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Gene Mutations in Adult Japanese Patients With Dilated Cardiomyopathy

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Background Some patients with dilated cardiomyopathy (DCM) have mutations of the genes that encode sarcomeric or cytoskeletal proteins of cardiomyocytes, but the prevalence of these mutations in Japan remains unclear.

Methods and Results A group of 99 unrelated adult patients with DCM (familial n=27, sporadic n=72) were screened for the following genes: cardiac β-myosin heavy chain, cardiac myosin-binding protein C (*MYBPC3*), regulatory and essential myosin light chains, α cardiac actin, α tropomyosin, cardiac troponin T, cardiac troponin I, cardiac troponin C, dystrophin, and lamin A/C. A mutation (R820Q) in *MYBPC3* was found in an aged patient. In addition, dystrophin mutations were identified in 3 male patients (2 with exon 45–48 deletion and 1 with exon 48–52 deletion). The prevalence of dystrophin mutations in male patients with DCM was 4.4% (3 of 68). No mutations involving amino acid changes were identified in the other genes.

Conclusions Although cases of adult patients with DCM caused by mutations of the genes encoding sarcomeric or cytoskeletal proteins of cardiomyocytes are infrequent in Japan, it may be advisable to screen older DCM patients for *MYBPC3* mutations, and male patients with familial DCM for dystrophin mutations. (*Circ J* 2005; **69:** 150–153)

Key Words: Dilated cardiomyopathy; Dystrophin; Myosin-binding protein C

Dilated cardiomyopathy (DCM) is a heterogeneous disease characterized by severe heart failure and sudden cardiac death, and the prevalence rate was reported to be 14.0 per 100,000 individuals in Japan[1] A familial form of disease has been noted in 20–35% of patients[2–4] and several disease-causing genes have been identified[5,6] including genes encoding sarcomeric proteins such as β-myosin heavy chain[7] cardiac troponin T[7] cardiac troponin I[8] cardiac actin[9] α-tropomyosin[10] and titin[11] genes encoding sarcolemmal and cytoskeletal proteins such as dystrophin[12] δ-sarcoglycan[13] β-sarcoglycan[14] desmin[15] and metavinculin[16] and genes encoding the nuclear membrane protein lamin A/C[17] In addition to causing DCM, mutations in these sarcomeric protein genes have been also reported to cause hypertrophic cardiomyopathy (HCM)[18] From these findings, it is hypothesized that other disease-causing genes in HCM, such as myosin-binding protein C, myosin ventricular regulatory light chain, and myosin ventricular essential light chain, may also cause DCM. However, there are few systematic studies of these gene mutations in Japanese patients with DCM, and the prevalence of disease-causing genes in patients with DCM in Japan is not well defined. The aim of the present study was to evaluate the prevalence of gene mutations in a population of adult patients with DCM in Japan.

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Methods

Patients

The study subjects comprised 99 unrelated probands with DCM (27 familial, 72 sporadic) aged over 20 years. All probands were identified at the Kanazawa University Hospital and affiliated hospitals. The diagnosis of DCM was based on the criteria of the Collaborative Research Group of the European Human and Capital Mobility Project on Familial Dilated Cardiomyopathy[19] (ie, echocardiographic demonstration of depressed systolic function of the left ventricle (LV) (LV ejection fraction (LVEF) <0.45 and/or fractional shortening <0.25) and a dilated LV (LV end-diastolic dimension >117% of the predicted value corrected for age and body surface area) in the absence of other cardiac or systemic causes). Accurate family histories for more than 2 generations were obtained from the probands, and the proband's relatives who provided informed consent were evaluated by electrocardiography, echocardiography, and blood sampling. The diagnosis of familial DCM was based on the same report.[19] Informed consent was obtained from all subjects in accordance with the guidelines of the Bioethical Committee on Medical Researches, School of Medicine, Kanazawa University.

Detection of Mutation

Deoxyribonucleic acid (DNA) was isolated from peripheral white blood cells of all subjects using a DNA extractor 341 Nucleic Acid Purification System (GENEPURE™, PE

Biosystems Foster City, CA, USA). In vitro amplification of genomic DNA was performed via polymerase chain reaction (PCR). Oligonucleotide primers were used to amplify exons of the following genes: β-myosin heavy chain (*MYH7*), myosin ventricular regulatory light chain 2 (*MYL2*), myosin ventricular essential light chain 1 (*MYL3*), cardiac myosin binding protein C (*MYBPC3*), cardiac α actin (*ACTC*), α-tropomyosin (*TPM1*), cardiac troponin T (*TNNT2*), cardiac troponin I (*TNNI3*), cardiac troponin C (*TNNC1*), and lamin A/C (*LMNA*). The multiplex PCR method[20,21] was used for the analysis of the dystrophin gene. Single-strand conformational polymorphism (SSCP) analysis of amplified DNA was then performed using a method described previously;[22] with a slight modification. Briefly, the PCR product (from 200 to 400 nucleotides) was heated to 95°C for 5 min and quenched on ice to produce almost complete denaturation. Strand separation was obtained using a 10% or a 10–20% gradient gel at a temperature of 4°C. Running conditions were a 20 mA constant current and 4°C. The total electrophoresis time was approximately 3 h. The conditions of electrophoresis (temperature and amperage) were adjusted as needed to give optimal separations for individual sequences. For abnormal SSCP patterns, PCR products were subcloned into the pCR2.1 vector using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA, USA). The nucleotide sequences of the cloned PCR products were determined on both strands by the dye terminator cycle sequencing method using an automated fluorescent sequencer (ABI PRISM™ 310 Genetic Analyzer, PE Biosystems). The following attributes were considered to indicate a disease-causing mutation as specified by Richard et al:[23] cosegregation with affected members in the family, absence of the mutation in 200 unrelated chromosomes of healthy adult controls, and the conservation of the mutated residue among species and isoforms.

Echocardiographic Evaluations

All patients underwent an echocardiographic study that included standard M-mode and 2-dimensional echocardiography. LV dimensions and septal and posterior wall thicknesses were measured at the level of the tips of the mitral valve leaflets. The LVEF was calculated as the difference in the end-diastolic and end-systolic volumes divided by the end-diastolic volume. Fractional shortening (FS) was calculated as the difference in the end-diastolic and end-systolic dimensions divided by the end-diastolic dimension.

Table 1 Baseline Characteristics of the Study Patients With Dilated Cardiomyopathy

Total	*99*
Male (%)	*68 (69%)*
Age (years)	*58.1±13.1*
Family history	*27 (27%)*
Syncope	*11 (11%)*
VT	*30 (30%)*
AF	*24 (24%)*
NYHA class	
I	*21*
II	*30*
III	*41*
IV	*7*
Echocardiography	
IVST (mm)	*9.5±2.2*
PWT (mm)	*9.7±2.0*
LVDd (mm)	*64.8±7.4*
LVDs (mm)	*55.8±8.2*
LVEF (%)	*29.1±9.8*
LVFS (%)	*14.1±5.1*
LAD (mm)	*43.0±8.2*

Data for age and echocardiographic measurements shown as mean±SD. VT, ventricular tachycardia; AF, atrial fibrillation; NYHA, New York Heart Association; IVST, interventricular septal thickness; PWT, posterior wall thickness; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; LAD, left atrial dimension.

Results

Baseline Characteristics of the Study Patients (Table 1)

Of the 99 patients, 68 were male and 31 were female, and the mean age of all patients was 58.1 years (range, 21–82). Occurrence was familial in 27 patients, but sporadic and non-familial in the other 72 patients. The LV end-diastolic dimension was 64.8±7.4 mm and the LV end-systolic dimension was 55.8±8.2 mm. The LVEF was 29.1±9.8% and FS was 14.1±5.1%.

Genetic Results

Synonymous mutations were identified in 17 patients in *MYH7* (14 with T63T, 2 with F244F, 4 with G354G, 1 with D376D, and 3 with I1009I, including 4 patients with 2 mutations and 1 with 4 mutations), in 4 patients in *TPM1* (A151A), in 31 patients in *MYL2* (G10G), and in 1 patient in *TNNT2* (I106I). Nonsynonymous mutations were identified in 4 patients, including 1 patient with a *MYBPC3* mutation, and 3 patients with dystrophin mutations (Table 2).

The patient with the *MYBPC3* mutation was a 71-year-old male who has been reported previously.[24] His LV end-diastolic dimension, end-systolic dimension, and FS values were 56 mm, 48 mm, and 14%, respectively.

Table 2 Clinical Findings in the DCM Patients With Gene Mutations

Case no.	*Age (years)*	*Sex*	*Gene*	*Mutation*	*Family history*	*CK (IU/L)*	*Conduction disturbance on ECG*	*Echocardiography*				
								IVST (mm)	*PWT (mm)*	*LVDd (mm)*	*LVDs (mm)*	*FS (%)*
1	*71*	*M*	*MYBPC3*	*R820Q*	*No*	*56*	*No*	*10*	*10*	*56*	*48*	*14*
2	*33*	*M*	*Dystrophin*	*Exons 45–48 del*	*Yes*	*754*	*LBBB*	*8*	*8*	*67*	*52*	*22*
3	*43*	*M*	*Dystrophin*	*Exons 48–52 del*	*Yes*	*96*	*LBBB*	*9*	*9*	*72*	*69*	*4*
4	*57*	*M*	*Dystrophin*	*Exons 45–48 del*	*Yes*	*438*	*RBBB*	*9*	*9*	*65*	*58*	*11*

CK, creatine phosphokinase; DCM, dilated cardiomyopathy; ECG, electrocardiogram; IVST, interventricular septal thickness; PWT, posterior wall thickness; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; FS, fractional shortening; del, deletion; LBBB, left bundle-branch block; RBBB, right bundle-branch block.

Dystrophin mutations were identified in 3 of 68 male patients (4.4%), 2 of whom had a deletion mutation of exons 45–48 and the other had a deletion mutation of exons 48–52. All these cases were in the familial subgroup. In each family unexpected sudden death had occurred in male patients at the age of approximately 30 years, and a male patient had died from heart failure in 1 family. All 3 probands had normal skeletal muscle activity, but 2 had increased serum creatine kinase (CK) concentrations. Conduction disturbances on ECG was found in all 3 patients, with left bundle-branch block noted in 2 and right bundle-branch block in 1 (Table 2).

No mutations involving amino acid change were identified in *MYH7*, *MYL2*, *MYL3*, *ACTC*, *TPM1*, *TNNT2*, *TNNI3*, *TNNC1*, or *LMNA*.

Discussion

The patient identified in the present study as having a *MYBPC3* mutation was the 71-year-old male who had been previously reported[24] and had been diagnosed clinically as having DCM. Genetic analysis revealed that he had an Arg820Gln mutation in *MYBPC3*, similar to some patients with HCM. Although it is not clear whether this patient had "burnt-out" HCM or had had DCM from the outset, the findings do suggest that screening for *MYBPC3* mutations may be advisable in older Japanese patients with DCM.[24] Ogimoto et al reported a patients with an *MYBPC3* mutation who presented with wall thinning and LV dilatation during long-term follow-up.[25] Because matrix disorganization may be associated with progression from HCM to DCM,[26] evaluation of matrix metalloproteinases and their tissue inhibitors as modifying factors, in addition to analysis of disease-causing gene mutations, may provide important information regarding the pathophysiology in patients with DCM.

Dystrophin mutations have been reported as a cause of X-linked DCM,[27] and the prevalence rate in patients with DCM has been reported to be 0–6.5%.[28,29] In an earlier Japanese study, Shiga et al reported that no mutation of the muscle promoter/first exon region of the dystrophin gene was identified in 92 patients with DCM,[30] but they did not study the other regions of the gene. In contrast, we identified mutations in exons 45–52 in the dystrophin gene in 3 of 68 male patients (4.4%) with DCM in the present study, which suggests that analysis for mutations in exons 45–52 in the dystrophin gene may be more important in Japanese patients with DCM.

Dystrophin mutations have been observed in both Duchenne and Becker muscular dystrophies. Frame-shift mutations are noted in Duchenne muscular dystrophy, whereas mutations without frame-shift are observed in Becker muscular dystrophy.[31] However, genotype–phenotype correlations in patients with dystrophin gene mutations have not been completely determined. To date, 16 different mutations have been reported in patients with DCM and Cohen and Muntoni[32] proposed the following classification: Group A to include mutations affecting transcription or splicing of the dystrophin gene at the 5' end of the gene, and Group B to include mutations in which specific protein domains of dystrophin are affected. Mutations identified in the present study would be categorized as Group B, and all of the present patients had a deletion of exon 48. Muntoni et al[33] and Melacini et al[34] reported that exon 49 was important for cardiac function, and it has also been proposed that intron 48 may contain sequences important to cardiac muscle function.[33] From these findings, we hypothesize that the regions including exons 48 and 49 and intron 48 may be necessary for maintaining cardiac function. In addition, less severe mutations of dystrophin, such as missense mutations, may be associated with the sporadic form of DCM.[35] The clinical characteristics of DCM caused by dystrophin mutations have X-linked inheritance and are associated with increased CK concentrations,[29,32] as seen in the present study. Epidemiologic studies in Japan have reported that the male-to-female ratio is 2.6 for DCM,[1] which is comparable with the current finding demonstrating X-linked inheritance in several patients with dystrophin mutations. When DCM patients are under investigation, especially those with increased CK concentrations and/or cardiac conduction disturbances, it is advisable to carry out screening for dystrophin mutations and this may also be true for sporadic cases.

No mutations with amino acid changes were identified in *MYH7*, *MYL2*, *MYL3*, *ACTC*, *TPM1*, *TNNT2*, *TNNI3*, *TNNC1*, or *LMNA*, which suggests that the prevalence of these mutations in adult patients with DCM may be very low in Japan. In children with DCM, however, it is hypothesized that more severe gene defects may exist, and that the prevalence of disease-causing gene mutations may differ from adult patients with DCM. Further investigations on this point are necessary.

Study Limitations

We did not evaluate other genes such as titin because of technical difficulties in surveying all 363 exons of this giant protein. In future studies it would be desirable to survey titin as well as the other disease-causing genes in order to determine the exact prevalence of gene mutations in Japanese patients with DCM.

The SSCP method is commonly used for screening for gene mutations, but its sensitivity for the detection of nucleotide substitutions is not 100%. Thus, some mutations may have been missed. Additional studies with more sensitive methods for the detection of mutations in these genes are necessary in order to confirm and clarify our results.

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Appendix 1

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