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A985G Polymorphism of the Endothelin-2 Gene and Atrial Fibrillation in Patients With Hypertrophic Cardiomyopathy

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Background It was recently suggested that the angiotensin-converting enzyme insertion/insertion genotype, which is considered to be protective against cardiovascular disease, was a significant risk factor for atrial fibrillation (AF) in patients with hypertrophic cardiomyopathy (HCM). The aim of this study was to investigate the association between the endothelin-2 (*EDN2*) A985G polymorphism and AF in patients with HCM.
Methods and Results The *EDN2* A985G polymorphism (rs 5800) was genotyped in 110 patients with HCM who had no clinically documented AF before medical treatment. The distribution of the *EDN2* genotypes (G/G, G/A, and A/A) was 77.3%, 19.1%, and 3.6%, respectively. The *EDN2* A allele frequency was 0.21 in 26 patients who subsequently developed AF during long-term follow-up and 0.11 in 84 patients who remained in sinus rhythm. The distribution of genotypes of the dominant *EDN2* A allele between the two groups was significantly different by chi-square analysis (42.3% vs 16.7%, p=0.014). In a multivariate model, the A985 allele of the *EDN2* gene was associated with increased adjusted risk for the occurrence of AF (p=0.018).
Conclusion The *EDN2* A985 allele, which is considered to be protective in cardiovascular disease, may be a risk factor for AF in patients with HCM. (*Circ J* 2007; **71:** 1932–1936)

Key Words: Atrial fibrillation; Endothelin-2 gene A985G polymorphism; Hypertrophic cardiomyopathy

Atrial fibrillation (AF) occurs in approximately 20% of patients with hypertrophic cardiomyopathy (HCM) and is widely recognized as a complication of HCM that is associated with substantial risk for heart failure, stroke, and even life-threatening arrhythmias[1–5] It is well known that hemodynamic changes followed by sympathetic or parasympathetic activation play an important role in the triggering of AF[6,7] Thomson et al reported that hypotension during central unloading might provide an additional or alternate trigger for arrhythmia in some patients with HCM[8] Because patients with HCM have a small left ventricular (LV) cavity because of myocardial hypertrophy, reduction of both venous return and intravascular volume leads to low cardiac output and several symptoms that are different from most other cardiovascular diseases. Therefore, paroxysmal AF caused by sympathetic activation preceded by acute hemodynamic changes may occur more frequently in HCM than in other structural heart diseases.

It is evident that a number of factors, genetic (polymorphisms) as well as environmental, affect the extent of LV hypertrophy in patients with HCM, although the disease is a monogenic disorder caused by mutations in at least 13 genes encoding sarcomeric proteins[9–13] Additional genetic factors, such as angiotensin-converting enzyme (ACE) genotypes, partially account for the variability in the phenotypic expression of the disease[14,15] We previously showed that a genotype that resulted in inhibition of the renin–angiotensin–aldosterone (RAA) system (the *ACE* insertion/insertion genotype) turned out to be a significant risk factor for AF in patients with HCM[16] contrary to the previous notion that the *ACE* deletion/deletion genotype of increased activation of the RAA system may be a predisposing factor for hypertension, AF, and other organic heart diseases[17–22]

Sharma et al demonstrated that the endothelin-2 (*EDN2*) gene is expressed in human atrial tissue and might mediate the development of hypertension[23] Recently, *EDN2* gene polymorphism, which contributes to modulation of cardiovascular physiology, has been reported to be associated with hemodynamic changes in patients with essential hypertension[24,25] Taking all these findings together, we hypothesized that the allele of *EDN2* that is protective for cardiovascular disease, considered to be the A985 allele, may contribute to the development of AF in patients with HCM. In the present study, we investigated the A985G polymorphism of *EDN2* to determine whether this polymorphism is associated with increased susceptibility to AF in HCM patients and analyzed the interaction between this polymorphism and the *ACE* insertion/deletion polymorphism.

Methods

Study Population

A series of 110 patients (86 men, mean age 53±13 years)

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diagnosed with HCM who gave informed consent for genetic analysis at the Ehime University Hospital were enrolled in the study. Analysis of DNA was performed between March 2000 and August 2000 using blood samples from patient volunteers. The study protocol was approved by the Ethics Committee of Ehime University School of Medicine. HCM was diagnosed on the basis of echocardiographic criteria defined as the presence of LV hypertrophy in the absence of other causes of hypertrophy. These patients also met the definition and classification proposed by the 1995 World Health Organization/International Society and Federation of Cardiology Task Force.[26] AF was diagnosed by 12-lead electrocardiography (ECG) and/or 24-h Holter monitoring at the follow-up visits. In particular, if patients had episodes of palpitation or dizziness, repeat Holter recordings were performed to detect AF or other tachyarrhythmias. Five patients who had prior documented permanent or paroxysmal AF were excluded. Patients with myocardial infarction and/or other significant heart problems, such as severe valvular heart disease, dilated phase HCM (defined as percentage fractional shortening <25%[27,28]), congenital heart disease, having an accessory pathway and bundle-branch block, were also excluded.

The incidence of AF during follow-up was assessed retrospectively. Two groups of patients were studied: Group A consisted of 26 patients who subsequently developed AF (paroxysmal or persistent AF 13 patients, permanent AF 13 patients) during a mean follow-up of 13±7 years; Group B consisted of 84 patients who remained in sinus rhythm (SR; follow-up period: 13±7 years). The onset of AF was associated with thromboembolic episodes in 4 (15%) of 26 patients. All previous cardiac studies in the patients were reviewed and the initial clinical status of the patients was determined on the basis of their medical records. All patients completed a standard questionnaire about their personal medical and family histories.

12-Lead ECG

The 12-lead ECGs were obtained using conventional lead positions and recorded at 25 mm/s; for analysis, all records were magnified by 200% to improve resolution. The QT interval and QRS duration were measured manually in the 6 precordial leads. The QT interval was measured from the onset of the QRS complex to the end of the T wave (ie, the return to the T–P baseline). When U waves were present, the QT interval was measured from the onset of the QRS complex to the nadir of the curve between the T and U waves. The QRS duration was defined as the maximal QRS length in any lead measured manually from the first to the last sharp deflection crossing the isoelectric line. The JT interval was calculated by subtracting QRS duration from QT interval in individual leads. The QT, QRS, and JT dispersions were defined as the difference between the maximum and minimum QT, QRS, and JT values, respectively. Values of the QT and JT intervals and dispersions were corrected for heart rate using Bazett's formula[29] (QTc and JTc, respectively).

Echocardiography

Two-dimensional (2-D) and M-mode echocardiography were performed using conventional methods. In summary, the end-diastolic LV wall thickness was recorded at the level of the mitral valve and papillary muscle in the septal and posterior walls, as well as in the lateral and posterior LV walls using short-axis 2-D images. The maximal wall thickness was assessed from the apical 4-chamber view. LV mass index (LV mass/body surface area) was calculated using a standard formula[30,31] LV outflow tract (LVOT) velocity was determined by continuous wave Doppler echocardiography, and the gradient was calculated using the modified Bernoulli equation. LVOT obstruction was defined as a pressure gradient ≥30 mmHg.[32] Apical HCM was defined as LV wall thickening confined to the most distal region of the LV apex below the papillary muscle level.

Determination of EDN2 and ACE Genotypes

Genomic DNA was extracted from peripheral blood samples by an extraction kit (Qiagen, Hilden, Germany). Similar to our previous study, we determined the *EDN2* polymorphism by the TaqMan polymerase chain reaction chemistry method (Roche Molecular Systems, Pleasanton, CA, USA).[33] The TaqMan probe is a fluorogenic probe that consists of an oligonucleotide labeled with both a fluorescent reporter dye and a quenched dye. The fluorescent reporter dye, such as VIC or FAM, is covalently linked to the 5' end of the nucleotide. Each of the reporters is quenched by a minor groove binder, typically located at the 3' end. The following primers were used for the A985G polymorphism (rs5800) in *EDN2*: a forward primer, 5'-ACA AAC CAG GAG CAA CCG TG-3'; a reverse primer, 5'-AGG GAA TGA GGG TGC AAG AA-3'; a G allele-specific probe, 5'-VIC-CCC TGG AGA CTG GA-MGB-3'; and an A allele-specific probe, 5'-FAM-CCT GGA GGC TGG AT-MGB-3'. The level of fluorescence of the polymerase chain reaction products was measured with the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). We validated the TaqMan method with more standard restriction fragment length polymorphism and sequencing. In addition, we genotyped the *ACE* insertion/deletion polymorphism as previously described.[16] The investigator who assessed the genotype was unaware of the clinical data of the study subjects.

Statistical Analysis

Summary data are expressed as mean±SD. Measured variables were compared between 2 groups with an unpaired Student's t-test. Categorical variables were compared by the chi-square test. Differences in the prevalence among groups and the Hardy-Weinberg equilibrium were analyzed by the chi-square method. To analyze the differences between A-allele carriers and non-carriers in *EDN2* or between D allele carriers and non-carriers in *ACE*, the *EDN2* A/A and G/A genotypes or the *ACE* insertion/deletion and deletion/deletion genotypes were pooled into 1 group. Variables with a p-value <0.05 in the univariate analysis were entered into multivariate logistic regression analysis to identify the independent predictive variables for AF. Odds ratios (OR) were estimated with 95% confidence intervals (CI) as measures of risk. A p-value <0.05 was considered statistically significant.

Results

Table 1 shows the clinical characteristics of the patients; no significant differences were found between the 2 groups in age, gender, body size, blood pressure, history of syncope, family history of HCM or sudden cardiac death, type of HCM (obstructive vs non-obstructive, presence or absence of apical hypertrophy), and their medical regimens. As shown in Table 2, the ECG data, except for QRS dispersion, were not significantly different between the 2 groups. QRS

Table 1 Patient Characteristics

	Group A (n=26)	Group B (n=84)	p value
Age at diagnosis, years	54±11	52±14	NS
Men, n (%)	22 (85)	64 (76)	NS
Height, cm	162±7	161±9	NS
Body weight, kg	65±10	63±13	NS
Systolic blood pressure, mmHg	135±20	136±19	NS
Diastolic blood pressure, mmHg	84±13	82±12	NS
History of syncope, n (%)	1 (4)	8 (10)	NS
Family history of SCD, n (%)	7 (27)	12 (14)	NS
Family history of HCM, n (%)	3 (12)	18 (21)	NS
HOCM, n (%)	6 (23)	15 (18)	NS
Apical HCM, n (%)	9 (35)	22 (26)	NS
Antiarrhythmic agents			
Class I, n (%)	7 (27)	12 (14)	NS
Class II, n (%)	14 (54)	34 (41)	NS
Class IV, n (%)	9 (35)	47 (56)	NS

Values are mean±SD.
SCD, sudden cardiac death; HCM, hypertrophic cardiomyopathy; HOCM, hypertrophic obstructive cardiomyopathy.

Table 2 Electrocardiographic and Echocardiographic Measurements

	Group A (n=26)	Group B (n=84)	p value
Electrocardiography			
QRS duration, ms	111±12	112±15	NS
QTc interval, ms	418±29	429±32	NS
JTc interval, ms	318±30	328±35	NS
QRS dispersion, ms	24±10	17±11	0.025
QTc dispersion, ms	29±32	37±17	NS
JTc dispersion, ms	42±27	35±19	NS
Echocardiography			
LAD, mm	40±8	37±5	0.011
LVDd, mm	47±6	46±4	NS
LVDs, ms	29±7	28±5	NS
IVS, mm	18±4	14±5	0.0002
PW, mm	11±2	10±3	NS
FS, %	39±8	40±7	NS
LVMi, g/m²	200±59	154±65	0.0006

Values are mean±SD.
LAD, left atrial dimension; LVDd, left ventricular dimension at end-diastole; LVDs, left ventricular dimension at end-systole; IVS, interventriclar septal wall thickness; PW, posterior wall thickness; FS, fractional shortening; LVMi, left ventricular mass index.

dispersion was significantly greater in group A than in group B patients (p=0.025). Left atrial dimension was significantly larger in group A than in group B (p=0.011) and the interventricular septal wall thickness was also significantly greater in group A than in group B (p=0.0002). Likewise, the LV mass index was significantly greater in group A than in group B (p=0.0006). LV dimensions at end-diastole and end-systole, posterior LV wall thickness, and fractional shortening of the LV were not significantly different between the 2 groups. The frequencies of the *EDN2* and *ACE* genotypes were virtually identical to those predicted by the Hardy-Weinberg equilibrium. The distribution of the *EDN2* genotypes (G/G, G/A, and A/A) was 77.3%, 19.1%, and 3.6%, respectively. The frequency for the *EDN2* A985 allele was 0.21 in group A and 0.11 in group B. There were significant differences between the 2 groups in the distribution of the *EDN2* genotypes (Table 3). Distribution of the *ACE* genotypes (deletion/deletion, deletion/insertion, and insertion/insertion) was 0%, 34.6%, 65.4% in group A and 16.7%, 48.8%, 34.5% in group B (p=0.0077). In a dominant

Table 3 Frequencies of the *EDN2* Genotypes and Alleles

	Group A (n=26)	Group B (n=84)	p value
Genotype frequency			
G/G, n (%)	15 (58)	70 (83)	
A/G, n (%)	11 (42)	10 (12)	0.002
A/A, n (%)	0 (0)	4 (5)	
A allele dominant model			
G/G, n (%)	15 (58)	70 (83)	0.014
A/G+A/A, n (%)	11 (42)	14 (17)	
Allele frequency			
G allele	0.79	0.89	0.087
A allele	0.21	0.11	

Table 4 Risk Factors for Atrial Fibrillation in Multiple Logistic Regression Analysis

	OR (95%CI)	p value
EDN2 A allele carrier	5.89 (1.36–25.6)	0.018
ACE I/I	7.61 (1.60–36.2)	0.011
QRS dispersion	1.11 (1.03–1.20)	0.008
LAD	1.07 (0.95–1.20)	NS
IVS	1.08 (0.88–1.32)	NS
LVMi	1.01 (0.99–1.03)	NS

OR, odds ratio; CI, confidence interval; ACE I/I, angiotensin-converting enzyme insertion/insertion. Other abbreviations as in Table 2.

deletion allele model (deletion/deletion and deletion/insertion genotypes vs insertion/insertion genotype), there was a significant difference between the 2 groups in genotypes (34.6% vs 65.4% in group A and 65.5% vs 34.5% in group B; p=0.011). Table 4 shows the OR for AF in patients with HCM determined by logistic regression analysis. The odds of AF in the *EDN2* A allele carrier were nearly 6-fold higher in the multivariate model after adjusting for left atrial diameter, interventricular wall thickness, and LV mass index. In addition, the insertion/insertion genotype of *ACE* and QRS dispersion were also significant independent risk factors for AF. *EDN2* A allele carriers had a 7-fold greater risk of AF if they had the insertion/insertion genotype of *ACE* (OR 7.02, 95%CI 2.05–24.0, p=0.0019).

Discussion

The main finding of this study is the significant association between the prevalence of AF and *EDN2* as well as *ACE* polymorphism in patients with HCM. In addition, QRS dispersion also emerged as an independent predictor of AF.

AF is the most common tachyarrhythmia and is associated with potentially life-threatening consequences in patients with HCM, including serious cerebrovascular events, heart failure, and even life-threatening arrhythmia[1–5] The prevalence of AF has been reported to be 10–28% in HCM at 7–10 years after the initial diagnosis[2,3,34–36] The incidence of AF in the present series was almost identical to that reported in previous studies. In our series, 19 (17%) patients were taking class Ia antiarrhythmic agents at the time of the first clinical evaluation, despite the fact that no evidence of AF had been documented in any patient. Class Ia antiarrhythmic agents are now regarded as standard therapy, not only for reducing the LV pressure gradient in patients with obstructive HCM[37–39] but also for improving LV diastolic dysfunction, even in patients with non-obstructive HCM[40] For that reason, HCM patients without any epi-

sodes of tachyarrhythmia are sometimes prescribed these drugs. Because the percentage of patients with taking class I antiarrhythmic agents did not differ significantly between group A and group B in the present study, it is unlikely that prophylactic use of these drugs contributed to the difference in incidence of AF.

The chromosomal localization of *EDN2* is 1p34 and the functional significance of the A985G polymorphism has not yet been demonstrated. However, variations within the 3'-UTR are known to affect mRNA stability[41] Therefore, A985G may affect the transcription and translation of *EDN2*. The HapMap data (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=5800) showed disparities in the frequency of the A985 allele between populations of different ethnicities, ranging from 4% to 31%. The frequency of the A allele in our HCM patients was almost identical to that of the Japanese population in the HapMap data. The A985 allele, which is possibly a protective polymorphism for cardiovascular disease, may be a risk factor for AF in patients with HCM. Although a previous study described an association between HCM and *EDN1*,[42] there are no previous reports showing that *EDN2* polymorphism increases the incidence of AF. Previously, we suggested that the *ACE* genotype may be a risk factor for AF in patients with HCM[16] and we postulated that the risk allele for AF in patients with HCM was the *ACE* insertion allele, despite the fact that that allele is protective in patients with other cardiovascular diseases. Because of the small size of the LV, because of hypertrophy, in HCM, reduction of venous return could easy lead to hypotension. In addition, an exaggerated fall in systemic vascular resistance because of impaired activation of the LV mechanoreceptors could also cause hypotension during exercise in HCM[8] These phenomena may induce sympathetic activation that might trigger AF. Thus, vasodilators such as ACE inhibitors, angiotensin-receptor blockers, calcium-channel blockers, and nitrates should be used with caution in patients with HCM, regardless of the presence of LVOT obstruction. In this scenario, we hypothesized that the possible protective allele of the *EDN2* polymorphism for cardiovascular disease, the A allele, might be related to AF susceptibility in HCM. Activation of the autonomic nervous system plays a major role in the initiation and perpetuation of AF[6] Cheung et al suggested that adrenergic activity may induce AF by increasing the spontaneous discharge rate of foci in the pulmonary veins[7] EDN2 constricts systemic veins and may help preserve venous return and prevent subsequent hypotension[43] As mentioned earlier, because acute hypotension followed by an increase in sympathetic nerve activity may easily occur in HCM, it is possible that a vasoconstrictor may have a protective effect against AF in HCM. However, the baseline diastolic blood pressure in the present patients was not significantly different between patients with and without AF, despite a difference in the *EDN2* A985G allele frequencies. This is not consistent with previous results,[24,25] which suggested that *EDN2* A985G polymorphism influenced the severity, rather than the initial development of hypertension. In the present study, patients with marked hypertension, which can lead to LV hypertrophy, were excluded and this may partly explain the discrepancy in results.

Interestingly, in our series QRS dispersion appeared to predict the incidence of AF. Because QRS dispersion is likely to represent inhomogeneity of ventricular depolarization, it may be a useful marker of ventricular tachyarrhythmia. However, there are no published data indicating a relationship between QRS dispersion and atrial tachyarrhythmias. Mazzoleni et al. reported a correlation between myocardial fibrosis and prolonged QRS duration[44] Furthermore, Yamaji et al found a relationship between the development of AF and marked fibrosis of the LV in HCM[45] In the present series, both the interventricular septal wall thickness and LV mass index were significantly greater in the AF group than in the SR group. Greater QRS dispersion may occur because of asymmetrical myocardial hypertrophy and inhomogenous distribution of ventricular myocardial fibrosis (often relevant to HCM). In addition, it is likely that inhomogeneity of myocardial fibrosis in the LV is linked with left atrial overload because of diastolic dysfunction and AF substrate development in HCM, although the precise mechanism is unknown.

In the present study, neither left atrial size nor age emerged as significant predictors in the multivariable analyses, although these parameters have been previously reported as significant predictors for AF in HCM patients! In that study, the left atrial diameter in patients who developed AF was nearly 50 mm, compared with 42 mm in those who remained in SR. In our study subjects, on the other hand, it was 41 mm in those who developed AF and 38 mm in those who remaining in SR. It must be noted that we excluded patients who had prior documented permanent or paroxysmal AF at the initial HCM diagnosis. Because the other study included patients who developed AF before the diagnosis, it is possible that the baseline characteristics of the present patient population were critically different from previous studies. Furthermore, it is reported that some HCM patients have AF in the absence of left atrial dilatation! which suggests that specific HCM-causing mutations and polymorphisms may predispose to AF, by causing an intrinsic atrial myopathy associated with prolonged and fragmented atrial conduction or by some unknown mechanism[46]

Study Limitations

The findings presented here must be viewed as preliminary because they are based on a single-center experience and a small number of patients. Furthermore, this study was conducted retrospectively and cannot be free from selection bias. We only genotyped individuals who gave blood between March 2000 and August 2000. It might be that those at high risk for cardiovascular events because of the severity of the disease had already died or discontinued their regular visits and therefore were excluded from this study. In addition, we did not measure cardiac output and other parameters that relate to vascular tone. Further molecular, biological, and clinical studies are needed to clarify the relationship between *EDN2* polymorphism and AF in HCM.

Conclusion

We demonstrated that carrying the A985 allele of *EDN2*, as well as the *ACE* insertion/insertion genotype, which protects against most cardiovascular diseases, plus dispersion of QRS duration may be risk factors of AF in patients with HCM.

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