High Incidence of Sudden Cardiac Death With Conduction Disturbances and Atrial Cardiomyopathy Caused by a Nonsense Mutation in the STA Gene

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Background — The STA gene encodes emerin and is one of the genes that is affected in Emery-Dreifuss muscular dystrophy (EDMD). Although it has been reported that EDMD caused by the STA gene mutation is associated with X-linked recessive inheritance, the genotype-phenotype correlations, with special reference to cardiac manifestations, are not well defined.

Methods and Results — We identified 16 carriers (7 male and 9 female) with a nonsense mutation in exon 6 of the STA gene in 2 EDMD families. Pacemakers were required for treatment of bradyarrhythmias in all 7 male carriers and in 2 of the 9 female carriers. In addition, 2 of the 9 female carriers displayed atrial fibrillation. In these 2 families, 3 males without pacemaker implantation, who were not tested genetically, had died suddenly. In these family members, the majority of carriers with the mutation had not been clinically diagnosed as having EDMD before genetic testing because of extremely mild or nonexistent skeletal myopathy.

Conclusions — EDMD caused by this mutation is characterized by atypical clinical features and incomplete penetrance of the clinical phenotype and may result in serious cardiac complications, including sudden death. Approaches to preventing possible sudden death in carriers with the STA gene mutation require further study. (Circulation. 2005;111:3352-3358.)

Key Words: cardiomyopathy ■ genetics ■ death, sudden ■ atrium ■ pacemakers

Emery-Dreifuss muscular dystrophy (EDMD) is characterized by genetic disorders, and the STA gene, which encodes emerin, is one of the causative genes. EDMD is a genetically heterogeneous disorder characterized by early contractures, slowly progressive muscle wasting and weakness, and cardiomyopathy with conduction block.1–3 The STA and LMNA genes are the disease-causing genes of EDMD. The former is associated with X-linked recessive inheritance4–6 and the latter with autosomal dominant, autosomal recessive, and sporadic forms of EDMD.7–9 However, genotype-phenotype correlations in patients with EDMD are not yet fully characterized. In particular, although cardiac involvement in EDMD can be a serious and important aspect of the disease in addition to skeletal muscle dystrophy,10,11 the spectrum of cardiac involvement has still not been fully determined.

We have recently identified 2 Japanese families with EDMD associated with a nonsense mutation in the STA gene. Some members of these families have had serious cardiac involvement, including sudden death, and have displayed an uncommon pattern of inheritance. The aim of this study was to report the (1) genetic analysis and (2) phenotypic variability, especially cardiac manifestations, in these families with EDMD.

Methods

Subjects
Two patients with EDMD were followed up at the Kanazawa University Hospital and its affiliated hospital. The diagnosis of EDMD was made according to clinical criteria agreed on by the ENMC consortium.12 The 2 probands with EDMD and their family members were studied clinically and genetically. Inform consent was obtained from all subjects in accordance with guidelines of the Bioethical Committee on Medical Research, School of Medicine, Kanazawa University. One of the 2 families (Figure 1A, Family 1) was the same one reported by Nagano et al.6 In addition, we evaluated their long-term clinical course retrospectively.

Genetic Studies
Genetic analysis was performed to determine whether the families had a mutation in the LMNA gene as well as the STA gene, because...
both are implicated in causing EDMD. Genomic DNA was purified from subjects’ white blood cells, after which in vitro amplification was performed by polymerase chain reaction (PCR). We sequenced 6 exons of the STA gene and 12 exons of the LMNA gene from the proband with use of an ABI PRISM 310 (PE Applied Biosystems), and the other family members were evaluated similarly. To confirm the presence of a nonsense mutation, restriction enzyme analysis was performed. When resolved on polyacrylamide gel, digestion of PCR products derived from the mutant allele with XspI gave rise to 262- and 87-bp fragments instead of 349 bp. To determine whether there was a possible common ancestral origin of the mutation in these 2 families, haplotype analysis of the 2 families was performed with the use of 4 microsatellite markers (DXS8091-DXS8103-DXS8061-[EDMD]-DXS1073) encompassing 6 megabases flanking the STA gene.

Clinical Evaluations
Evaluation of the phenotype was completed before determination of the genotype. All probands and family members underwent 12-lead ECGs, and the majority also had M-mode and 2-dimensional echocardiography. They also underwent 24-hour Holter monitoring (and electrophysiological testing when indicated). Echocardiography was performed according to conventional techniques. Standard transthoracic M-mode and 2-dimensional echocardiographic studies were performed to identify and quantify morphological features of the atrium and ventricle, according to criteria established by the American Society of Echocardiography. Fractional shortening was calculated as the difference in end-diastolic and end-systolic dimensions divided by the end-diastolic dimension. The apical 4-chamber view was used for measurement of left and right atria. We defined right atrial enlargement as being present when the major axis was >45 mm and/or the minor axis was >37 mm.

Immunohistochemistry
Biopsy specimens from the left deltoid muscle were frozen in LN2 and stored at ≤−80°C until use. Emerin was detected by the avidin-biotin-peroxidase complex method after methanol fixation on 6-μm-thick cryosections with use of a commercial monoclonal antibody (Novocastra) diluted 1:50 in phosphate-buffered saline plus 10% appropriate species serum.

Results
Genetic Results
In both probands (Figure 1A, Family 1, II-2; Figure 2, Family 2, IV-17), sequence analysis of the STA gene revealed a mutation leading to a single base substitution (1735 G→A) in exon 6 (Figure 1B). Relatives of these 2 probands were studied further, totaling 27 members from these 2 families (Figures 1A and 2). Of these 27 individuals, 16 (7 males, mean±SD age, 45.7±12.6 years; 9 females, 51.8±12.4 years) had the same mutation in the STA gene. Seven male carriers with this mutation showed both 262- and 87-bp fragments by PCR–restriction fragment length polymorphism (RFLP) analysis. In contrast, 9 female carriers showed 349-bp fragments in addition to 262- and 87-bp fragments (Figure 1A), indicating heterozygosity for the mutation. This mutation is predicted to result in a premature stop codon at 226 in the C-terminal hydrophobic tail of emerin. Sequence analysis of all 6 exons of the STA gene and all 12 exons of the LMNA gene did not show any other mutation. Although the same mutation in the STA gene was detected in these 2

![Image](http://circ.ahajournals.org/)

**Figure 1.** Mutation analysis and immunohistochemistry. A, Pedigree of Family 1 (above) and PCR-RFLP analysis (below). Circle indicates female; squares, males. Closed and open symbols indicate clinically affected and unaffected individuals, respectively. Plus and minus signs indicate presence and absence of mutation, respectively. Haplotypes are listed (top to bottom) for markers DXS8091, DXS8103, DXS8061, and DXS1073. Boxed haplotypes indicate affected haplotypes. PCR-RFLP analysis was carried out by digestion of PCR products with XspI, which generated polymorphic restriction fragments of 349, 262, and/or 87 bp. B, DNA sequence analysis in proband (left) and his mother (right). Left chromatogram shows single nucleotide transition from G to A at position 1735 of STA. Right chromatogram shows heterozygosity for mutation. C, Immunohistochemistry of skeletal muscle (deltoid muscle) of proband (left) and control (right) with anti-emerin polyclonal antibody. Emerin is not detectable in nuclear membranes of muscle biopsy section of proband compared with control.
families, family histories, verified for 4 generations, did not reveal a common ancestry. In haplotype analysis, however, the disease gene haplotype (20, 24, 22, 21) was identical in the 2 families, suggesting that they were ancestrally related and that EDMD caused by this mutation may have been the consequence of a founder effect (Figure 1A and Figure 2).

Clinical Results and Long-Term Clinical Course

Thirty-three individuals in the 2 families underwent clinical evaluation. In Family 1 (Figure 1A), the proband (II-2) and his brother (II-1) had complete atrioventricular block and sinus node dysfunction at ages 14 and 29, respectively, and they had undergone pacemaker implantation. Their mother (I-2) had atrial fibrillation by ECG. Their father (I-1) had a normal ECG and normal neurological findings. In Family 2 (Figure 2), 8 members (II-9, III-10, III-11, III-13, III-24, IV-9, IV-10, and IV-17) had pacemaker implantation for conduction abnormalities and sinus node dysfunction. Three subjects (Family 2, II-6, III-26, and IV-21) had died suddenly at the ages of 40, 27, and 37 years, respectively, although they had no prior cardiac symptoms, including syncopal episodes. One individual (IV-21) had died suddenly while playing baseball, but the exact circumstances at the time of death were unknown in the other 2 individuals. Sudden death did not occur among any of the patients with pacemaker implantation. The Table summarizes the clinical, ECG, Holter monitoring, and echocardiographic changes of the 16 carriers for a period ranging from 1 to 23 years. All of the 7 male carriers had cardiac involvement, and their first cardiac manifestation occurred at age 10 to 37 years (mean age, 20.9 years). Of the 9 female carriers, 3 had cardiac manifestations, but these occurred at an older age than in the male carriers. The other female carriers (Family 2, III-16, III-20, IV-11, IV-12, IV-13, and IV-20) had no morbid manifestations. Among these 16 patients, skeletal muscle involvement was present in 3 males (18.8%), 1 of whom (Family 1, II-1) had typical neuromuscular disorders, including early joint contractures in elbows, Achilles tendons, and posterior neck and decrease in muscle strength in the humeroperoneal distribution.6 The other 2 males (Family 1, II-2 and Family 2, IV-17) showed contractures of the posterior neck without apparent muscle weakness or atrophy. None of the female carriers displayed neuromuscular abnormalities. One affected male (Family 1, II-2) suddenly developed transient left hemiplegia at 37 years of age. A computed tomography scan of the brain showed evidence of cerebral infarction. Transesophageal echocardiography showed spontaneous echo contrast in the left atrium, but no thrombus was detected. Left atrial appendage blood-flow velocity was decreased. This transient ischemic attack appeared to be caused by thromboembolism.

Figure 2. Pedigree of family 2. See legend to Figure 1A for explanation of pedigree and haplotype analysis. Alleles in parentheses represent inferred genotypes. PM indicates pacemaker implantation; SCD, sudden cardiac death.
By ECG, atrioventricular block with low-amplitude and bifid P waves was documented in all 7 males and occurred at an early age (mean ± SD, 29.9 ± 8.9 years; range, 14 to 42). In addition, 4 of them also had sinus node dysfunction (Figure 3). Subsequently the ECG showed junctional escape rhythm without obvious P waves, indicating atrial standstill in 3 of the 7 affected males (Figure 3). All male carriers had undergone pacemaker implantation at a mean age of 32.1 years (range, 14 to 44), and pacemaker dependency was recorded in all cases at the time of the most recent examination. With regard to female carriers, 2 (Family 1, I-2 and Family 2, III-10) had chronic atrial fibrillation (Figure 3), and 2 (Family 2, II-9 and III-24) had undergone pacemaker implantation. By Holter recording, neither couplets of ventricular ectopy nor life-threatening ventricular tachyarrhythmias, such as tachycardia or fibrillation, were found in the 7 affected individuals who had this procedure. Pacemaker failure was not found among affected individuals with pacemaker implantation. On echocardiographic examination, the most characteristic finding was atrial dilatation. All male carriers had right atrial dilatation, which increased with age, and 2 of them also had left atrial dilatation (ie, biatrial dilatation). No female carriers had right atrial dilatation. One female carrier had left atrial dilatation, but this did not show progression with age. Two male carriers also had left ventricular dilatation (left ventricular end-diastolic dimension >55 mm) but had normal left ventricular contractility. None of the patients demonstrated features suggestive of dilated cardiomyopathy.

Electrophysiological Study
In 2 patients (Family 1, II-2 and Family 2, III-11), electrophysiological studies were performed and revealed that atrial electrical activity measured with intracardiac electrodes was absent in the right atrium. Atrial pacing with stimuli up to 5 V failed to stimulate the right atrium. From these findings, they were thought to have persistent atrial standstill, which is characterized by the absence of atrial activity on surface and intracavitary ECGs, absence of atrial mechanical activity, and inability to electrically stimulate the atria.

Pathological Studies
To test for the presence of emerin in nuclei, we performed immunohistochemical analysis of emerin in the proband’s skeletal muscle with use of a polyclonal antibody. As shown in Figure 1C, a deficiency in immunostaining of the nuclear membranes was found, whereas a normal control specimen showed positive immunostaining of nuclear membranes.

Discussion
In this study, we have demonstrated that patients with EDMD caused by an STA gene mutation are characterized by atypical clinical features and incomplete penetrance of the clinical phenotype and that this resulted in serious cardiac complications, including sudden death. 

The STA gene encodes nuclear protein emerin on chromosome Xq28, and EDMD caused by an STA gene mutation is associated with X-linked recessive inheritance. In Family 2, however, familial conduction system disease with atrial cardiomyopathy appeared to be inherited in an autosomal dominant manner, and the majority of mutation carriers were not clinically diagnosed as having EDMD before genetic testing. This was probably because the majority of affected family members showed few signs of skeletal muscle involvement, and the individuals who displayed cardiac manifestations, such as atrial fibrillation and atrial dilatation, were female. In addition, these cardiac manifestations in females occurred at an older age than in males. Although the mutation carriers did not present with a typical EDMD phenotype, Subject (IV-17) in Family 2 showed contractures of the posterior neck. This fact prompted us to screen for mutations in the STA and LMNA genes, and we detected a nonsense mutation in the STA gene. These results suggest that cardiac manifestations resulting from this mutation may show X-linked dominant transmission with incomplete and age-related penetrance and that screening for this mutation may be useful in evaluating the genetic background of patients with cardiac conduction disturbances and atrial cardiomyopathy. In addition to atrial dilatation, 2 male carriers also had left ventricular dilatation, although this may have been associated with either VVI pacing or the STA gene mutation.

Emerin is a ubiquitous protein located on the cytoplasmic surface of the inner nuclear membrane. Structural analysis predicts that the hydrophobic transmembrane region is required for insertion into the inner nuclear membrane, and unstable membrane insertion may be the cause of the complete loss or great reduction in emerin levels. The mutation in this study at position 226 of the transmembrane region of emerin may cause instability in the protein responsible for integration into the membrane. Additionally, immunohistochemical analysis of emerin in the proband of Family 1 in this study showed a complete lack of emerin. Although no biopsies from female carriers in this study were available, it has been reported that
immunohistochemical analysis of skin fibroblasts or exfoliative buccal cells in female carriers reveals a mosaic pattern of expression of emerin. From these findings, it is possible that cardiac manifestations may occur not only in male carriers but also in female carriers, because this nonsense mutation in the STA gene could also cause greatly reduced emerin levels owing to possible dominant negative effects of the mutation. In the present study, 2 female carriers presented with atrial fibrillation, but several female carriers without cardiac manifestations were also found. Manilal et al. have suggested that the wide range of emerin expression observed in female carriers, deviating considerably from the 50% predicted by random X inactivation, is due to skewed expression of emerin mRNA from the normal and mutated allele. Therefore, female carriers may show differential expression between subjects because of the skewed inactivation of the normal X chromosome occurring in cardiac muscle and conduction system cells of the affected atrium.

The mechanisms by which the STA gene mutation causes conduction disturbances are unclear, but the following possibilities are hypothesized. In heart and cultured rat cardiomyocytes, emerin is associated with intercalated discs in addition to specific localization at the inner nuclear membrane. This specific localization to cardiac desmosomes and fasciae adherents could account for the characteristic conduction defects in EDMD. The gap junction is a region in the intercalated disc where cells are in electrical and functional contact with each other. The lack of or decrease in emerin in the heart may alter electrical resistance in addition to cardiomyocyte adhesion and may lead to conduction delay or block.

EDMD patients sometimes experience sudden death without prior cardiac symptoms. In the present study, 3 subjects in Family 2 died suddenly. Although the etiology of sudden death in carriers with the STA gene mutation is unknown, it is hypothesized that bradyarrhythmias such as sinus arrest or advanced atrioventricular block may play a

<table>
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<th>Family No.</th>
<th>Case</th>
<th>Sex</th>
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<th>LAD, mm</th>
<th>RA Dilatation</th>
<th>EDD, mm</th>
<th>ESD, mm</th>
<th>FS, %</th>
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LAD indicates left atrial diameter; RA, right atrium; EDD, left ventricular end-diastolic dimension; ESD, left ventricular end-systolic dimension; FS, left ventricular fractional shortening; HR, heart rate; AF, atrial fibrillation; AVB, atrioventricular block; N/A, not available; SND, sinus node dysfunction; AS, atrial standstill; SR, sinus rhythm; U/D, undetermined; ?, unknown; +, presence; and –, absence.

*Age at examination.
†Age at onset of cardiac manifestations.
‡Age at pacemaker implantation.
§Findings during right ventricular pacing.
role, because they are noted in the majority of mutation carriers. In addition, no case of sudden death was observed among patients after pacemaker insertion. From these findings, it is speculated that sudden cardiac death in patients with the STA gene mutation might be prevented by prophylactic pacemaker implantation. However, the number of affected subjects in this study was small, and the cause of death in the 2 persons who died suddenly was undefined. Moreover, mutations of the LMNA gene, another disease-causing gene of EDMD, can cause sudden death despite pacemaker implantation, presumably from ventricular arrhythmias. Therefore, an implantable cardioverter-defibrillator may be required for prevention of sudden death in some patients with the STA gene mutation.

In conclusion, our results provide evidence that this mutation shows features of X-linked dominant transmission with both incomplete and age-related penetrance. Patients who display conduction disturbances or atrial cardiomyopathy, even when skeletal myopathy is absent, are possible candidates for this mutation, and it may prove worthwhile to screen such individuals for this condition. Consideration should be given to the possibility that prophylactic pacemaker implantation or implantable cardioverter-defibrillator therapy may be needed in some patients to prevent sudden death in carriers with the STA gene mutation.

References


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