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Brugada syndrome in spinal and bulbar muscular atrophy

Amane Araki, MD
Masahisa Katsuno, MD
Keisuke Suzuki, MD
Haruhiko Banno, MD
Noriaki Suga, MD
Atsushi Hashizume, MD
Tomoo Mano, MD
Yasuhiro Hijikata, MD
Hideaki Nakatsuji, MD
Hirohisa Watanabe, MD
Masahiko Yamamoto, MD
Takeru Makiyama, MD
Seiko Ohno, MD
Megumi Fukuyama, MD
Shin-ichiro Morimoto, MD
Minoru Horie, MD
Gen Sobue, MD

Correspondence to
Dr. Katsuno:
ka2no@med.nagoya-u.ac.jp
or Dr. Sobue:
sobueg@med.nagoya-u.ac.jp

ABSTRACT

Objective: The aim of this study was to clarify myocardial involvement and its clinical implications in subjects with spinal and bulbar muscular atrophy (SBMA), a neuromuscular disease affecting both neuronal and nonneuronal tissues.

Methods: Two independent cardiologists evaluated ECGs from a total of 144 consecutive subjects with SBMA. We performed immunohistochemical, immunoblot, and quantitative real-time PCR analyses of autopsied myocardium.

Results: Abnormal ECGs were detected in 70 (48.6%) of 144 subjects. The most frequent findings were ST-segment abnormalities in V_{1-3} (19.4%), followed by ST-segment abnormalities in V_{5-6} (18.1%). We detected Brugada-type ECGs in 17 of 28 subjects with ST-segment abnormalities in V_{1-3}. Of those, one subject presented with syncope that required an implantable cardioverter defibrillator and led to eventual sudden death, and another subject also died suddenly. No subjects with Brugada-type ECGs had mutations in *SCN5A*, *CACNA1C*, or *CACNB2* genes. In autopsied cases, we detected nuclear accumulation of the mutant androgen receptor protein and decreased expression levels of *SCN5A* in the myocardium.

Conclusions: Subjects with SBMA often show Brugada-type ECG. The accumulation of the pathogenic androgen receptor may have a role in the myocardial involvement in SBMA. **Neurology® 2014;82:1813-1821**

GLOSSARY

AR = androgen receptor; ***CACNA1C*** = calcium channel, voltage-dependent, L type, α 1C subunit; ***CACNB2*** = calcium channel, voltage-dependent, β 2 subunit; **GAPDH** = glyceraldehyde-3-phosphate dehydrogenase; ***HEY2*** = hes-related family bHLH transcription factor with YRPW motif 2; **SBMA** = spinal and bulbar muscular atrophy; ***SCN5A*** = sodium channel, voltage-gated, type V, α subunit; ***SCN10A*** = sodium channel, voltage-gated, type X, α subunit.

Spinal and bulbar muscular atrophy (SBMA), or Kennedy disease, is a hereditary neuromuscular disease caused by the expansion of a trinucleotide CAG repeat in the androgen receptor (*AR*) gene.[1,2] SBMA occurs predominately in adult males.[3,4] The accumulation of the pathogenic AR proteins in the nucleus of motor neurons is central to the pathogenesis of this disease.[5]

In addition to neuromuscular deficits, nonneuronal symptoms such as gynecomastia, muscle cramps, hypertension, hyperlipidemia, and liver dysfunction are often observed in patients with SBMA.[4,6] While gynecomastia and other symptoms of androgen insensitivity appear to result from the partial loss of AR function, the remaining nonneuronal symptoms are likely due to toxicity of the pathogenic AR in these tissues.[7,8] In particular, the elevated serum levels of creatine kinase together with histopathologic findings of myopathic changes indicate the role of skeletal muscle damage in the pathogenesis of SBMA.[9–11]

Although the nuclear accumulation of the pathogenic AR protein is observed in autopsied myocardium,[7] clinical signs of cardiac disease have not been fully evaluated.[12] In an effort to elucidate the myocardial pathology and its clinical implication in SBMA, we analyzed electrophysiologic, histopathologic, and biochemical properties of the myocardium of SBMA, and

Supplemental data at Neurology.org

From the Department of Neurology (A.A., M.K., K.S., H.B., N.S., A.H., T. Mano, Y.H., H.N., H.W., G.S.), Nagoya University Graduate School of Medicine; Institute for Advanced Research (H.B.), Nagoya University; Department of Speech Pathology and Audiology (M.Y.), Aichi-Gakuin University School of Health Science, Nisshin; Department of Cardiovascular Medicine (T. Makiyama), Kyoto University Graduate School of Medicine; Department of Cardiovascular and Respiratory Medicine (S.O., M.F., M.H.), Shiga University of Medical Science, Ohtsu; and Division of Cardiology (S.-i.M.), Department of Internal Medicine, Fujita Health University School of Medicine, Toyoake, Japan.

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found that subjects with SBMA may show Brugada-type ECG, which is an ECG representation of a coved or saddle-back–type ST-segment elevation in ≥1 lead among the right precordial leads V_{1-3}, and is associated with sudden death.[13,14]

METHODS

Patients. Subjects were 144 consecutive Japanese subjects with SBMA (aged 52.1 ± 10.1 years). We included genetically confirmed male Japanese subjects with more than one of the following symptoms: muscle weakness, muscle atrophy, or bulbar palsy. The subjects were excluded if they met any of the following criteria: (1) severe complications such as malignancy; (2) other neurologic complications; (3) had taken hormonal agents within 48 weeks before informed consent; or (4) participated in any other clinical trials before informed consent. All subjects were outpatients and were followed in Nagoya University Hospital. The data were collected between February 2002 and August 2012.

Evaluation of ECG. Standard 12-lead ECG was recorded from all of the participants and evaluated by 2 independent cardiologists. The diagnosis of the Brugada-type ECG or Brugada syndrome was made on the basis of the second consensus conference.[14] In the subjects with Brugada-type abnormality on standard 12-lead ECG, we also examined ECG in which the right precordial leads were placed to the third intercostal spaces for classifying 3 types of Brugada-type ECGs. We did not perform the sodium channel blocker challenge test in subjects with type 2 or 3 Brugada-type ECG.

Evaluation of disease severity and serologic parameters. We evaluated disease severity using the revised Amyotrophic Lateral Sclerosis Functional Rating Scale, a validated questionnaire-based scale that measures physical function performing activity of daily living as described previously.[15] We defined the onset of disease as the time when muscle weakness began, but not when tremor of the fingers appeared. Serum levels of testosterone were measured by radioimmunoassay in the subjects who were evaluated until 2006 and by chemiluminescent immune assay in the others.

Histopathology. We analyzed autopsied myocardial specimens of 7 genetically diagnosed subjects with SBMA and 4 sex- and age-matched control subjects (aged 66.7 ± 9.7 and 69.5 ± 5.8 years at the time of death, respectively) using immunohistochemistry. Pathologic diagnoses of the controls were malignant lymphoma, dementia with Lewy bodies, corticobasal degeneration, and progressive supranuclear palsy. The myocardia were excised at autopsy and fixed immediately in 10% buffered formalin solution. Sections (6 μm) were deparaffinized, treated with microwave heating for 10 minutes in 10 mM citrate buffer at pH 6.0, and incubated with primary antibodies. Subsequent staining procedures were performed using the Envision+ kit (Dako, Glostrup, Denmark) as described previously.[16] For anti-polyglutamine staining, sections were treated with 98% formic acid at room temperature for 3 minutes before the antigen retrieval with the microwave. We used the following primary antibodies: AR (N-20, 1:1,000; Santa Cruz Biotechnology, Dallas, TX); SCN5A (sc-22758, 1:1,000; Santa Cruz); and polyglutamine 1C2 (MAB1574, 1:20,000; Millipore Corp., Billerica, MA).

Immunoblotting. We analyzed autopsied myocardial specimens of 4 genetically diagnosed subjects with SBMA and 4 sex- and age-matched control subjects (aged 66.8 ± 11.0 and 69.5 ± 5.8 years at the time of death, respectively) using immunoblotting. Autopsied myocardial specimens were homogenized in CelLytic lysis buffer (Sigma-Aldrich, St. Louis, MO) containing a phosphatase inhibitor cocktail (Sigma-Aldrich) and a protease inhibitor cocktail (Thermo Scientific, Waltham, MA). Homogenates were then centrifuged at 2,500*g* for 10 minutes at 4°C. The supernatant fractions were separated on 5% to 20% SDS–polyacrylamide gel electrophoresis gels and transferred to Hybond-P membranes (GE Healthcare, Buckinghamshire, UK). Primary antibody binding was probed using horseradish peroxidase–conjugated secondary antibodies (GE Healthcare) at a dilution of 1:5,000, and bands were detected using the ECL prime kit (GE Healthcare). The immunoblots were digitalized (LAS-3000 imaging system; Fujifilm), signal intensities were quantified with Image Gauge software version 4.22 (Fujifilm), and the means ± SEM were expressed in arbitrary units. The following primary antibodies were used: anti-SCN5A (sc-23174, 1:1,000; Santa Cruz) and anti-GAPDH (MAB374, 1:5,000; Millipore).

Quantitative real-time PCR. Quantification of messenger RNA levels was determined by real-time PCR as described previously.[17] Briefly, total RNA was extracted from autopsied myocardium using RNeasy Fibrous Tissue Midi Kit (Qiagen, Venlo, Limburg). The extracted RNA was reverse-transcribed into first-strand complementary DNA using SuperScript III reverse transcriptase (Invitrogen). Detailed methods for real-time PCR are described in appendix e-1 on the *Neurology*® Web site at Neurology.org.

Genetic analysis. Genomic DNA was extracted from peripheral blood of subjects with SBMA using conventional techniques. PCR amplification of the CAG repeat in the *AR* gene was performed using a fluorescein-labeled forward primer (5′-TCCAGAATCTGTTCCAGAGCGTGC-3′) and a nonlabeled reverse primer (5′-TGGCCTCGCTCAGGATGTCTTTAAG-3′). Detailed PCR conditions were described previously.[18] Aliquots of PCR products were combined with loading dye and separated by electrophoresis with an autoread sequencer (SQ-5500; Hitachi Electronics Engineering, Tokyo, Japan). In subjects with Brugada-type ECGs, we screened for sodium channel, voltage-gated, type V, α subunit (*SCN5A*), calcium channel, voltage-dependent, L type, α 1C subunit (*CACNA1C*), and calcium channel, voltage-dependent, β 2 subunit (*CACNB2*) mutations, which are representative genes linked to Brugada syndrome, using a high-resolution melting method or denaturing high-performance liquid chromatography (WAVE system model 3500; Transgenomic, Omaha, NE) and subsequent direct sequencing, as previously reported.[19,20]

Standard protocol approvals, registrations, and patient consents. This study adhered to the Ethics Guidelines for Human Genome/Gene Analysis Research and those for Epidemiological Studies endorsed by the Japanese government, and was approved by the Ethics Committees of Nagoya University Graduate School of Medicine, Kyoto University Graduate School of Medicine, and Shiga University of Medical Science. All participants provided written informed consent. The collection of autopsied human tissues and their use for this study were also approved by the Ethics Committee of Nagoya University Graduate School of Medicine, and written informed consent was obtained from the subjects' next of kin. Experimental procedures involving human subjects were conducted in conformance with the principles expressed in the Declaration of Helsinki.

Table 1 Clinical features and blood chemical values of subjects with SBMA

	Mean ± SD	Range	No.
Age at examination, y	52.1 ± 10.1	27-75	144
Duration from onset, y	8.7 ± 5.1	1-25	144
Age at onset, y	43.5 ± 9.7	22-69	144
CAG repeat size in *AR* gene	48.1 ± 3.3	40-57	144
ALSFRS-R (normal score = 48)	42.4 ± 3.9	29-48	144
Systolic blood pressure, mm Hg	130.0 ± 18.9	92-190	144
Diastolic blood pressure, mm Hg	81.5 ± 11.8	53-115	144
Hemoglobin A1c (NGSP), %	5.8 ± 0.8	4.6-9.3	144
Total cholesterol, mg/dL	213.9 ± 36.7	140-342	144
Triglyceride, mg/dL	175.2 ± 103.5	49-651	144
Sodium, mEq/L	140.8 ± 1.9	136-146	144
Potassium, mEq/L	4.3 ± 0.4	3.4-5.8	144
Testosterone (RIA), ng/mL	6.5 ± 1.8	2.9-10.2	68
Testosterone (CLIA), ng/mL	7.8 ± 3.0	3.4-15.0	75

Abbreviations: ALSFRS-R = revised Amyotrophic Lateral Sclerosis Functional Rating Scale; AR = androgen receptor; CLIA = chemiluminescent immune assay; NGSP = National Glycohemoglobin Standardization Program; RIA = radioimmunoassay; SBMA = spinal and bulbar muscular atrophy.
Serum levels of testosterone were measured by RIA in the subjects evaluated until 2006 and by CLIA in the others.

Statistical analysis. All data are presented as means ± SD. Intergroup differences in categorical and continuous variables were assessed using the χ^2 test and unpaired t test, respectively. A p value <0.05 was considered to indicate significance. Calculations were performed using the statistical software package SPSS 20.0J (SPSS Japan, Tokyo, Japan).

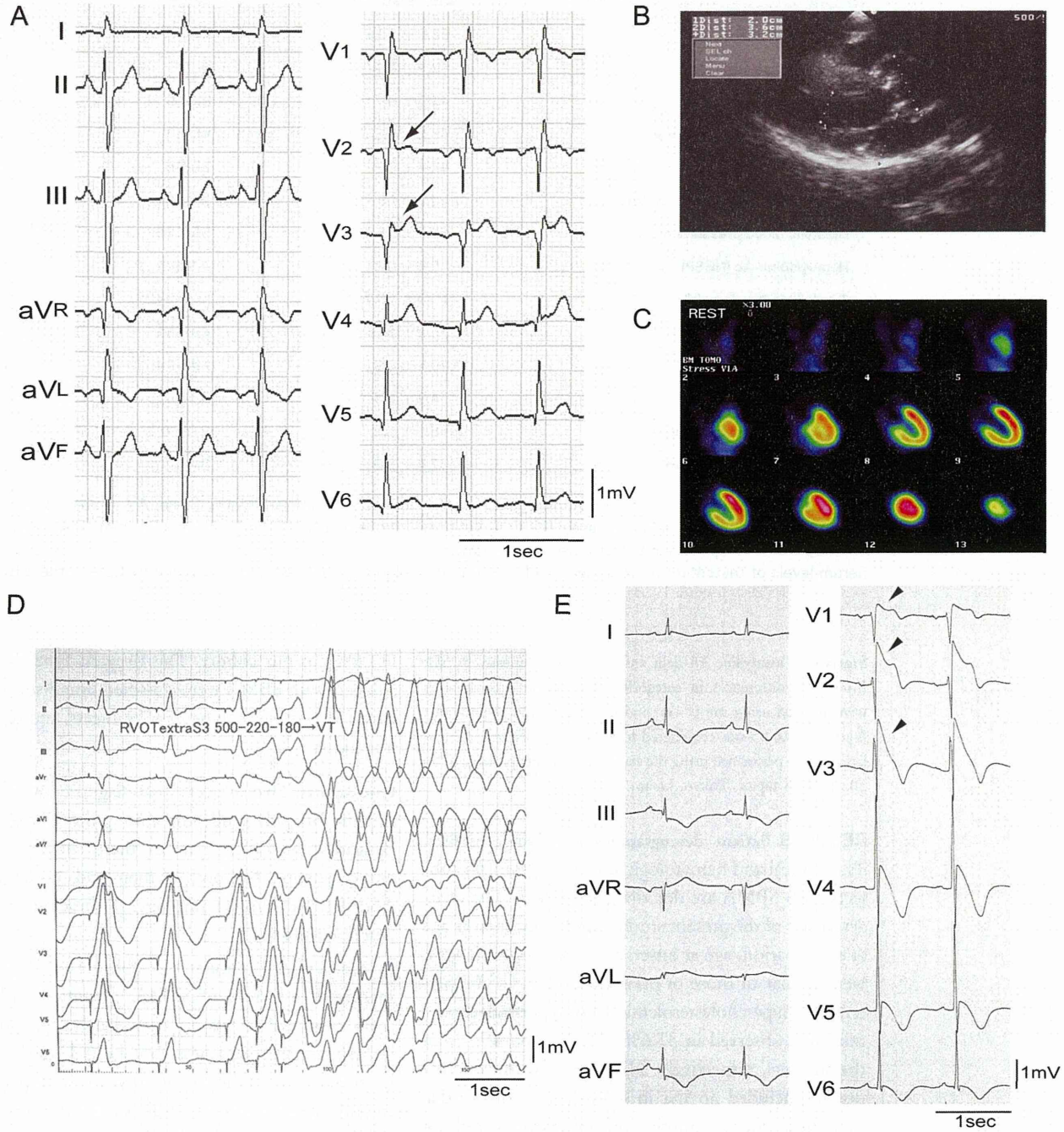
RESULTS Patient demographics and blood profiles. The clinical and hematologic features of the 144 subjects with SBMA are described in table 1. The characteristics of the present study population, such as age at examination, age at onset, and CAG repeat length, were similar to those of previous studies.[21,22] Hypertension, hypercholesterolemia, and hypertriglyceridemia were observed in 37.6%, 41.7%, and 49.3% of the subjects, respectively. Histories of cardiovascular disease included angina in 4 cases, and myocardial infarction, aortic regurgitation, bradycardia, pulmonary artery stenosis with ventricular septal defect, arrhythmia, cardiac hypertrophy, or aortic dissection in one case each.

ECG abnormalities. Seventy (48.6%) of 144 subjects showed an abnormal ECG. The most frequent findings were ST-segment abnormalities in V_{1-3} (19.4%), followed by ST-segment abnormalities in I, aVL, V_{5-6} (18.1%), left ventricular hypertrophies (9.7%), ST-segment abnormalities in II, aVF (7.6%), and right bundle branch blocks (5.6%). Among the ST-segment abnormalities in right precordial leads (V_{1-3}), the most common feature was Brugada-type ECGs, which were detected in 17 of 144 subjects (11.8%) in our cohort. The Brugada-type ECGs in subjects with SBMA were classified into 3 subtypes[13]: type 1 in 6 subjects, type 2 in 7 subjects, and type 3 in 4 subjects.

Case reports. Two subjects were diagnosed with symptomatic Brugada syndrome among the 17 cases with Brugada-type ECG. One of these subjects experienced syncope, and both of them had sudden death. The detailed clinical findings of these subjects are described below.

Case 1. Case 1 (patient 1 in table e-1), a 75-year-old man with a history of SBMA, presented with syncope. His medical history included benign prostatic hyperplasia and glaucoma, but he did not have any heart diseases. At the age of 40 years, he experienced the first syncope and medical examination revealed a right bundle branch block. He noticed muscle weakness of his limbs at age 65 years and was diagnosed with SBMA by genetic screening (CAG repeat length was 46). He had his second syncope after drinking at the age of 78 years but regained consciousness 5 minutes later. His ECG showed type 2 Brugada ECG (figure 1A). Neither his echocardiogram nor iodine-123-β-methyl iodophenyl pentadecanoic acid myocardial scintigram demonstrated any detectable left ventricular dysfunction (figure 1, B and C). There were no abnormalities in heart coronary arteries with contrast, but the electrophysiologic study induced ventricular tachycardia by extra-stimuli from the right ventricular outflow tract, in which the basic

Figure 1 Representative cases of SBMA and Brugada pattern ECG



(A–D) Standard ECG (A), echocardiogram (B), ^{123}I-BMIPP myocardial scintigram (C), and ECG in the electrophysiologic study from the RVOT (D) of case 1. The arrows indicate the saddle-back–type ST-segment elevation that is characteristic of type 2 Brugada ECG (A). (E) Standard ECG of case 2 recorded just before death. The arrowheads indicate the coved-type ST-segment elevation, indicating type 1 Brugada ECG. ^{123}I-BMIPP = iodine-123-β-methyl iodophenyl pentadecanoic acid; RVOT = right ventricular outflow tract; SBMA = spinal and bulbar muscular atrophy.

ventricular stimulation period was 500 milliseconds, and 2 consecutive stimulation periods were 220 and 180 milliseconds (figure 1D). He received an implantable cardioverter defibrillator under the diagnosis of Brugada syndrome, but it was removed 2 years later because of a bacterial infection. At age 82 years, he was hospitalized for pneumonia and dehydration, and died suddenly when he was sleeping at night. His last temperature was 37.5°C. His sodium and potassium levels on the date of death were 164 and 3.6 mEq/L (normal ranges were 138–146 and 3.6–4.9 mEq/L), respectively. No autopsy was performed on this subject.

Case 2. Case 2 (patient 2 in table e-1), a 68-year-old man with a history of SBMA, presented with sudden death. His medical history was unremarkable except for high blood pressure, diabetes mellitus, traumatic subarachnoid hemorrhage, and spasmodic deafness.

He noticed dysphagia and muscle weakness of his limbs at the age of 50 years. He was diagnosed with SBMA by genetic screening (CAG repeat length was 50). At age 70 years, he suddenly lost consciousness at the moment when he moved to a dining table with the assistance of his family at night, following loss of appetite for 1 week. His sodium and potassium levels were 115 and 3.8 mEq/L, respectively. His ECG showed pulseless electrical activity with ST elevations in V_{1-4} (type 1 Brugada ECG) (figure 1E). Despite cardiopulmonary resuscitations, he died 23 minutes after the onset of unconsciousness. A mild bronchial pneumonia, a mild ischemia-related heart change, and arteriosclerosis of coronary arteries were indicated at autopsy, but histopathologic analysis did not identify the direct cause of death. Acute heart failure due to arrhythmia was suspected as the cause of death.

Clinical features of the subjects with Brugada-type ECGs. Longer CAG repeat sizes and higher serum testosterone levels tended to occur in subjects with Brugada-type ECGs compared with those without Brugada-type ECGs, but there was no statistically significant difference between the groups in clinical features and blood chemical values (table 2). PQ and QRS intervals were also equivalent between the groups. The details of 17 subjects with SBMA and Brugada-type ECG are shown in table e-1. One of 14 subjects who underwent echocardiography had a right ventricular overload and mild tricuspid regurgitation, whereas no evidence of myocardial dysfunction was found in the remaining 13 subjects. None of the 17 subjects with Brugada-type ECGs had family histories of sudden cardiac deaths at an age younger than 45 years, symptomatic bradycardia, or coved-type ECGs in their family members. Genetic analysis demonstrated no known gene mutations in conjunction with Brugada syndrome in the 17 subjects, including *SCN5A*, *CACNA1C*, and *CACNB2* genes.

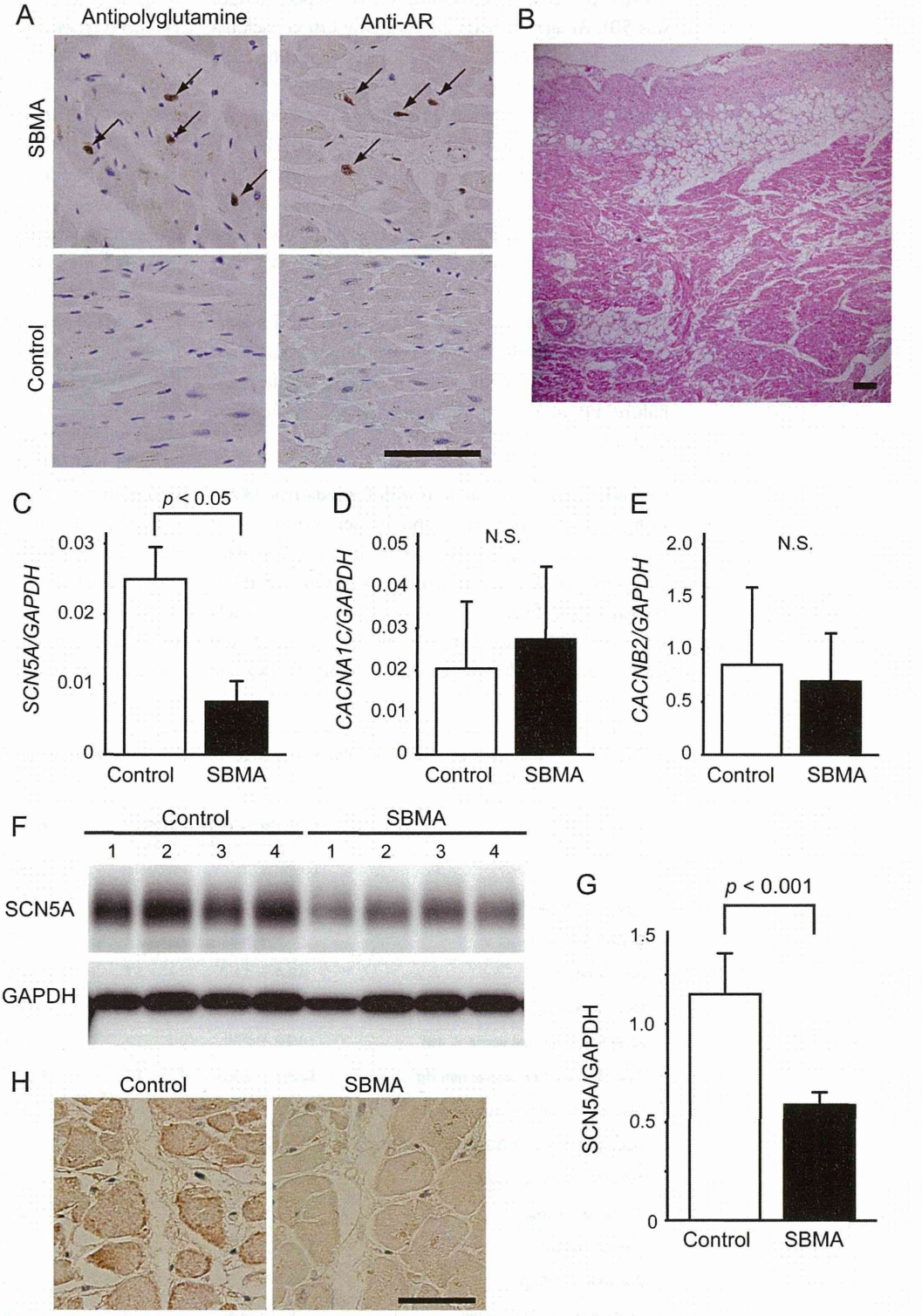
Histopathologic and biochemical study. Histopathologic analyses of the myocardium revealed nuclear accumulation of the pathogenic AR in all 7 autopsied subjects with SBMA (figure 2A), of which one had symptomatic Brugada syndrome as described above (case 2). Hematoxylin & eosin staining showed no detectable abnormalities, except for one subject with increased fat tissue within the right ventricle myocardium, which was reported previously in subjects with Brugada syndrome (figure 2B).[23] To elucidate the molecular basis of Brugada-type ECG in SBMA, we

Table 2 Comparison of clinical, blood chemical, and ECG findings of subjects with SBMA with or without Brugada ECG

	With Brugada-type ECG		Without Brugada-type ECG		
	Mean ± SD	No.	Mean ± SD	No.	p
Age at examination, y	51.5 ± 13.6	17	52.2 ± 9.6	127	NS
Duration from onset, y	8.2 ± 5.2	17	8.7 ± 5.1	127	NS
Age at onset, y	43.4 ± 11.0	17	43.5 ± 9.5	127	NS
CAG repeat size in *AR* gene	49.5 ± 3.9	17	47.9 ± 3.2	127	NS
ALSFRS-R (normal score = 48)	43.2 ± 2.7	17	42.3 ± 4.0	127	NS
Systolic blood pressure, mm Hg	133.1 ± 28.0	17	129.6 ± 17.5	127	NS
Diastolic blood pressure, mm Hg	83.1 ± 14.9	17	81.3 ± 11.4	127	NS
Hemoglobin A1c (NGSP), %	6.0 ± 1.0	17	5.8 ± 0.8	127	NS
Total cholesterol, mg/dL	204.4 ± 40.8	17	215.1 ± 36.1	127	NS
Triglyceride, mg/dL	146.2 ± 70.8	17	179.1 ± 106.7	127	NS
Sodium, mEq/L	140.7 ± 2.3	17	140.9 ± 1.8	127	NS
Potassium, mEq/L	4.3 ± 0.5	17	4.3 ± 0.3	127	NS
Testosterone (RIA), ng/mL	6.3 ± 2.4	8	6.6 ± 1.8	60	NS
Testosterone (CLIA), ng/mL	9.5 ± 3.2	9	7.6 ± 2.9	66	NS
Heart rate, bpm	77.9 ± 17.6	17	72.4 ± 12.5	127	NS
PQ interval, ms	163 ± 17	17	157 ± 21	127	NS
QRS interval, ms	107 ± 13	17	104 ± 11	127	NS

Abbreviations: ALSFRS-R = revised Amyotrophic Lateral Sclerosis Functional Rating Scale; AR = androgen receptor; bpm = beats per minute; CLIA = chemiluminescent immune assay; NGSP = National Glycohemoglobin Standardization Program; NS = not significant; RIA = radioimmunoassay; SBMA = spinal and bulbar muscular atrophy.
Serum levels of testosterone were measured by RIA in the subjects evaluated until 2006 and by CLIA in the others.

Figure 2 Histopathologic and biochemical features of myocardium from subjects with SBMA



(A) Immunohistochemistry using antibodies against AR and expanded polyglutamine and hematoxylin & eosin staining of the myocardium of representative subjects. Arrows indicate the nuclear accumulation of the pathogenic AR in SBMA. (B) Fat deposition in the right ventricle myocardium in one subject with SBMA. (C–E) The messenger RNA levels of *CACNA1C* (C), *CACNB2* (D), and *SCN5A* (E) measured with quantitative real-time PCR. (F) Immunoblots of SCN5A and GAPDH in myocardium from SBMA and control subjects. (G) Quantification of anti-SCN5A immunoreactive bands. The amount of protein is demonstrated as the ratio for GAPDH. (H) Immunohistochemistry using antibodies against SCN5A in the myocardium from SBMA and control subjects. Scale bars indicate 50 μm. AR = androgen receptor; *CACNA1C* = calcium channel, voltage-dependent, L type, α 1C subunit; *CACNB2* = calcium channel, voltage-dependent, β 2 subunit; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; SBMA = spinal and bulbar muscular atrophy; *SCN5A* = sodium channel, voltage-gated, type V, α subunit.

investigated the gene expression of ion channels that are firmly linked to Brugada syndrome. The results of quantitative real-time PCR showed a marked decrease in messenger RNA levels of *SCN5A* but not *CACNA1C* or *CACNB2* in the myocardium of subjects with SBMA (figure 2, C–E). Decreased protein levels of SCN5A were also detected by immunoblot (figure 2, F and G) and immunohistochemistry (figure 2H).

DISCUSSION In the present study, we demonstrated that subjects with SBMA exhibit several forms of ECG abnormalities. The most striking observation in our cohort is that Brugada-type ECG was observed in more than 10% of the subjects with SBMA. Of those, 2 subjects reported symptomatic Brugada syndrome, leading to sudden death. Although sudden death by neuronal loss in the intermediolateral nucleus was reported in amyotrophic lateral sclerosis,[24] there are no reports of Brugada syndrome, suggesting that this type of ECG appears to be specific to SBMA.

Brugada syndrome may cause sudden death by ventricular fibrillation at an average age of 41 years, without structural heart problems.[14,25] This syndrome may be responsible for 4% to 12% of all sudden deaths, and at least 20% of sudden deaths in patients with structurally normal hearts. Brugada-type ECG is observed frequently in asymptomatic patients.[26] Given that the frequency of Brugada-type ECG is reported to be 0.15% to 1.22% in Japanese community-based populations,[27,28] our study suggests a very high prevalence of Brugada-type ECGs in SBMA and implies a link between the pathogenesis of the disorders, although we cannot fully exclude the possibility of a chance association of these 2 conditions. In this regard, it is intriguing that SBMA and Brugada syndrome share common clinical features. Both diseases are characterized by male predominance. SBMA affects males exclusively, whereas females with homozygous mutation of the *AR* gene manifest no neuromuscular phenotype.[29] Similarly, Brugada syndrome is known to show a high male predominance (80%–90%).[14] The pathologic process of SBMA is dependent on circulating levels of testosterone,[5,30–32] and high testosterone levels increase the risk of Brugada syndrome via intensifying ionic current imbalance in the myocardium.[33] These commonalities may underlie the high prevalence of Brugada-type ECGs in SBMA. Although another epidemiologic study in 25 European patients with SBMA showed no ECG abnormalities,[34] the discrepancy between this and our studies may be attributable to the difference in the sample size and ethnic background. Brugada syndrome is more common in Asian than Caucasian populations,[14,25,35] which may contribute to the high occurrence of Brugada-type ECGs in Japanese subjects with SBMA in the present study.

Typical Brugada-type ST elevation (J point elevation) results from an ion current imbalance across cardiac cell membrane, which is induced by the malfunction of cardiac ion channels governing the action potential. Mutations in genes encoding these ion channels or their modulating proteins have been shown to be causative of Brugada syndrome. Among them, mutations in *SCN5A*, which encodes the α subunit of cardiac sodium channel, have been reported in 10% to 30% of patients with Brugada syndrome.[14] Although much less frequent, several other causative genes have also been identified, including the L-type calcium channel (*CACNA1C* and *CACNB2*), glycerol-3-phosphate dehydrogenase 1-like channel, and potassium channels. Recently, a genome-wide association analysis also identified susceptibility loci at *SCN5A*, *SCN10A*, and the *HEY2* genes for Brugada syndrome.[36] Although none of our subjects with Brugada-type ECG had mutations in *SCN5A*, *CACNA1C*, or *CACNB2*, histopathologic and biochemical analyses indicated downregulation of *SCN5A* gene expression in the myocardium of subjects with SBMA. Given that the mutations in the *SCN5A* gene are shown to culminate in functional loss of the sodium channels,[37] decreased expression of this gene in the myocardium may be associated with the pathogenesis of myocardial involvement in SBMA. Nonneuronal cells, such as hepatocytes and epithelial cells of scrotal skin, show the accumulation of the pathogenic AR, which, at least partially, corresponds to clinical symptoms and findings of SBMA.[8] Nuclear accumulation of the pathogenic AR induces transcriptional dysregulation, leading to altered expression of several genes in skeletal muscles, as well as the spinal cord.[38,39] Taken together, gene expression abnormalities due to the nuclear accumulation of pathogenic AR may result in myocardial dysfunction in SBMA.

In the present study, we detected no differences in clinical and genetic features between SBMA subjects with and without Brugada-type ECG, but severe hyponatremia in the subject with symptomatic Brugada syndrome (case 2) suggests that patients with SBMA have the potential risk of Brugada syndrome at basal conditions and that certain triggers (such as hyponatremia) may enhance the electrophysiologic deficits in the myocardium and manifest Brugada-type ECGs or fatal ventricular arrhythmias. This hypothesis is consistent with the observation that Brugada ECG patterns may be modulated by numerous factors, including the autonomic nervous system, altered levels of electrolytes, heavy meals, and high body temperature.[26] In particular, hypokalemia in conjunction with hyponatremia is reported to

precipitate Brugada syndrome, as shown in the present study.[40]

Although respiratory tract infections due to bulbar palsy are common at advanced stages of SBMA, the cause of death is not determined in a certain population.[21] Our results indicate that sudden death due to fatal arrhythmias associated with Brugada syndrome may occur in patients with SBMA. Because subjects with Brugada type 1 ECG are often symptomatic and at a high risk of sudden death, it is important to examine ECGs in patients with SBMA and to carefully manage subjects with Brugada-type ECG abnormalities.

AUTHOR CONTRIBUTIONS

A.A.: drafting the manuscript, analysis/interpretation of the data, acquisition of data, statistical analysis, research project execution, study design and concept. M.K.: revising the manuscript, analysis/interpretation of the data, acquisition of data, statistical analysis, research project execution, study design and concept. K.S., H.B., N.S., A.H., T. Mano, and Y.H.: analysis/interpretation of the data, acquisition of data. H.N.: analysis/interpretation of the data. H.W.: analysis/interpretation of the data, research project execution. M.Y.: analysis/interpretation of the data, acquisition of data. T. Makiyama, S.O., and M.F.: acquisition of data, analysis/interpretation of the data. S.M.: analysis/interpretation of the data, research project execution. M.H.: analysis/interpretation of the data, research project execution, study design and concept. G.S.: research project organization, research project execution, revising the manuscript, interpretation of the data, statistical analysis, study design and concept.

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Paeoniflorin eliminates a mutant AR via NF-YA-dependent proteolysis in spinal and bulbar muscular atrophy

Genki Tohnai[1,†], Hiroaki Adachi[1,†,*], Masahisa Katsuno[1], Hideki Doi[1], Shinjiro Matsumoto[1], Naohide Kondo[1], Yu Miyazaki[1], Madoka Iida[1], Hideaki Nakatsuji[1], Qiang Qiang[1], Ying Ding[1], Hirohisa Watanabe[1], Masahiko Yamamoto[2], Kenzo Ohtsuka[3] and Gen Sobue[1,*]

[1]Department of Neurology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan, [2]Department of Speech Pathology and Audiology, Aichi-Gakuin University School of Health Science, 12 Araike, Iwasaki-cho, Nisshin 470-0195, Japan and [3]Laboratory of Cell and Stress Biology, Department of Environmental Biology, Chubu University, 1200 Matsumoto-cho, Kasugai, Aichi 487-8501, Japan

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The accumulation of abnormal proteins is a common characteristic of neurodegenerative diseases. This accumulation reflects a severe disturbance of cellular homeostasis in pathogenic protein clearance. Here, we demonstrated that the activation of the two major proteolytic machineries, the molecular chaperone–ubiquitin proteasome system (UPS) and the autophagy system, were simultaneously enhanced by paeoniflorin (PF), a major component of *Paeonia* plants, and exerted therapeutic effects in models of spinal and bulbar muscular atrophy (SBMA). PF significantly increased the expression of nuclear factor-YA (NF-YA), which strongly upregulated the molecules involved in the proteolytic machinery [molecular chaperones, carboxyl terminus of Hsc70-interacting protein and transcription factor EB], which thus mitigated the behavioral and pathological impairments in an SBMA mouse model through the upregulation of pathogenic androgen receptor protein clearance in motor neurons and muscles. These findings demonstrated that PF is able to enhance both the UPS and autophagy systems by upregulating the expression of NF-YA, which promotes therapeutic effects in an SBMA model.

INTRODUCTION

Polyglutamine (polyQ) diseases are inherited neurodegenerative disorders caused by the expansion of a trinucleotide CAG repeat in the causative genes. To date, nine disorders have been identified as polyQ diseases (1). The expanded polyQ stretch is thought to confer a toxic gain of function to the mutant protein. Spinal and bulbar muscular atrophy (SBMA) is a motor neuron disease caused by a polyQ tract within the androgen receptor (AR) (2). SBMA is characterized by motor neuron loss in the spinal cord and brainstem that is accompanied by diffuse nuclear accumulation and nuclear inclusions (NIs) of the mutant AR in motor neurons and specific visceral organs (3,4). SBMA patients also show myogenic changes together with the neurogenic atrophy according to muscle biopsies (5). SBMA patients gradually develop progressive weakness of the bulbar and extremity muscles, and muscle strength and function are inversely correlated with the CAG repeat length, age and duration of weakness (6,7). Nuclear and cytoplasmic inclusions are common pathological features in polyQ diseases. The inclusions are colocalized with many components of molecular chaperones, the ubiquitin proteasome system (UPS) and the autophagy system, raising the possibility that this proteolytic machinery may actively degrade components of these inclusions (8). Molecular chaperones facilitate the refolding and proteolysis of toxic,

* To whom correspondence should be addressed at: Department of Neurology, Nagoya University Graduate School of Medicine, 65, Tsurumai-Cho, Showa-Ku, Nagoya, 466-8550, Japan. Tel: +81 527442391; Fax: +81 527442394; Email: hadachi-ns@umin.org (H.A.); Department of Neurology, Nagoya University Graduate School of Medicine, 65, Tsurumai-Cho, Showa-Ku, Nagoya, 466-8550, Japan. Tel: +81 527442385; Fax: +81 527442384; Email: sobueg@med.nagoya-u.ac.jp (G.S.)
†The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors.