosis or marked hypertension at the time of clinical diagnosis. In this study, the borderline LV hypertrophy criterion (LV wall thickness 13–14 mm) was not applicable because genetic examinations were not performed in this cohort. Asymmetric hypertrophy was originally applied to patients with conventional septal hypertrophy; however, the pattern or distribution of LV hypertrophy was not taken into account as per the latest recommendation.[1] Thus, asymmetric hypertrophy is determined if the LV thickness ratio of maximum to minimum in the same cross-section exceeds 1.3.

RV Biopsy and Histopathological Analysis

RV endomyocardial biopsy was performed in this cohort because of (1) differential diagnoses for other cardiomyopathies, such as amyloidosis, Fabry's disease, sarcoidosis, or hypertensive heart disease; (2) atypical progression of LV dysfunction; or (3) new-onset HF despite preserved left ventricular ejection fraction (LVEF). We excluded patients younger than 20 years old because myocardial features may change with age. We also excluded male and female patients older than 75 and 80 years old, respectively. Patients with coexisting valvular diseases responsible for cardiomyopathy were also excluded.[2]

A total of 238 patients were clinically diagnosed and pathologically confirmed to have HCM (including 114 HCM with overt LV dysfunction[10] defined as LVEF <50%); 53 patients were excluded because their tissue samples (Masson's staining) had deteriorated over time. Finally, 185 patients (mean age 57±14 years, 62% male) were evaluated. This study was approved by the institutional ethics committee (M24-071).

Biopsy samples were obtained from the endocardium at the right interventricular septum using disposable biopsy forceps (Toyokura Ika Kogyo Co, Ltd, Tokyo, Japan) by the transvenous approach via the femoral vein or the right jugular vein, as described elsewhere.[13] The detailed tissue sample preparation methods are described in **Supplementary File 1**. The extent of tissue fibrosis was automatically calculated by the area of fibrosis (%) in the total area of the Masson's trichrome sample using a digital microscope (Aperio Scanscope, Aperio Technology, Vista, CA, USA) (**Figure S1**), which has been utilized for calculating myocardial fibrosis elsewhere.[12] The degree of myocardial disarray was graded from 0 to 5, as described in **Table S1**). Separate from the quantitative risk assessment, tissue fibrosis was qualitatively classified into 3 degrees: mild (<10% area of fibrosis in specimens), moderate (10–20%), and severe (>20%), as previously reported[14] for further risk stratification, with and without other prognostic factors.

Electrophysiological Analysis

A standard 12-lead ECG was recorded in all patients. The SAECG was recorded from the X, Y, and Z orthogonal leads. LP was defined as present when at least 2 of the following 3 criteria were positive: filtered QRS duration (fQRS) >120 ms; root-mean-square voltage in the terminal 40 ms (RMS40) <18 μV; and duration of the low amplitude signal <40 μV (LAS40) >38 ms. The detailed electrophysiological protocol is shown in **Supplementary File 1**.

Echocardiography

After patients with significant valvular disease were excluded, the echocardiographic measurements were performed as follows: the end-diastolic and end-systolic dimensions were measured on the parasternal view at the level of papillary muscles and the left atrial size was measured on the parasternal long-axis view. Measurement of maximum wall thickness and definition of asymmetric hypertrophy were described above.

Hemodynamic Study

All patients underwent catheterization for hemodynamic evaluation. The LVEF was measured using left ventriculography, CMR imaging, or radio nuclear imaging. All patients were examined by right heart catheterization to assess hemodynamics. Coronary angiography was performed in all patients during their first hospitalization for diagnosis or within the year prior.

CMR-LGE Analysis

Of the 185 total patients, 60 underwent CMR using the gadolinium-enhanced imaging technique. The detailed CMR protocol and its LGE analysis were described previously[15] and are described in **Supplementary File 1**. In brief, CMR was performed on a 1.5-T MR scanner (Magnetom Sonata, Siemens, Erlangen, Germany) and LGE used a segmented inversion-recovery (IR) prepared true-FISP sequence with ECG triggering at 2, 5, 10, and 20 min after the administration of 0.15 mmol/kg of gadolinium-DTPA (Magnevist, Bayer Schering Pharma, Berlin, Germany). For quantification of LV mass, we semi-automatically traced the LV endocardial and epicardial contours at end-diastole in each short-axis slice of 7 sections using customized software (Ziostation2; Ziosoft Inc, Tokyo, Japan). A region of interest (ROI) was selected within the normal remote myocardium to generate the mean and standard deviation (SD) for the various SDs. The mass of LGE (%LGE) was automatically calculated with the same software as regions exhibiting a signal intensity above a predetermined threshold (4 SD above the mean signal intensity of apparently normal myocardium).[12]

Follow-up

Patient follow-up began on the day of biopsy. Patients were tracked through outpatient visits every 1–3 months or were followed at ICD check-ups every 6 months. The endpoint of the study was lethal arrhythmic events defined as sustained VT or VF, ICD appropriate discharge, or aborted SCD during the follow-up period. SCD was diagnosed if the patient underwent a sudden collapse within 1 h of onset of symptoms without any previous cardiac manifestation.

Table 1. Baseline Characteristics of Patients With HCM

	Total (n=185)	Tissue fibrosis			P value
		Mild (n=58)	Moderate (n=78)	Severe (n=49)	
Age	57±14	59±12	56±13	57±16	NS
Sex (% male)	114 (62)	34 (59)	53 (68)	27 (55)	NS
Family history of SCD, n (%)	16 (9)	4 (7)	6 (8)	6 (12)	NS
Hypertension, n (%)	72 (40)	24 (42)	31 (40)	17 (36)	NS
Diabetes mellitus, n (%)	31 (17)	11 (20)	12 (15)	8 (17)	NS
Atrial fibrillation, n (%)	70 (38)	16 (28)	21 (27)	18 (37)	NS
Syncope, n (%)	41 (22)	9 (16)	18 (23)	14 (29)	NS
Prior NSVT, n (%)	55 (30)	13 (22)	25 (32)	17 (36)	NS
Prior sustained VT/VF, n (%)	26 (14)	5 (9)	9 (12)	12 (24)	NS
Prior hospitalization, n (%)	76 (41)	15 (26)	35 (45)	26 (53)*	0.01 vs. mild*
Echo and hemodynamic parameters					
LVEF, %	47±19	45±19	48±19	47±20	NS
Max. wall thickness, mm	17±6	16±6	17±6	18±6	NS
Wall thickness >30 mm, n (%)	5 (3)	2 (3)	2 (3)	1 (2)	NS
Asymmetric hypertrophy, n (%)	82 (44)	28 (48)	31 (40)	23 (47)	NS
Max. PG >30 mmHg, n (%)	41 (22)	9 (16)	23 (29)	9 (18)	NS
BNP, pg/ml (IQR)	256 (137–506)	221 (116–470)	278 (126–502)	272 (175–613)	NS
PCWP, mmHg	12±7	10±6	12±6	13±7*	0.03 vs. mild*
Pathological parameters					
Myocyte diameter, μm	21±5	20±4	21±5	22±4	NS
Myocardial disarray, grade: 0–5	2.6±1.3	2.4±1.4	2.6±1.1	2.7±1.3	NS
ECG and electrophysiology					
QRS duration, ms	119±30	114±24	121±27	122±39	NS
QTc interval, ms	454±67	452±72	446±52	468±81	NS
LAS40, ms	32±22	28±16	30±17	41±29*	0.03 vs. mild*
fQRS, ms	120±30	114±28	122±30	123±34	NS
RMS voltage, μV	63±64	67±58	64±63	57±71	NS
LP(+) by SAECG, n/total N (%)	30/123 (24)	8/38 (21)	9/53 (17)	13/32 (41)	NS
CMR parameters (n=60)					
LV mass, g	165±54	158±58	179±54	148±45	NS
LGE % LV mass (4SD), %	31±18	32±19	28±18	35±15	NS
Medication, treatment					
ICD/CRT-D at diagnosis, n (%)	5 (3)	0 (0)	3 (4)	2 (4)	NS
β-blocker, n (%)	68 (37)	16 (28)	34 (44)	8 (16)	NS
ACEI/ARB, n (%)	79 (43)	26 (45)	31 (40)	22 (45)	NS
Amiodarone, n (%)	14 (8)	1 (2)	5 (6)	8 (16)*	0.01 vs. mild*
Other antiarrhythmics, n (%)	43 (23)	10 (17)	20 (26)	12 (24)	NS

*Statistically difference between mild and severe. ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BNP, B-type natriuretic peptide; CMR, cardiac magnetic resonance imaging; CRT-D, cardiac resynchronization therapy with defibrillator; fQRS, total filtered QRS duration; HCM, hypertrophic cardiomyopathy; ICD, implantable cardioverter-defibrillator; LAS40, duration of the low amplitude signal <40μV; LP(+), positive late potential; LVEF, left ventricular ejection fraction; NS, not significant; NSVT, nonsustained ventricular tachycardia; PCWP, pulmonary capillary wedge pressure; PG, pressure gradient in left ventricle; RMS, root-mean-square; SAECG, signal-averaged electrocardiogram; SCD, sudden cardiac death; VT/VF, ventricular tachycardia/ventricular fibrillation.

Statistical Analysis

Continuous variables are expressed as the mean±SD, median (interquartile range of 25–75%), or n (%). Comparison among the 3 groups was made using Tukey's method for continuous variables to adjust multiplicity, applying $P<0.05$ as the significance level. Bonferroni's method was used for categorical variables, applying $P<0.016$ among the 3 groups as the significance level. Survival curves were calculated by the Kaplan-Meier method using the log-rank test for group comparison among the extent of graded tissue fibrosis (<10%, 10–20%, and >20%). All variables with a P-value <0.05 in the univariate analysis were considered candidates for inclusion in the multivariate analysis. Cox proportional hazard regression adjustment was performed to calculate the hazard ratio (HR) in the multivariate analysis. All analyses were performed with JMP version 9 software (SAS Institute Inc, Cary, NC, USA).

Results

Baseline Characteristics

The baseline characteristics of the 185 patients are shown in **Table 1**; 76 (41%) patients had a history of hospitalization for HF or arrhythmia, and 26 (14%) had a history of VT/VF, in which nonsustained VT was not included. ICD or cardiac resynchronization therapy with defibrillator (CRT-D) was undertaken in 5 patients at baseline. The baseline LVEF, pul-

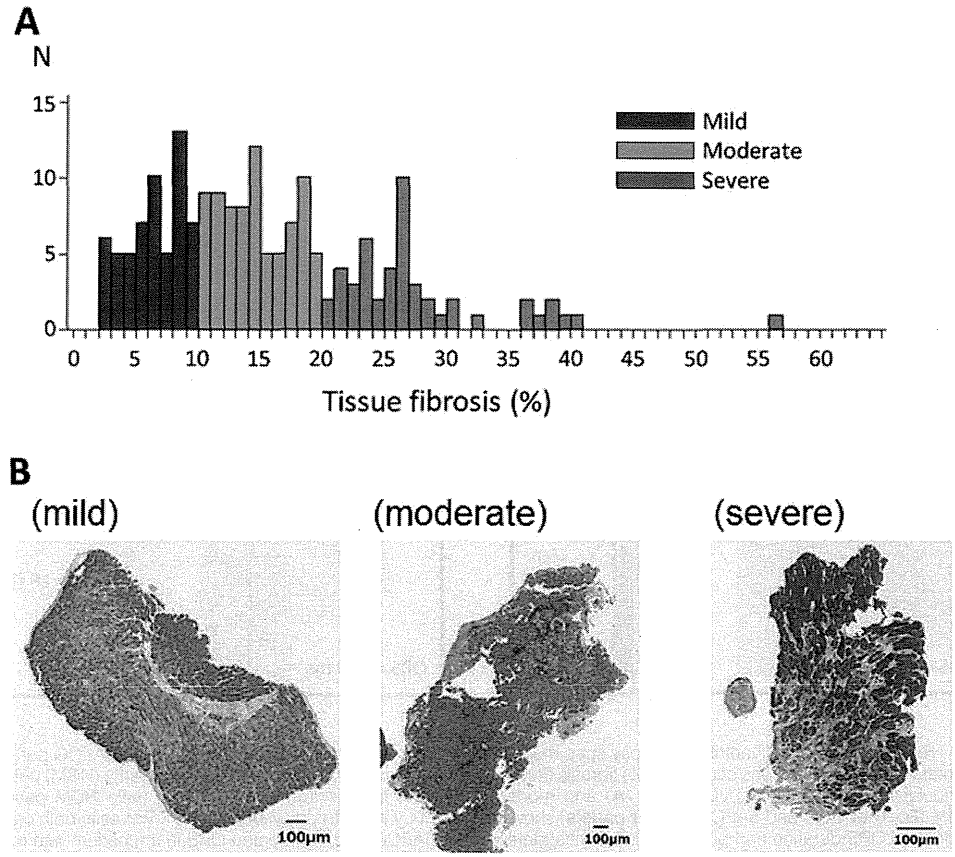

Figure 1. Quantitative fibrotic change in right ventricular (RV) biopsy specimens from hypertrophic cardiomyopathy patients with mild, moderate or severe fibrosis. (**A**) Distribution of fibrosis (%) in all samples. (**B**) Representative RV biopsy specimens stained by Masson's trichrome showing mild (3.9% of area), moderate (18.2%) or severe (31.7%) fibrosis, which represents the fibrosis of the entire area, including endomyocardial thickening and perivascular fibrosis.

monary capillary wedge pressure (PCWP), B-type natriuretic peptide (BNP) concentration, and maximum LV wall thickness were 47±19%, 12±7 mmHg, 256 (IQR: 137–506) pg/ml, and 17±6 mm, respectively, at the time of biopsy.

Clinical and Histopathological Changes

The average tissue area was 2.34±1.38 mm^2. In the tissue sample measurements, the fibrosis ratio (% area) was 15.7±9.8% and the distribution of fibrotic change in all samples is shown in **Figure 1A**. The level of fibrosis was classified as mild (<10%; n=58), moderate (10–20%; n=78), or severe (>20%; n=49). A representative tissue sample of each group is shown in **Figure 1B**.

As shown in **Table 1**, no significant correlation was found among the groups for age, sex, history of hypertension, diabetes mellitus, atrial fibrillation, or other conventional risk factors, including family history of SCD, syncope, maximum wall thickness, and pressure gradient. A history of hospitalization for HF or arrhythmia was more common in patients with severe (n=26, 53%) tissue fibrosis compared with mild (n=15, 26%) tissue fibrosis (P=0.01).

LVEF and plasma BNP were not associated with the degree of fibrosis at the time of diagnosis. On the other hand, PCWP was higher in patients with severe fibrosis compared with mild fibrosis (P=0.03). The mean myocyte diameter (21±5 μm) and degree of myocardial disarray (2.6±1.3) in the total cohort did not differ among the groups.

Conduction Abnormality and Lethal Ventricular Arrhythmias

Figure 2 shows representative tissue samples of mild (6.8%) and moderate (16.6%) fibrotic change in HCM patients. Although LVEF and QRS duration on ECG were comparable in these 2 patients, a longer filtered QRS duration, LAS40, and thus positive LP were detected in the patient with moderate fibrosis. Although not all patients underwent SAECG (n=123), increased fibrosis (%area) was mildly associated with longer LAS40 (r^2=0.07, P<0.01) (**Figure S2**). LAS40 was larger in patients with severe fibrosis compared with mild fibrosis (P<0.05) (**Table 1**).

Next, we compared the degree of tissue fibrosis and the development of lethal ventricular arrhythmias. As shown in **Figure 3A**, the degree of fibrosis at the time of HCM diagno-

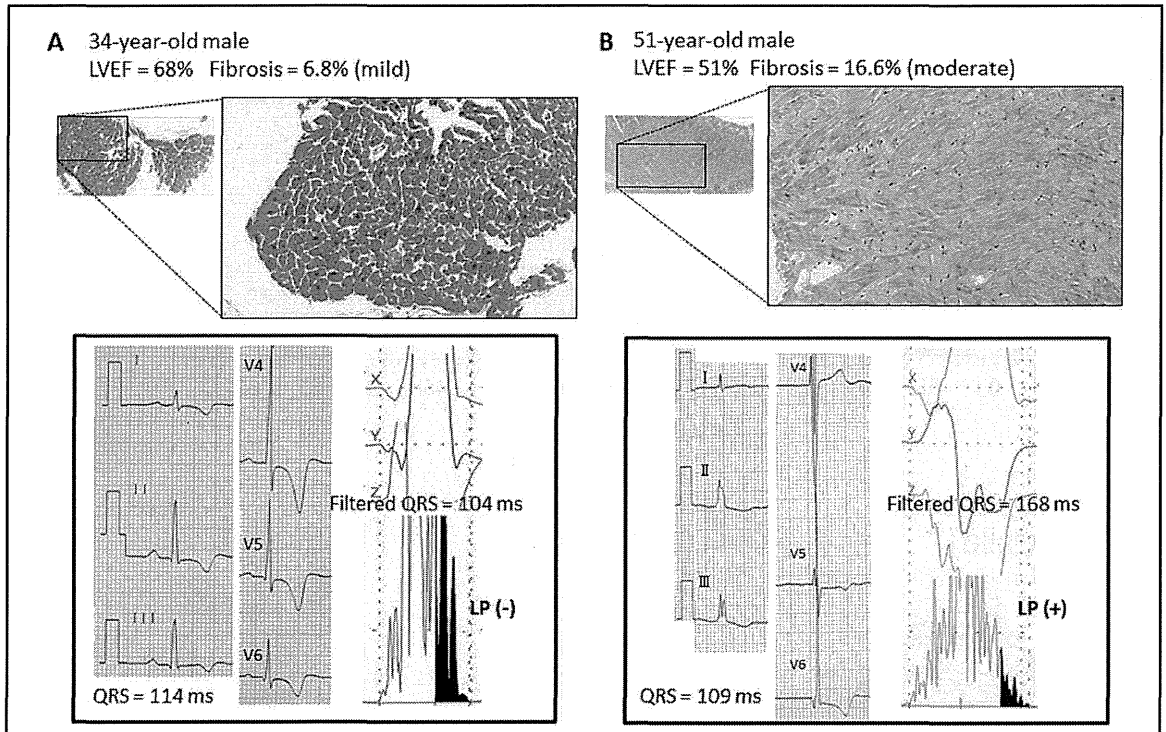

Figure 2. Representative right ventricular biopsy specimens of mild or moderate fibrosis and the ECG and SAECG parameters. Representative biopsy specimens of mild (6.8%) fibrosis from a 34-year-old male hypertrophic cardiomyopathy (HCM) patient with left ventricular ejection fraction (LVEF)=68% (**A**) and moderate (16.6%) fibrosis from a 51-year-old male HCM patient with LVEF=51% (**B**). Their ECG and SAECG (**Lower panels**) show significant LV hypertrophy with inverted T-waves in both cases, but a longer filtered QRS duration and positive late potential detected by SAECG and fragmented QRS in the patient with moderate fibrosis compared with the patient with mild fibrosis. SAECG, signal-averaged ECG.

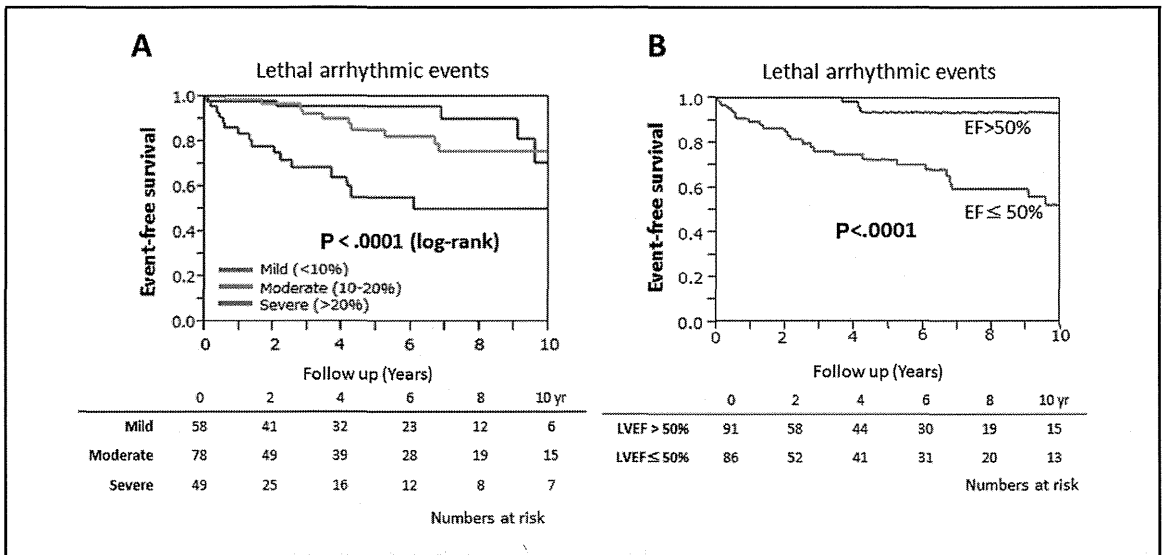

Figure 3. Lethal arrhythmic events and degree of tissue fibrosis or left ventricular ejection fraction (LVEF). Kaplan-Meier unadjusted estimates of freedom from lethal arrhythmic events or sudden cardiac death according to the degree of fibrotic change (**A**) or LVEF (**B**) in 185 patients with hypertrophic cardiomyopathy.

Table 2. Tissue Fibrosis and Lethal Ventricular Arrhythmias in Patients With HCM

	Tissue fibrosis			P value
	Mild (n=58)	Moderate (n=78)	Severe (n=49)	
ICD or CRT-D, n (%)	8 (15)	19 (24)	19 (39)*	0.009 vs. mild*
Prior sustained VT/VF, n (%)	5 (9)	9 (12)	12 (24)	NS
Sustained VT, n	4	6	8	
Spontaneous VF, n	1	3	4	
Subsequent VT/VF or SCD, n (%)	5 (9)	10 (13)	16 (33)*,†	0.006 vs. mild* 0.02 vs. moderate†
Sustained VT without ICD, n	0	3	5	
Spontaneous VF without ICD, n	1	5	1	
SCD without ICD, n	2	1	0	
Appropriate ICD discharge, n	2	1	10	
Total VT/VF or SCD, n (%)	8 (14)	18 (23)	20 (41)*	0.003 vs. mild*

*Statistically significant difference between mild and severe. †Statistically significant difference between moderate and severe. Abbreviations as in Table 1.

Table 3. Univariate and Multivariate Analysis for Lethal Arrhythmic Events in Patients With HCM (n=31/185)

	Univariate analysis			Multivariate analysis		
	HR	95% CI	P value	HR	95% CI	P value
Age (/year)	0.97	0.95–1.00	0.05			
Male sex	1.56	0.74–3.58	0.24			
Tissue fibrosis (/10%)	2.57	1.56–4.39	0.0002	1.65	1.19–2.28	0.003
Cell diameter (/μm)	1.07	1.00–1.14	0.04	0.99	0.90–1.07	0.82
Disarray (/1 grade)	1.05	0.79–1.38	0.75			
Max. wall thickness (/mm)	0.98	0.92–1.04	0.53			
Asymmetric hypertrophy	0.85	0.37–1.91	0.70			
LGE %LV mass (4 SD) (/%)	1.04	1.00–1.10	0.06			
LVEF (/10%)	0.67	0.54–0.82	<0.0001	0.64	0.48–0.84	0.001
PCWP (/mmHg)	1.04	0.99–1.09	0.12			
LAS40 (/5ms)	1.14	1.07–1.20	0.0002			
fQRS (/10ms)	1.15	1.04–1.25	0.008			
RMS voltage (/μV)	0.99	0.97–1.00	0.002			
Positive LP by SAECG	5.11	2.29–11.5	0.0001	3.14	1.06–8.61	0.04
QRS duration (/10ms)	1.16	1.06–1.25	0.001	0.94	0.82–1.06	0.32
QTc interval (/10ms)	0.76	0.95–1.06	0.76			

CI, confidence interval; HR, hazard ratio; LGE, late gadolinium enhancement by CMR. Other abbreviations as in Table 1.

sis was significantly associated with subsequent lethal ventricular arrhythmias. During the 5±4 year follow-up period, 31 patients had lethal arrhythmic events (15 cases of sustained VT or VF, 3 of SCD, and 13 of appropriate ICD discharge). These events occurred in 5 of 58 (9%) patients with mild fibrosis, in 10 of 78 (13%) patients with moderate fibrosis, and in 16 of 49 (33%) patients with severe fibrosis (HR: 5.43, 95% confidence interval (CI): 2.12–16.6; P=0.0003; severe vs. mild). The total number of patients with lethal arrhythmic events, including prior and subsequent VT/VF or SCD, was larger in the group of patients with severe fibrosis (n=20, 41%) compared with mild (n=8, 14%) or moderate (n=18, 23%) fibrosis (P=0.003, severe vs. mild) (**Table 2**). On the other hand, as shown in **Figure 3B**, patients with lower LVEF (≤50%) had a higher risk of lethal arrhythmic events than those with preserved LVEF (P<0.0001).

CMR-LGE Analysis

Of the 185 clinically diagnosed and pathologically confirmed HCM patients, CMR was performed in 60 to show fibrotic change by LGE analysis. The LV mass of LGE (LGE %LV mass index) was calculated as the region exhibiting a signal intensity >4 SD. The averaged LGE %LV mass was 31±18% (range 2–68%). There was no correlation between tissue fibrosis from biopsy and LGE %LV mass by CMR-LGE (**Figure S3A**). Only in the severe fibrosis group was a significant correlation (P<0.05) observed between tissue fibrosis and the LGE %LV mass from CMR-LGE (**Figure S3B**).

Univariate and Multivariate Analyses

As shown in **Table 3**, univariate analysis revealed that a gradual increase of tissue fibrosis as well as cell diameter, LVEF, LAS40, fQRS, RMS voltage (ie, positive LP) by SAECG, and QRS duration on the 12-lead ECG were associated with subsequent lethal arrhythmic events, including VT/VF, ICD appropriate discharge, and SCD in HCM patients. Furthermore, multivariate analysis revealed that patients with a higher level of tissue fibrosis (HR: 1.65, 95% CI: 1.19–2.28; P=0.003

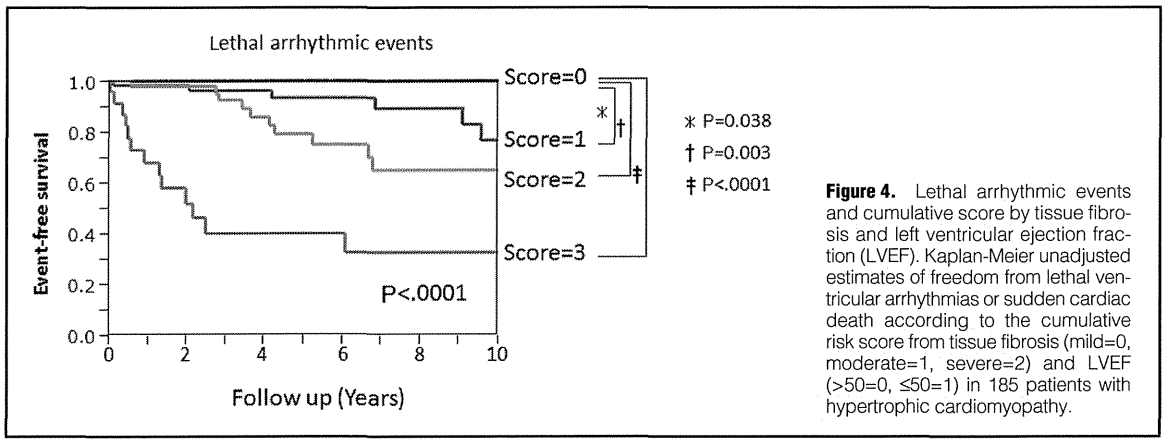

Figure 4. Lethal arrhythmic events and cumulative score by tissue fibrosis and left ventricular ejection fraction (LVEF). Kaplan-Meier unadjusted estimates of freedom from lethal ventricular arrhythmias or sudden cardiac death according to the cumulative risk score from tissue fibrosis (mild=0, moderate=1, severe=2) and LVEF (>50=0, ≤50=1) in 185 patients with hypertrophic cardiomyopathy.

per 10% increase), lower LVEF (HR: 0.64, 95% CI: 0.48–0.84; P=0.001 per 10% increase), and positive SAECG (HR: 3.14, 95% CI: 1.06–8.61; P=0.04) were prognostic in predicting future lethal arrhythmias.

Risk Stratification

Positive LP was only found in 24% of patients with a SAECG recording (**Table 1**), so LP had a higher specificity but a lower sensitivity for composite cardiac events in this study. To assess the predictive value of classification schemes that estimate lethal arrhythmic events in patients with HCM, we defined the combined risk score (0–3) formed by the sum of each independent risk factor: the degree of tissue fibrosis (mild=0, moderate=1, and severe=2) and LVEF (>50%=0, ≤50%=1). Patients with higher scores tended to have a greater risk of lethal arrhythmic events (**Figure 4**).

Discussion

New Findings

To the best of our knowledge, this is the first study to demonstrate the prognostic value of fibrotic change in tissue samples by biopsy quantitatively examined in a significant number of HCM patients. The severity of fibrosis in myocardial biopsy, a positive LP on SAECG, and lower LVEF were associated with a greater risk of lethal arrhythmic events in HCM patients. These findings provide novel insight into lethal ventricular arrhythmias and a new approach to estimating the prognosis of HCM patients.

Clinical Significance of Fibrosis in HCM

Numerous postmortem studies have demonstrated that myocardial fibrosis (interstitial or replacement) in HCM patients is distinct from that observed in patients with coronary artery disease or dilated cardiomyopathy.[16,17] A key mechanism involved in adverse outcomes in HCM is believed to be myocardial fibrosis, which is a pathological hallmark of the condition,[18] and can be identified by biopsy.[19,20] Recent studies of HCM patients suggest that the extent of fibrosis as measured by CMR correlates with histologically proven myocardial scarring[11] and is associated with worse prognosis,[10] including arrhythmic events.[6,9,21] However, in this study, fibrotic change (LGE %LV mass) by CMR-LGE did not reach statistical significance for the prediction of lethal arrhythmic events (HR=1.04, 95% CI: 1.00–1.10, P=0.06) (**Table 3**). To the best of our knowledge, only a few reports have compared CMR and histopathology with a focus on fibrosis;[11,12,22] segments containing >15% collagen were more likely to show LGE. However, the LGE technique cannot be used to visualize diffuse fibrosis[23] and it should be noted that the averaged fibrosis in this study was 15.7±9.8%, which may be difficult to detect by CMR-LGE. No significant relationship was observed between LGE %LV mass by CMR-LGE and tissue fibrosis in myocardial biopsy, especially in cases of mild or moderate fibrotic HCM (**Figure S3B**).

In this study, the severity of fibrosis, a positive LP, and lower LVEF were significantly associated with prognosis, especially for subsequent lethal arrhythmic events (**Table 3**, **Figure 3**). However, disarray was not correlated to the prognosis of patients aged between 20 and 75 (male) or 80 (female) years. These findings are consistent with a previous study that found that the prevalence of disarray was high in HCM patients who died suddenly before 21 years of age.[22] Thus, myocardial disarray may play an important role in the prognosis of younger HCM patients.

Promotion of Conduction Abnormality and VT/VF by Myocardial Fibrosis

Fibrous tissue promotes re-entrant ventricular arrhythmias and contributes to increased ventricular stiffness. In a coculture model, increased myofibroblast/myocyte area decreased conduction velocity and degenerated a spiral re-entry into multiple waves, like a VF.[24] Thus, increased myocardial fibrosis and disarray in HCM usually decreases excitation propagation, leading to a conduction delay or block, a substrate of re-entrant arrhythmias. SAECG can noninvasively evaluate a delayed potential as a substrate of ventricular arrhythmias in several diseases, although a previous study suggested that SAECG was not always useful for identifying HCM patients with VT or SCD.[5] Positive LP was found in only 24% of the present patients who underwent SAECG recording (**Table 1**), so LP had a higher specificity but a lower sensitivity for composite cardiac events in this study. The electrophysiological consequence of this substrate has been well demonstrated by Schumacher et al.[25] LV regional extensive hypertrophy and myocardial scarring are associated with local conduction delay and conduction block, which may contribute to the increased incidence of VT/VF in patients with HCM.

A prolonged QRS duration on 12-lead ECG is associated with an increased risk of cardiovascular death by HF and cardiomyopathy, including in HCM.[26] Kamiyama et al reported

that QRS duration on the 12-lead ECG was much longer in patients with dilated HCM compared with patients with dilated cardiomyopathy.[27] Kawara et al reported the correlation of conduction delay with a fibrotic tissue pattern in chronic diseased myocardium, including HCM, particularly in areas of patchy fibrosis.[28] In this study, QRS duration on standard 12-lead ECG was associated with lethal arrhythmic events only in the univariate analysis (**Table 3**); however, the severity of fibrosis in the tissue samples was weakly associated with a longer delayed potential (LAS40) (**Figure S2**). These findings suggest that the increased fibrosis in HCM associated with longer QRS duration and positive LP represented by prolonged delayed potential detected by SAECG indicates an abnormal conduction delay and may contribute at least in part to the increased incidence of lethal ventricular arrhythmias or SCD.

Prognositic Effect of Tissue Fibrosis and Its Potential for Risk Stratification

Sudden unexpected death is a well-recognized and devastating consequence of HCM. A previous cohort study[29] demonstrated that an appropriate ICD shock was delivered at a rate of 5.6%/year in HCM patients (n=506, mean age 42±17) during 3.7±3-year follow-up. It is of note that patients treated with ICD primarily for prevention also showed a substantial appropriate intervention rate (reported to be 4%/year). Thus, identifying patients with HCM who are at highest risk of SCD is a major problem. The conventional risk factors for the primary prevention of SCD in HCM are family history of SCD, unexplained syncope, multiple-repetitive nonsustained VT, abnormal exercise blood pressure response, or massive LV hypertrophy.[1] However, no significant difference was observed among patients with 1, 2, or ≥3 of these parameters with respect to the likelihood of appropriate ICD discharge.[29] Therefore, this risk stratification cannot always guide SCD prevention in precise terms for each HCM patient, and SCD is also known to occur in patients without any of the aforementioned risk factors.

Myocardial fibrosis measured by LGE-CMR was recently used as an independent predictor of adverse outcome in HCM patients.[9,14] However, LGE-CMR imaging mainly detects focal fibrosis and does not detect microscopic diffuse fibrosis. In contrast, CMR-T1 mapping may quantify diffuse as well as focal fibrosis.[30] Histopathological features related to unstable electrophysiological substrate may lead to lethal ventricular tachyarrhythmias and SCD.[31] In this study, we directly quantified the fibrotic changes in tissue samples and assessed its relevance to the long-term prognosis in HCM patients. These pathophysiologic changes may represent both micro-level and global fibrosis in HCM. Thus, increased fibrosis in the tissue samples of RV biopsy, as well as positive SAECG, QRS duration, and lower LVEF, can lead to VT/VF.

Study Limitations

Although this was a single-center, retrospective study, all patients that were enrolled underwent a biopsy of the RV septum after being admitted to the hospital. RV biopsy was not routinely performed in HCM patients, but might be recommended in HCM patients with increasing LV diameter and reducing LV contractions, which are likely related to increased fibrotic change.[31] This cohort was slightly biased and had a poorer prognosis than general, asymptomatic HCM patients. As such, it remains unclear whether these findings are applicable to asymptomatic HCM patients. Second, no genetic testing data were obtained in this study, and genetic disorders may affect the prognosis. Third, the endomyocardial biopsy was performed from the RV septum, but not the LV, and does not represent the entire heart; thus, only a limited number of samples could be evaluated. As such, there is a possibility that the results underestimated the overall fibrosis. Despite these limitations, this study demonstrated the clinical significance of tissue fibrosis and the physiological parameters for patients with HCM who are at risk of adverse cardiac events.

Conclusions

Fibrotic changes observed in tissue samples from RV biopsies play an important role in the development of lethal ventricular arrhythmias in HCM patients with impaired systolic function. When combined with the LV systolic function, the extent of tissue fibrosis may assist in the risk stratification of HCM patients.

Acknowledgments

This work was supported by grants from the Ministry of Health, Labour, and Welfare of Japan (2010-145); a Grant-in-Aid for Scientific Research on Innovative Areas (22136011 A02, Aiba); a Grant-in-Aid for Scientific Research (C) (24591086 Aiba) from MEXT of Japan; a Research Grant for Cardiovascular Diseases (H24–033 Shimizu, Aiba) from the Ministry of Health, Labour, and Welfare of Japan; and an Intramural Research Fund for Cardiovascular Diseases of the National Cerebral and Cardiovascular Center (26-6-6 Wada).

Disclosures

Conflict of Interests: None.

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Supplementary Files

Supplementary File 1

Methods

Table S1. Grade of myocardial disarray

Figure S1. (**A**,**B**) Representative biopsy samples from a single patient used to calculate the blue (fibrosis) area, in which the fibrosis (%area) was calculated by simply circling all tissue areas and then automatically calculating the ratio of blue in the total area.

Figure S2. Relationship between tissue fibrosis (%-area) from a right ventricular biopsy and duration of low amplitude signal <40 μV (LAS40, ms) by signal-averaged ECG (SAECG) in patients with hypertrophic cardiomyopathy.

Figure S3. (**A**) Relationship between CMR-LGE %LV mass and tissue fibrosis by myocardial biopsy. (**B**) Sub-analysis of the relationship by degree of tissue fibrosis; mild (<10%), moderate (10–20%) and severe (>20%).

Please find supplementary file(s);
http://dx.doi.org/10.1253/circj.CJ-15-0104

Circulation Journal
Official Journal of the Japanese Circulation Society
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EDITORIAL

Clinical Features of Long QT Syndrome in Children

Naokata Sumitomo, MD, PhD

Long QT syndrome (LQTS) is a genetic channelopathy with prolonged ventricular repolarization of the myocardial cells, associated with severe cardiac events such as syncope, aborted cardiac arrest, and sudden cardiac death.[1] Children with LQTS have more serious risk factors than adults. As noted in a consensus report, patients with syncope or cardiac arrest before the age of 7 years, or patients who have syncope or cardiac arrest in the first year of life, are thought to be at high risk.[2] This Editorial Comment focuses on the recent issues of LQTS in children.

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Prevalence of LQTS in Infants

The prevalence of congenital LQTS in infants is reported to be 1:2534 (0.039%) in Caucasians,[3] and 4:4285 (0.093%) in Japanese.[4] The reasonable cutoff value of the long QT interval in infants is reported to be 470 ms (31 of 43,080; 0.07%[3] or 5 of 4,285; 0.12%[4]), and 460 ms (59 of 43,080; 0.14%[3], or 8 of 4,285; 0.19%[4]), respectively, in those studies.

The genetic variant was identified in 12 of 28 neonates (43%),[3] and in 1 of 3 infants (33%)[4] with a QTc interval >470 ms. *KCNQ1* mutations were found in 8 patients,[3] and *KCNH2* mutations were found in 4[3] and 1[4] patients, respectively. On Okinawa island, 17 of 23 children were identified as gene-positive LQTS by school-based ECG screening; 14 of them had *SCN5A* E1784K mutations.[5] The prevalence of LQT3 is much higher than on any other island in Japan.

ECG Screening for Infants

The efficacy of ECG screening of infants and children is controversial. Saul et al reported that ECG screening is cost-effective in preventing sudden infant death syndrome, as well as sudden death in childhood, using a cutoff value of the QTc interval of 460 ms,[6] but Skinner and Van Hare reported that ECG screening is an unreliable diagnostic tool, and should only be performed to detect probands and to screen family members because most of the deaths from LQTS occur in patients who have had previous symptoms.[7]

ECG Characteristics of LQTS in Fetuses and Neonates

The characteristic features of the ECG in fetal and neonatal LQTS are sinus bradycardia or atrioventricular block (AVB).[8–10] The baseline fetal heart rate in sinus rhythm is significantly lower in fetal LQTS (range 90–144 beats/min, mean 118.9± 13.3 beats/min) than in normal fetuses (range 125–147 beats/min, mean 141.0±9.4 beats/min; P<0.0001).[8] LQTS is strongly suggested if the fetal heart rate is less than the 3rd percentile.[11] In addition to a low baseline heart rate, nonreactive heart rate patterns are also suggestive in LQTS fetuses and may be explained by lower-than-normal right sympathetic cardiac activity or a blunted response to a sympathetic drive, as seen postnatally.[8]

LQTS with 2:1 AVB is commonly observed during the fetal and neonatal periods,[9,10] but rarely observed in childhood and adulthood. In neonates with LQT2 and LQT3, 2:1 AVB was observed in 55% and 83% of the patients, respectively (**Figure 1**).[10] AVB may be caused by functional block of the ventricle, because of prolongation of the ventricular refractory period, and in the majority of patients the atrioventricular conduction returns with a significant decrease in the QTc interval during the follow-up period.[8] That may explain the rare occurrence of AVB in older children and adults with LQTS.

Prognosis of LQTS in Children

Previous reports showed that males with LQTS before the age of 15 years have a significant increased risk of syncope, aborted cardiac arrest, and sudden cardiac death.[12] However, the risk of these cardiac events is inverted after the age of 14 years.[12] Comparing the genotype of LQTS carriers, LQT1 females have a significantly lower risk of cardiac events than LQT1 males ≤15 years old (hazard ratio (HR) 0.58; P=0.005), but a significantly higher risk of cardiac events than males between the ages of 16 and 40 years (HR 3.35; P=0.007) (**Figure 2**).[13] LQT2 and LQT3 children show no significant differences between male and female carriers. Females with LQT2 have a significantly higher risk of a first cardiac event than males between the ages of 16 and 40 years (HR 3.71; P=0.010).[13] During 0–12 years old, males with LQT1 have the highest rate of a first syncope episode (P<0.001), but within the age range of 13–20 years, LQT2 females experience the highest rate of both first and subsequent syncope events (P<0.001 and P=0.01).[14]

In this issue of the Journal, Ozawa et al[15] report that the LQT2 phenotype presents with more frequent cardiac arrests or repetitive torsade de points (TdP) episodes than the LQT1 phenotype. They also demonstrate that LQT2 females have a repeat TdP episode within a short time period after a prior TdP episode, especially after puberty. As they note in their literature, the effect of estrogen, which prolongs the action potential duration (APD) through the inhibition of I_{Kr}, may contribute to the high occurrence of TdP in LQT2 females after adolescence.

A high occurrence of cardiac events in LQT1 males during childhood is also reported.[13–15] Male children may be more vigorous than females, which may result in them having more

Received January 19, 2016; accepted January 19, 2016; released online February 1, 2016
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ISSN-1346-9843 doi:10.1253/circj.CJ-16-0046

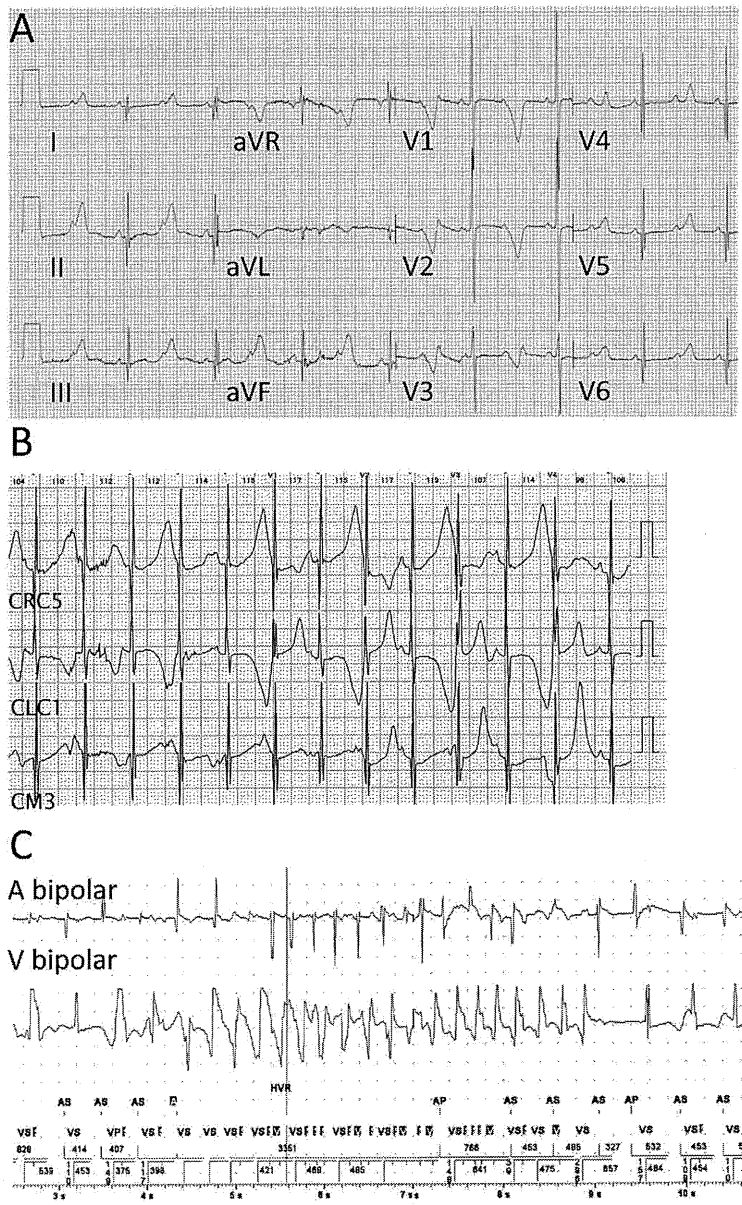

Figure 1. ECG of a 1-year-old male infant with long QT syndrome. (**A**) 2:1 atrioventricular block (AVB). The QT interval is prolonged to 630ms, and the QTc interval is also prolonged to 685ms. Prolongation of the ventricular refractory period resulted in 2:1 AVB with a ventricular rate of 71 beats/min. (**B**) Holter recording at 4 years old, shows 1:1 atrioventricular conduction with overt T wave alternance. (**C**) Recording from a pacemaker shows typical torsade de pointes in the same boy at 4 years old.

frequent cardiac events than females. Testosterone shortens the APD through enhancement of I_{Ks}, which may explain the decrease in the cardiac events after adolescence in LQT1 males.

Recent Issues of LQTS in Children

Attention deficit/hyperactivity disorder (ADHD) is a relatively common disease and is prevalent in approximately 11% of children. ADHD-directed stimulant therapy (methylphenidate, dextroamphetamine/amphetamine, lisdexamfetamine, and dexmethylphenidate) is relatively contraindicated in patients with LQTS because of the possibility of LQTS-triggered cardiac events. During a mean follow-up of 7.9±5.4 years after the initiation of ADHD medication, the cumulative probability of cardiac events in the ADHD treatment group was 62% vs. 28% in the matched LQTS control group (P<0.001).[16] Time-dependent use of ADHD medication is associated with an increased risk of cardiac events (HR 3.07; P=0.03).[16] Syncope is the most common symptom and the risk is higher in males.

Acknowledgments

Remuneration to the author for attending meetings, and presentations, being payment for the time and effort of attending the activity, by Ono Pharmaceutical Co Ltd and Medtronic Co Ltd.

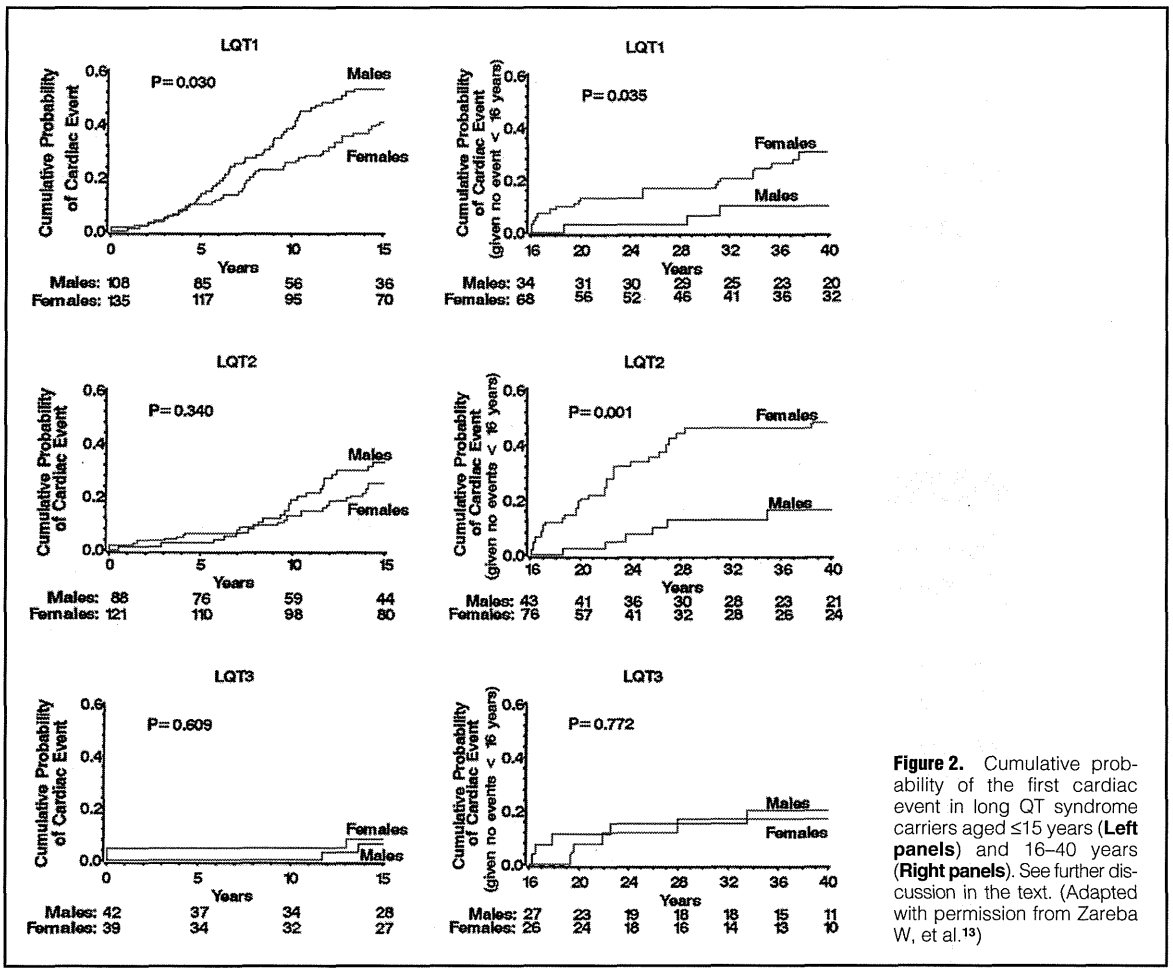

Figure 2. Cumulative probability of the first cardiac event in long QT syndrome carriers aged ≤15 years (**Left panels**) and 16–40 years (**Right panels**). See further discussion in the text. (Adapted with permission from Zareba W, et al.[13])

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JACC: CLINICAL ELECTROPHYSIOLOGY VOL. ■, NO. ■, 2016

ISSN 2405-500X/$36.00
PUBLISHED BY ELSEVIER INC. http://dx.doi.org/10.1016/j.jacep.2015.10.010

Comorbid Epilepsy and Developmental Disorders in Congenial Long QT Syndrome With Life-Threatening Perinatal Arrhythmias

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ABSTRACT

OBJECTIVES Given the association of LQTS and neurological disorders, we speculated that the more severe LQTS phenotype, perinatal LQTS, would exhibit more frequent comorbid neurodevelopmental anomalies than LQTS without perinatal arrhythmias (nonperinatal LQTS).

BACKGROUND Congenital long QT syndrome with life-threatening perinatal arrhythmias (perinatal LQTS) has a poor life prognosis.

METHODS Twenty-one consecutive LQTS patients diagnosed before 1 year of age at our institution and 3 previously reported perinatal LQTS patients with neurological seizures were enrolled. In total, the clinical course was evaluated in 24 patients.

RESULTS Among 21 infantile LQTS patients, 5 of 6 with perinatal LQTS (83%) were diagnosed with epilepsy and 4 (67%) with developmental disorders, but none with nonperinatal LQTS were. The total development quotient by Kinder Infant Development Scale scores was 17 to 72 (median 67) in 5 epileptic perinatal LQTS. In the 8 perinatal LQTS patients with neurological disorders, including 3 previously reported cases, epileptic seizures occurred at 2 days to 2.5 years of age and 5 had developmental disorders. Mutations in these 8 patients were located in the transmembrane loop of *KCNH2*, and D3/S4-S5 linker, D4/S4, or the D4/S6 segment of *SCN5A*.

CONCLUSIONS A high comorbidity of neurodevelopmental anomalies was observed in perinatal LQTS. Mutations in patients with neurological comorbidities were in loci linked to LQTS with a severe cardiac phenotype. These observations indicate the possibility that neurological disorders in perinatal LQTS are manifested as neurological phenotypes associated with severe cardiac phenotypes, while we could not completely exclude another possibility that those were caused by a brain perfusion injury. (J Am Coll Cardiol EP 2016;■:■–■) © 2016 by the American College of Cardiology Foundation.

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Manuscript received September 10, 2015; revised manuscript received October 22, 2015, accepted October 29, 2015.

ABBREVIATIONS AND ACRONYMS

AVB = atrioventricular block

CT = computed tomography

DQ = development quotient

ECG = electrocardiogram

EEG = electroencephalogram

KIDS = Kinder Infant Development Scale

LQTS = long QT syndrome

MRI = magnetic resonance imaging

QTc = corrected QT

TdP = torsade de pointes

Congenital long QT syndrome (LQTS) patients who experience aborted cardiac arrest in the first year of life are at very high risk for near-fatal or fatal cardiac events during the next 10 years of life (1). Especially, LQTS cases with torsade de pointes (TdP) and 2:1 atrioventricular block (AVB) during the perinatal period have poorer prognoses than LQTS cases without these arrhythmias (2-5). Current therapies, such as β-blockers, mexiletine, and pacemaker device implantations, have reduced the mortality and resulted in relatively favorable prognoses for perinatal LQTS (1,3,6). However, aborted cardiac arrest and sudden death still occur in this group despite treatment (1,3,4).

As with LQTS, Mendelian epilepsies and cardiac arrhythmias may also arise from mutations in ion channels or related signaling molecules, some due to mutations in the same genes associated with LQTS (7). In the brain, as in the myocardium, inherited dysfunction of ion channels (channelopathies) can destabilize excitable tissue, leading to paroxysmal clinical events (7). The possible association of epilepsy arising from the same channelopathies as LQTS was recently examined (8-10). Abnormal cortical electroencephalographic (EEG) activity was identified more frequently in subjects with LQTS secondary to potassium channel mutations than in healthy controls (8). In addition, 15% of the patients with LQTS who presented with seizures or seizure-like episodes had EEG-identified epileptiform activity (9). Furthermore, mutations in *KCNH2* or *SCN5A* were identified in 6 of 68 patients with sudden unexpected death in epilepsy (10).

A comorbidity of epilepsy and/or developmental disorders has been observed in perinatal LQTS patients who survived life-threatening ventricular arrhythmias. However, to the best of our knowledge, only 3 case reports have been previously published (11-13). Therefore, we hypothesized that perinatal LQTS patients, the most severe phenotype of LQTS (1,3,4), would have higher incidences of neurological manifestations of channelopathies, such as epilepsy or developmental disorders. In this study, we evaluated the clinical and neurological findings in infantile LQTS patients with or without perinatal arrhythmias.

METHODS

PATIENTS. Twenty-four consecutive patients diagnosed with LQTS before 1 year of age at the National Cerebral and Cardiovascular Center from November 1998 to August 2015 were considered for this study. LQTS was diagnosed by genetic testing or a corrected QT (QTc) interval ≥470 ms with a family history of LQTS, calculated with Bazett's formula (14) on the resting electrocardiogram (ECG). Three patients who were less than 1 year old at the last follow-up were excluded and the remaining 21 were enrolled in this study. Four sibling pairs were included, and 1 patient was previously described (Patient #3) (15). Among the 21 patients, 6 had life-threatening arrhythmic events during the perinatal period, such as TdP or 2:1 AVB due to QTc prolongation. We classified these 6 patients as perinatal LQTS, and the other 15 as nonperinatal LQTS. Further, we added the data of the clinical features and genetic analyses from 3 previously reported cases with perinatal LQTS and epileptic seizures (11-13). A total of 24 patients were examined. We assert that all procedures contributing to this work complied with the relevant national guidelines on human experimentation (Japan) and with the Helsinki Declaration of 1975 (as revised in 2008), and were approved by the institutional ethics committees (M25-132).

CLINICAL CHARACTERISTICS. The following parameters were assessed: gender, age at the initial presentation, family history of LQTS, gene mutations, ECG findings at the initial presentation, syncope or life-threatening arrhythmias during follow-up, medical treatments for LQTS, comorbid epilepsy, developmental outcomes, and other neurological disorders. Syncope was distinguished from epileptic seizures by a rapid onset without warning, shorter duration, and no postictal phase.

The ECG findings at the initial presentation were compared between 9 perinatal and 15 nonperinatal LQTS patients, including the 3 previously reported cases. The incidence of life-threatening arrhythmias, epilepsy, and developmental disorders during the follow-up was evaluated in our 6 perinatal LQTS and 15 nonperinatal LQTS patients.

DEVELOPMENTAL OUTCOME. The developmental outcomes were assessed using the Kinder Infant Development Scale (KIDS) (16,17) in 14 patients from our institution. In the perinatal LQTS group, the KIDS was available only in 5 patients with comorbid epileptic seizures. KIDS type B, C, and T were used as appropriate. Type B was designed for assessing infant children 12 to 23 months of age. It included 142 items and yields subscales for 9 developmental domains: physical motor, manipulation, language reception, language expression, concept, social relationships with children, social relationships with adults, training, and feeding. Type C was designed for

children 36 to 83 months of age. It included 133 items and yield subscales for the same developmental domains except for feeding. Type T was designed for assessing developmentally delayed children 36 to 83 months of age. It included 282 items and yield subscales for the 9 developmental domains of type B. Type T was also useful for assessing severe developmentally delayed children of up to 12 years old. Each item was scored as pass (1 point) or fail (0) by the parents and the scores were summed for each subscale. The overall developmental age from the total score and those for all subscales were determined using a conversion chart (16). Development quotients (DQs) for the total and all subscales were then calculated using the following formula.

$$DQ = \text{development age/calendar age} \times 100$$

A total DQ under 70 was defined as a developmental disorder. DQs were compared between 5 epileptic perinatal LQTS and 9 nonperinatal LQTS patients.

NEUROLOGICAL EVALUATION. Epilepsy was diagnosed by pediatric neurologists, based on the definitions of a seizure and epilepsy by the Task Force of the International League Against Epilepsy in 2005 (18). An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain. Epilepsy is a disorder of the brain characterized by and enduring a predisposition to generate epileptic seizures, and by the neurobiologic, cognitive, psychological, and social consequence of this condition. The definition of epilepsy requires the occurrence of at least 1 epileptic seizure. We evaluated the neurological examination, blood tests, and electroencephalograms to diagnose epilepsy in all our patients with clinical seizures. We eliminated the possibility of

TABLE 1 Clinical Characteristics of LQTS Patients Under 1 Year of Age

Patient #	Sex	Genotype	Gene	Mutation	Age at the Initial Presentation (days)	HR (beats/min)	QTc (ms)	Medications at the Initial Presentation	Clinical Presentation	Arrhythmias During the Neonatal Period
1	M	LQT3	*SCN5A*	N406K	2	120	566	-	Frequent PVC	TdP
2	F	LQT2	*KCNH2*	T623I	0	93	606	Transplacental (BB, Ver, Mg)	FH	2:1AVB
3	M	LQT3	*SCN5A*	G1631D	0	150	538	-	Fetal TdP	TdP
4	M	LQT3	*SCN5A*	P1332L	1	158	584	-	NSVT	TdP, VT
5	M	LQT2	*KCNH2*	S624R	0	58	686	Transplacental (Mex, Mg)	Fetal TdP, fetal AVB	2:1 AVB
6	F	LQT2	*KCNH2*	T613M	0	115	582	Transplacental (BB, Mex, Mg)	Fetal TdP	Wenckebach AVB
7	M	LQT1	*KCNQ1*	A341V	57	150	506	-	FH	-
8	M	LQT3	*SCN5A*	N406K	60	136	452	-	FH	-
9	M	LQT1	*KCNQ1*	A341V	0	97	559	Transplacental (BB)	FH	-
10	F	LQT2	*KCNH2*	W563C	0	143	494	Transplacental (Mg)	FH	-
11	M	LQT1	*KCNQ1*	G325R	0	115	582	Transplacental (BB)	FH	-
12	F	LQT2	*KCNH2*	T65P	321	130	472	-	FH	-
13	F	Unidentified	-	-	0	120	566	Transplacental (Mg)	FH Bradycardia	-
14	M	LQT7	*KCNJ2*	G300V	0	136	543	Transplacental (BB, Ver)	FH	-
15	M	LQT1	*KCNQ1*	R174C	0	136	513	Transplacental (BB)	FH	-
16	M	LQT7	*KCNJ2*	G300V	0	120	509	Transplacental (BB)	FH	-
17	M	LQT2	*KCNH2*	W563C	0	125	520	Transplacental (BB)	FH	-
18	M	LQT1	*KCNQ1*	A344E	38	150	493	-	FH	-
19	F	LQT1	*KCNQ1*	L563P	316	120	453	-	FH	-
20	M	LQT2	*KCNH2*	T65P	0	136	482	Transplacental (BB)	FH	-
21	F	LQT1	*KCNQ1*	R174H	0	120	509	Transplacental (BB)	FH	-
22*	M	LQT3 LQT6	*SCN5A* *KCNE2*	R1623Q I57T	0	N/A	535	N/A	Irregular fetal heart rhythm	TdP
23*	M	LQT3	*SCN5A*	M1766L	1	81	513	N/A	Irregular fetal heart rhythm	TdP
24*	F	LQT2	*KCNH2*	T613M	0	67	607	N/A	Fetal bradycardia	2:1 AVB, TdP

Patients #1 and #8, #10 and #17, #12 and #20, and #14 and #16 were siblings. *Patients #22, #23, and #24 were previously reported cases (11-13).

AVB = atrioventricular block; BB = β-blocker; FH = family history; HR = heart rate; LQTS = long QT syndrome; Mex = mexiletine; Mg = magnesium; N/A = not available; NSVT = nonsustained ventricular tachycardia; PVC = premature ventricular contraction; TdP = torsade de pointes; Ver = verapamil; VT = ventricular tachycardia.