Impact of Delta-Sarcoglycan Gene Polymorphism on the Occurrence of Coronary Spastic Angina in Japanese Patients With Hypertrophic Cardiomyopathy

Tsuyoshi Honda, MD; Seigo Sugiyama, MD; Tomohiro Sakamoto, MD; Koichi Kaikita, MD; Hisao Ogawa, MD

Background Patients with hypertrophic cardiomyopathy (HCM) frequently complain of angina-like symptoms in the absence of organic coronary stenoses. Coronary spasm might cause myocardial ischemia in HCM patients. Delta-sarcoglycan plays a crucial role in the pathogenesis of HCM and coronary spasm in a mouse model.

Methods and Results This is a retrospective, single-center study with a small sample size. Seventy patients with HCM underwent coronary angiography and received acetylcholine provocation test. Coronary risk factors and 5'-untranslated region (UTR) G to C polymorphism on delta-sarcoglycan gene (n=64) were evaluated in the HCM patients. In 31 (44.3%) of 70 HCM patients, coronary spasm was induced by the provocation. None of the coronary risk factors was significantly different between the coronary spasm group and the non-coronary spasm group. The 5'-UTR gene polymorphism was associated with the occurrence of coronary spasm with an additive effect on the coexistence (p=0.025). Multiple logistic regression analysis showed that the C allele of 5'-UTR polymorphism was a significant risk factor for coronary spasm in patients with HCM (odds ratio, 3.1; 95% confidence interval, 1.0 to 9.5; p=0.045) that was independent of traditional coronary risk factors.

Conclusions The 5'-UTR polymorphism on delta-sarcoglycan gene was associated with coronary spasm in Japanese patients with HCM. (Circ J 2007; 71: 1263–1267)

Key Words: Coronary spastic angina; Delta-sarcoglycan; Gene polymorphism; Hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is characterized by left and/or right ventricular hypertrophy and hypercontraction without known cause for the hypertrophy. Patients with HCM often complain of angina-like chest pain and uncomfortable chest sensations in the absence of significant coronary artery stenoses. Myocardial ischemia as a result of coronary artery disease in patients with HCM is thought to affect cardiovascular events, including premature sudden death. Coronary artery spasm is considered to play an important role in the pathogenesis of myocardial ischemia in patients without organic coronary artery stenoses and myocardial infarction, in the absence of significant obstructive coronary atheromatous lesion, is a common finding in patients with HCM. Chronic ischemic myocardial damage probably contributes causally to the clinical deterioration of patients with HCM, leading to ventricular dilatation and progressive fatal cardiac failure through myocardial fibrosis and micro myocardial necrosis, thus screening for the presence of coronary artery disease, including coronary spasm, is clinically important in HCM patients. Several reports have shown that HCM seems to coexist with coronary spastic angina (CSA) in Japanese patients.

Mutations in sarcomeric contractile protein genes have been reported to cause familial HCM. Suzuki et al reported the coexistence of familial HCM and CSA in 2 Japanese brothers, suggesting a possibility that some genetic factors might be associated with the coexistence of HCM and CSA? It has been reported that mutation of the delta-sarcoglycan (SGCD) gene contributes to pathogenesis of both HCM and coronary artery spasm in an animal model. Thus, it is possible that the SGCD gene might be associated with the coexistence of CSA and HCM in humans. In the present study, we examined the association between the SGCD gene polymorphism and coronary spasm in Japanese patients with HCM.

Methods

Study Population

This is a retrospective, single-center study investigated at Kumamoto University Hospital. The diagnosis of HCM was made by echocardiography and left ventriculography on the basis of the definitions of the World Health Organization/International Society and Federation of Cardiology. A total of 100 patients with HCM were referred to the Department of Cardiovascular Medicine at Kumamoto University Hospital. They were primarily enrolled in the present study, but 30 patients were excluded from the study for the following criteria. Twelve patients with elevated left ventricular outflow tract pressure gradient (LVOTPG) > 30 mmHg at rest were excluded because of increased risk of the acetylcholine provocation test. Eighteen patients with significant obstructive coronary atherosclerotic lesions causing myocardial ischemia were also excluded because of the risk of performing percutaneous coronary intervention. Diagnostic coro-
nary angiography including the acetylcholine provocation testing was performed in 70 patients with HCM (Table 1).

This study protocol was approved by the Ethics Committee of Graduate School of Medical Sciences, Kumamoto University and informed consent was obtained from all participants in the study.

**Analysis of Coronary Risk Factors for Coronary Artery Disease**

The traditional risk factors for coronary artery disease used for statistical analysis in this study were hypertension, lipid profiles (total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C) and triglyceride (TG)), cigarette smoking habit, body mass index and diabetes mellitus (DM).

Hypertension was defined as a systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg or antihypertensive medication. Cigarette smoking was defined as ≥10 cigarettes/day for 10 years. Serum TC, LDL-C, HDL-C, TG and blood glucose were measured in the fasting state on the next day after admission. DM was defined as a fasting blood glucose ≥126 mg/dl and/or a blood glucose ≥200 mg/dl at 2 h after a 75 g oral glucose load or antidiabetic medication.

**Coronary Angiographic Study**

All cardiovascular medications except sublingual nitroglycerin were stopped for at least 4 days before the coronary angiographic study; nitroglycerin was also stopped at least 2 h before the angiography. Coronary angiographic examinations (Judkins technique) were done in the morning when patients were in the fasting state. Coronary angiograms were obtained initially to evaluate the presence of coronary obstructive atherosclerosis. Then, intracoronary injection of acetylcholine was performed, as reported previously.17–19 Briefly, incremental doses (50 μg, 100 μg) of acetylcholine were injected into the left main coronary artery and the time interval between each injection was ≥5 min. Left coronary angiography was performed when the ST segment changes or chest pain, or both developed or 1–2 min after initiation of each injection. Next, an acetylcholine dose of 50 μg was injected into the right coronary artery and right coronary angiography was performed in the same manner. CSA was defined as total occlusion or severe vasoconstriction (>90%) of the coronary artery associated with chest pain and ischemic ST segment elevation or depression on the electrocardiogram after intracoronary injection of acetylcholine as described previously.17–19

**Screening for 5'-Untranslated Region (UTR) G to C Polymorphism of SGCD Gene by the Allele Specific Real-Time Polymerase Chain Reaction Assay**

We investigated 5’-UTR G to C polymorphism on SGCD gene in 64 HCM patients who gave informed consent for genetic analyses. Genomic DNA was extracted from white blood cells. We examined 5’-UTR G to C polymorphism on SGCD gene by the specific assay (assy number C, 26840118_10, TaqMan® SNP Genotyping Assays, Applied Biosystems) on an ABI PRISM Genetic Analyzer 7900 (TaqMan, Applied Biosystems, Foster City, CA, USA). The single-nucleotide polymorphism (SNP) was confirmed using db SNP at the website of the National Center for Biotechnology Information and Applied Biosystems Celera Discovery System with the following SNP ID: rs13170573.

**Statistical Analysis**

Results of normally distributed continuous variables are expressed as the mean values ± SD and those for continuous variables with skewed distribution are expressed as the median values (interquartile range). Continuous variables were compared by 2-tailed unpaired t-tests and the Mann–Whitney U-test, as appropriate. Categorical variables were compared by chi-squared analysis with Fisher’s exact probability. This cohort was in agreement with a Hardy–Weinberg equilibrium. Univariate logistic regression analysis with traditional coronary risk factors and SGCD gene polymorphism was performed to determine significant risk factors for CSA in patients with HCM. To analyze the differences between C allele carriers and non-carriers, the C/C and C/G genotypes were pooled into 1 group. Association between polymorphisms and CSA status was tested by logistic regression analysis controlling for age, gender and cigarette smoking. Statistical significance was defined as p<0.05.

**Results**

**Clinical Characteristics in Patients With HCM**

More than half of the patients (54.3%) with HCM presented an apical pattern of left ventricular hypertrophy, which was classified into Maron classification V (Table 1).

**Comparisons of Clinical Characteristics in HCM Patients With or Without CSA**

In 31 (44.3%) of 70 HCM patients, CSA was induced by the acetylcholine provocation test. None of the traditional coronary risk factors including cigarette smoking was significantly different between the CSA group and the non-CSA group. The Maron classification was not significantly different between the CSA group and the non-CSA group in patients with HCM (Table 1).

On admission, half of the study patients were treated with medications (calcium-channel blockers: 17.1%, nitrates: 7.1%, ß-blockers: 5.7%, angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers: 14.3%) (Table 1).

**Comparisons of Genotype in HCM Patients With or Without CSA**

In screening for 5'-UTR SGCD gene polymorphism, the frequency of GG, CG, and CC genotype in HCM patients with CSA was 17 (54.8%), 12 (38.7%) and 2 (6.5%), respectively. However, the frequency of GG, CG, and CC genotypes in HCM patients without CSA was 26 (78.8%), 7 (21.2%) and 0 (0%), respectively. There was a significant association between the SGCD gene polymorphism and the presence of CSA in the HCM patients with an additive effect on the coexistence (p=0.025, Table 2). Multiple logistic regression analysis using age, gender, cigarette smoking and the SGCD gene polymorphism showed that the C allele of 5'-UTR SGCD gene polymorphism (odds ratio, 3.1; p=0.045, confidence interval, 1.03 to 9.48) was the most predictive independent risk factor for coronary spasms in patients with HCM (Table 3).

**Discussion**

We report for the first time that the 5'-UTR G to C polymorphism on SGCD gene was significantly associated with CSA in Japanese patients with HCM. In addition, multiple
logistic regression analysis showed that the SGCD gene polymorphism was a significant independent risk factor for CSA in HCM patients. These results suggest that SGCD might be partly responsible for the occurrence of coronary spasm in Japanese patients with HCM.

Generally, approximately 50% to 70% of the cases of familial HCM are thought to be because of mutations in the genes encoding sarcomere proteins. A case report on the coexistence of HCM and CSA in a Japanese family suggested that the possible pathogenesis of both coronary...
spasm and cardiomyopathy might potentially originate from the same genetic involvement. It has been reported that mutation of SGCD gene causes both HCM and coronary artery spasm in an animal model. The precise molecular mechanisms involving the pathogenesis of CSA in SGCD deficient mice are still uncertain. It has been shown that tissue damage from cardiac degeneration might affect surrounding myocardial microcirculation by local inflammation. Wheeler et al have indicated that inflammatory infiltrate and cytokine release derived from focally degenerating cardiac myocytes in the sarcoglycan deficient mouse hearts, might lead to vasospasm. Furthermore, myocardial ischemia because of vasospasm might cause tissue damage and then it could form a vicious cycle. These reports have suggested that SGCD might potentially influence the coexistence of HCM and CSA in humans. In the present study, we showed that the 5’-UTR polymorphism on SGCD gene could play an important role on the occurrence of CSA in Japanese patients with HCM. Although we did not determine the mechanisms by which 5’-UTR polymorphism on SGCD gene influences the development of CSA and HCM, the gene variation in 5’-UTR might affect transcriptional activity in the SGCD gene. Actually, there is a case report of the coexistence of HCM and CSA in a Japanese lineage, thus evaluation of the SGCD gene polymorphism in this family might be valuable to investigate the genetic mechanism of CSA in patients with HCM. Screening HCM patients for SGCD gene polymorphism might also be a useful clinical strategy to evaluate the genetic risk of CSA.

It has been reported that CSA was generally associated with cigarette smoking. However, there were no differences in coronary risk factors between HCM patients with or without CSA, indicating that the pathogenesis of the coexistence of HCM and CSA might be unique as compared with the pathogenesis of classical CSA reported in previous studies.

However, mutations in the SGCD gene have been shown to cause dilated cardiomyopathy in humans. It has been reported that CSA might cause heart failure similar to dilated cardiomyopathy. These reports suggested that myocardial ischemia because of coronary spasm in HCM might play a potential role for the conversion of HCM to dilated cardiomyopathy. The transition from HCM to the dilated state is a critical prognostic factor in patients with HCM. Previously, several reports have shown that vasodilating agents, including calcium-channel blockers and nitrates, could improve cardiac dysfunction as a result of CSA. Because calcium-channel blockers improved vascular dysfunction and prevented progression of cardiomyopathy in sarcoglycan-deficient animals, calcium-channel blockers could be clinically effective medications for Japanese HCM patients. Coronary spasm might be a crucial event leading to myocardial ischemia, heart failure and cardiac sudden death in patients with HCM. Because of the frequent coexistence of HCM and CSA in Japanese, it is recommended that patients with HCM should be practically evaluated with cardiac catheterization including the acetylcholine provocation test. In the clinical setting, ß-blockers and calcium-channel blockers have been commonly administered to HCM patients to improve ventricular diastolic compliance and symptoms. On the basis of our results of the present study, we would recommend that calcium-channel blockers could be administered first to patients with HCM to induce coronary vasodilation and to prevent the aggravation of coronary spasm by ß-blockers.

According to the HapMap project, the frequency of GG, CG, and CC genotypes on SGCD gene in Japanese samples collected in the Tokyo metropolitan area was 68.9%, 31.1% and 0%. The frequency of the C allele in our HCM patients with CSA was higher than in the general Japanese population.

It has been reported that endothelial NO synthase (eNOS) gene polymorphisms, such as Glu298Asp and T-786>C, are significantly associated with coronary spasm. Ogmoto et al showed that eNOS gene polymorphism (Glu298Asp) was significantly associated with the coexistent HCM and CSA. The association between eNOS gene polymorphism (T-786>C) and the coexistence of HCM and CSA is very interesting, but this might be beyond the present study. Future research is needed for further investigation.

The first limitation of this study was the small size of the patients groups at the single center and might be affected by bias of administered patients. Our findings need to be confirmed in a large cohort of patients with HCM. The second is that our study population does not represent all patients with HCM. We excluded patients with hypertrophic obstructive cardiomyopathy (HOCM) from the present study. Thus, the coexistence of HOCM and CSA remains unknown. Finally, we could not evaluate whether vasodilators, such as calcium-channel blockers and nitrates, improved prognosis of HCM patients with coronary spasm, because all of them were treated with vasodilators. The results of this study might be limited to Japanese HCM patients with LVOTG ≥30 mmHg at rest.

In conclusion, we found that the occurrence of coronary spasm induced by the acetylcholine provocation test was frequent in the present study population and 5’-UTR G to C polymorphism on SGCD gene was frequent in the HCM patients with coronary spasm compared with those without coronary spasm. Determining SGCD gene polymorphism might be clinically useful to evaluate the genetic risk of coronary spasm in Japanese patients with HCM, providing us helpful information to understand the complicated pathophysiology.

Acknowledgements

The authors thank Megumi Tsukamoto for the excellent technical support. This study was supported by a Grant-in-Aid for Scientific Research (B-17390232 and C-1750753) from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and the Smoking Research Foundation Grant for Biomedical Research, Japan.

References

SGCD on the Occurrence of CSA in HCM