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CLINICAL PERSPECTIVE

Calmodulin is an essential Ca^{2+}-binding protein that transduces intracellular Ca^{2+} signals to influence activity of ion channels, kinases, and other target proteins necessary for cardiac function. The essential nature of calmodulin is emphasized by its conservation and redundancy among vertebrates and in the recent discovery of calmodulin mutations in life-threatening genetic arrhythmia syndromes. In humans, 3 unique genes (*CALM1*, *CALM2*, *CALM3*) encode for identical calmodulin proteins, and mutations have been described in 2 of these genes (*CALM1*, *CALM2*) associated with either congenital long-QT syndrome or catecholaminergic polymorphic ventricular tachycardia. Previously, only 1 mutation was identified in *CALM2* and here we contribute additional genotype–phenotype correlation with discovery of 5 novel mutations in this gene. All mutations were de novo, altered highly conserved amino acid residues within Ca^{2+}-binding domains located in the carboxyl-terminal half of calmodulin and caused significant impairment of ion binding. Phenotypes of the mutation-positive subjects shared prolonged QTc intervals and a tendency for cardiac events (eg, syncope, cardiac arrest) to occur during physical exertion. Two of the 5 novel *CALM2* mutation-positive subjects also exhibited features consistent with catecholaminergic polymorphic ventricular tachycardia. Importantly, all subjects with *CALM2* mutations received β-adrenergic receptor blockers, and this therapy was successful in preventing life-threatening exertion-triggered arrhythmias. Calmodulin gene mutations should be sought in pediatric cases of long-QT syndrome and catecholaminergic polymorphic ventricular tachycardia for whom other genetic candidates have been excluded.

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Novel Calmodulin Mutations Associated With Congenital Arrhythmia Susceptibility

Naomasa Makita, Nobue Yagihara, Lia Crotti, Christopher N. Johnson, Britt-Maria Beckmann, Michelle S. Roh, Daichi Shigemizu, Peter Lichtner, Taisuke Ishikawa, Takeshi Aiba, Tessa Homfray, Elijah R. Behr, Didier Klug, Isabelle Denjoy, Elisa Mastantuono, Daniel Theisen, Tatsuhiko Tsunoda, Wataru Satake, Tatsushi Toda, Hidewaki Nakagawa, Yukiomi Tsuji, Takeshi Tsuchiya, Hirokazu Yamamoto, Yoshihiro Miyamoto, Naoto Endo, Akinori Kimura, Kouichi Ozaki, Hideki Motomura, Kenji Suda, Toshihiro Tanaka, Peter J. Schwartz, Thomas Meitinger, Stefan Kääb, Pascale Guicheney, Wataru Shimizu, Zahurul A. Bhuiyan, Hiroshi Watanabe, Walter J. Chazin and Alfred L. George, Jr

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Original Article

Novel Mutation in the α-Myosin Heavy Chain Gene Is Associated With Sick Sinus Syndrome

Taisuke Ishikawa, DVM, PhD; Chuanchau J. Jou, DO, PhD; Akihiko Nogami, MD, PhD; Shinya Kowase, MD; Cammon B. Arrington, MD, PhD; Spencer M. Barnett, BS; Daniel T. Harrell, BS; Takuro Arimura, DVM, PhD; Yukiomi Tsuji, MD, PhD; Akinori Kimura, MD, PhD; Naomasa Makita, MD, PhD

Background—Recent genome-wide association studies have demonstrated an association between *MYH6*, the gene encoding α-myosin heavy chain (α-MHC), and sinus node function in the general population. Moreover, a rare *MYH6* variant, R721W, predisposing susceptibility to sick sinus syndrome has been identified. However, the existence of disease-causing *MYH6* mutations for familial sick sinus syndrome and their underlying mechanisms remain unknown.

Methods and Results—We screened 9 genotype-negative probands with sick sinus syndrome families for mutations in *MYH6* and identified an in-frame 3-bp deletion predicted to delete one residue (delE933) at the highly conserved coiled-coil structure within the binding motif to myosin-binding protein C in one patient. Co-immunoprecipitation analysis revealed enhanced binding of delE933 α-MHC to myosin-binding protein C. Irregular fluorescent speckles retained in the cytoplasm with substantially disrupted sarcomere striation were observed in neonatal rat cardiomyocytes transfected with α-MHC mutants carrying delE933 or R721W. In addition to the sarcomere impairments, delE933 α-MHC exhibited electrophysiological abnormalities both in vitro and in vivo. The atrial cardiomyocyte cell line HL-1 stably expressing delE933 α-MHC showed a significantly slower conduction velocity on multielectrode array than those of wild-type α-MHC or control plasmid transfected cells. Furthermore, targeted morpholino knockdown of *MYH6* in zebrafish significantly reduced the heart rate, which was rescued by coexpressed wild-type human α-MHC but not by delE933 α-MHC.

Conclusions—The novel *MYH6* mutation delE933 causes both structural damage of the sarcomere and functional impairments on atrial action propagation. This report reinforces the relevance of *MYH6* for sinus node function and identifies a novel pathophysiology underlying familial sick sinus syndrome. **(*Circ Arrhythm Electrophysiol.* 2015;8:00-00. DOI: 10.1161/CIRCEP.114.002534.)**

Key words: genetics ■ MYH6 ■ myosin heavy chain ■ sick sinus syndrome ■ sinus node dysfunction

Sick sinus syndrome (SSS) is a common arrhythmia often associated with aging, structural heart diseases, or surgical injury, but can also occur in a familial form.[1] Several studies have demonstrated genetic mutations in both sporadic and familial cases of SSS.[2–4] Affected ion channel or ion channel–associated genes identified to date include sodium channel, Nav1.5 (*SCN5A*),[2] ankyrin-B (*ANK2*),[3] and hyperpolarization-activated channel (*HCN4*).[4] Mutations in *HCN4* result in sinus node dysfunction caused by a reduction of the pacemaker current, whereas *SCN5A* mutations lead to conduction delay within the sinus node or exit block.[5]

MYH6 and *MYH7* encode the homologous myosin heavy chain (MHC) isoforms α-MHC and β-MHC, respectively, in cardiomyocytes, which play pivotal roles in the organization of sarcomeric structures and muscle contraction.[6–8] *MYH7* is predominantly expressed in the adult ventricle, whereas *MYH6* is mainly expressed in the fetal heart and adult atrium.[9] *MYH7* is a well-established causative gene with over 300 mutations responsible for hypertrophic cardiomyopathy and dilated cardiomyopathy,[10,11] whereas more limited *MYH6* mutations have been reported in cardiomyopathy[12,13] and congenital heart disease, such as atrial septal defect.[7,14–17] On the contrary, recent genome-wide association studies demonstrated that a common nonsynonymous variant A1101V in *MYH6* was associated with an increased resting heart rate,[17–19] whereas another rare nonsynonymous variant (resulting in R721W) was associated

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From the Department of Molecular Physiology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki (T.I., D.T.H., Y.T., N.M.); Department of Molecular Pathogenesis, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan (T.I., T.A., A.K.); Division of Pediatric Cardiology, University of Utah, Salt Lake City, UT (C.J.J., C.B.A., S.M.B.); Cardiovascular Division, University of Tsukuba, Tsukuba (A.N.); Department of Heart Rhythm Management, Yokohama Rosai Hospital, Yokohama (A.N., S.K.); and Department of Veterinary Medicine, Kagoshima University, Kagoshima, Japan (T.A.).

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Correspondence to Naomasa Makita, MD, PhD, Department of Molecular Physiology, Nagasaki University, Graduate School of Biomedical Sciences, 1-12-4 Sakamoto, Nagasaki 852–8523, Japan. E-mail makitan@nagasaki-u.ac.jp or Akinori Kimura, MD, PhD, Department of Molecular Pathogenesis, Medical Research Institute, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113–8510, Japan. E-mail akitis@mri.tmd.ac.jp

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WHAT IS KNOWN

- Sick sinus syndrome (SSS) is often associated with aging and structural heart diseases, but it may occur in a familial form.
- Recent genome-wide association studies uncovered MYH6 encoding atrial myosin heavy chain as a susceptibility gene for heart rate and SSS; however, its underlying mechanisms and the existence of causative mutations for SSS remain unknown.
- Here, we report a novel *MYH6* mutation delE933 in an SSS patient who has a family history of SSS.

WHAT THE STUDY ADDS

- When expressed in cardiomyocytes, delE933-*MYH6* impaired the atrial action potential propagation and disrupted sarcomere integrity consistent with the R721W-*MYH6*, a high risk genetic predisposition for SSS demonstrated in Icelanders.
- Our data reinforces the relevance of *MYH6* of sinus node function and suggested that structural damages of the sarcomere and functional impairments on atrial action potential propagation may underlie familial SSS with *MYH6* mutations.

with a high risk of SSS.[20] Moreover, heterozygous zebrafish carrying the *MYH6* mutation N695K (*MYH6*$^{hu/423/+}$) displayed partial atrial contractile defects.[21] Based on these observations, it is conceivable that some *MYH6* variations impair AQ8 the sarcomere structure or function of the atrium, which in turn would cause electrophysiological abnormalities and sinus node dysfunction. However, it remains to be elucidated whether (1) *MYH6* is the causative gene for familial SSS and (2) the genetic variations of *MYH6* associated with SSS confer pacemaker dysfunction through structural damage of the sarcomere of the atrial muscle surrounding the sinus node or AQ9 by functional impairment of the pacemaker channel or sodium channel. The present study identified a novel *MYH6* mutation in one SSS proband and investigated the means by which this could confer sinus node dysfunction.

Methods

Genetic Screening of *MYH6* Mutations

We previously performed genetic screening of mutations in *SCN5A* and *HCN4* in 15 probands afflicted with familial SSS and found 6 distinct *SCN5A* mutations.[22] In this study, we enrolled 9 SSS families out of this cohort, which were free from *SCN5A* or *HCN4* mutations. Age at diagnosis of the probands (3 male and 6 female) ranged from 3 to 65 years old (44.6±21.8 years old; mean±SD).

Genomic DNA was extracted from peripheral blood of each subject using standard methods. Coding regions of *MYH6* were amplified by polymerase chain reaction using exon-flanking intronic primers (Table I in the Data Supplement). Direct DNA sequencing was performed using ABI 3130 genetic analyzers (Life Technologies, Carlsbad, CA). Mutations were validated by the analysis of unrelated 400 healthy Japanese individuals and dbSNP, 1000 Genome Project, AQ10 Exome Variant Server, and Human Genetic Variation Database (HGVD, Japanese variation database, http://www.genome.med.kyoto-u.ac.jp/SnpDB/). All probands and family members who participated in the study gave their written informed consent in accordance with the Declaration of Helsinki. The research protocol was approved by the Ethics Review Committee of Nagasaki University and the Ethics Review Committee of Medical Research Institute, Tokyo Medical and Dental University.

Alignment of Amino Acid Sequences and Structural Prediction of α-MHC

Amino acid sequence of human α-MHC was aligned using NCBI AQ11 HomoloGene program with those of other species, and the phylogenetic conservations were testified among human MHC isoforms (the GenBank accession number of each gene is listed in Tables II and III in the Data Supplement). Alterations of the coiled-coil structure of the α-MHC were predicted in silico by using SWISS-MODEL (http://swissmodel.expasy.org/) and visualized by a software RasTop (http://www.geneinfinity.org/rastop/).

Plasmids and cRNA preparation

A 5.8 kb cDNA fragment of human *MYH6* was obtained by reverse transcription-polymerase chain reaction from human heart RNA using a primer pair *MYH6*-F-EcoRV and *MYH6*-R-Sal1 (Table I in the Data Supplement) and was cloned into pEGFP-C1 (Takara Bio, Shiga, Japan) to make green fluorescent protein (GFP)-tagged *MYH6* plasmid (pEGFP-*MYH6*). Mutant *MYH6* plasmids of R721W (c.2161C>T) and delE933 (c.2797_2799delGAG) were constructed using an overlap-extension polymerase chain reaction strategy.

To assess the binding affinity of the mutant S2 region of α-MHC to myosin-binding protein C (MyBP-C) on the basis of the previous report,[8] cDNAs corresponding to the binding regions for human α-MHC (S2 region; aa. 884–965 of NP_002462) and human MyBP-C (C1C2 region; aa. 256–363 of NP_000247) were amplified and cloned into the c-myc-tag plasmid pCMV-Tag3B (Takara Bio; pCMV3B-*MYH6*-S2) and the pEGFP-C1 (pEGFP-*MYBPC3*-C1C2), respectively. All constructs were sequenced to ensure that no errors were introduced.

For the zebrafish experiments, wild-type (WT) and mutant *MYH6* cDNA fragments were, respectively, cloned into pIRES2-EGFP vector (Takara Bio; pIRES2-EGFP-*MYH6*) and pCS2+ vector[23] (pCS2-*MYH6*) by using specific primer pairs (Table I in the Data Supplement). cRNAs of human *MYH6* were synthesized using the mMessage mMachine in vitro transcription kit (Life Technologies) and purified as described previously.[24] Purified mutant cRNAs were sequenced by the University of Utah sequencing core facility.

Coimmunoprecipitation Assay

HeLa cells were cotransfected with pEGFP-*MYBPC3*-C1C2 and pCMV3B-*MYH6*-S2 using Transfectin lipid reagent (BioRad, Hercules, CA). After 48 hours of the transfection, cells were lysed in TNE buf- AQ12 fer (1% Nonidet P-40, 1 mmol/L EDTA, 150 mmol/L NaCl, and 10 mmol/L Tris-HCl, pH 7.8) containing Protease Inhibitor Cocktail. Total cellular lysate was obtained by centrifugation at 13 000*g* for 5 minutes, and its protein concentration was measured by BCA protein assay (Thermo Fisher Scientific, Waltham, MA). Coimmunoprecipitation assay was performed using equal amount of cellular lysate with goat anti-myc polyclonal antibody (Sigma-Aldrich, St. Louis, MO) using the Catch and Release version 2.0 reversible immunoprecipitation system (Millipore, Billerica, MA). Immunoprecipitates were separated on a 9% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. After blocking with 5% skim milk in PBS, membranes were incubated with primary anti-GFP monoclonal antibody (1:100, Santa Cruz Biotechnology, Dallas, TX) overnight at 4°C and rabbit-anti mouse IgG HRP-conjugated antibody (Dako, Grostrup, Denmark) AQ13 for 1 hour at RT. Signals were visualized by Immobilon Western Chemiluminescent HRP Substrate (Millipore) and Luminescent Image Analyzer LAS-3000 mini (Fujifilm, Tokyo, Japan).

Immunofluorescence Study

Immunohistological study was performed using neonatal rat ventricular cardiomyocytes prepared from 1-day-old Sprague–Dawley rats as described previously.[25] Briefly, neonatal rat ventricular cardiomyocytes (4×10^4) were transfected with WT or mutant pEGFP-*MYH6* plasmid with Lipofectamine LTX. Twenty-four hours later, the cells were fixed with 100% ethanol, stained by primary mouse anti-α-actinin antibody (1:100, Sigma-Aldrich) overnight at 4°C and visualized with secondary Alexa Fluor 568 goat antimouse IgG antibody (1:500, Life Technologies). The fluorescent images were analyzed using LSM510 laser-scanning confocal microscope with a 63× oil immersion objective lens (Carl-Zeiss Microscopy, Jena, Germany).

All care and treatment of animals were in accordance with the guidelines for the Care and Use of Laboratory Animals published by the National Institute of Health (NIH Publication, eighth edition 2011) and subjected to prior approval by the animal protection authorities of Nagasaki University and Tokyo Medical and Dental University.

Action Potential Propagation Velocity Measurements in HL-1 Cells Stably Expressing Human *MYH6*

The mouse atrial cardiomyocyte cell line HL-1 (4×10^5), gift from Dr Claycomb, was cultured as previously described.[26] Cells were transfected with 2 μg of linearized pIRES2-EGFP-*MYH6* plasmids of WT or delE933 or pIRES2-EGFP plasmid and 4 μl of Lipofectamine LTX (Life Technologies) according to the manufacturer's instructions. Forty-eight hours after transfection, cells were cultured in the presence of 400 μg/mL G418 (Life Technologies) for 4 weeks to establish stable cell lines.

Stable HL-1 cells (1×10^5 cells) expressing WT-*MYH6*, delE933-*MYH6*, or mock pIRES2-EGFP were plated on 8×8 planner multielectrode arrays (array size 1 mm×1 mm; electrode diameter 50 μm; Alpha MED Scientific Inc., Osaka, Japan) precoated with gelatin and fibronectin (Sigma-Aldrich). Seventy-two hours later, a single stimulus of 10 μA was applied on a designated point to initiate spontaneous beating spontaneous, and electric field potentials were recorded for 1 minute. Action potential propagation velocity was calculated by averaging the velocities between the stimulation point and the remaining 63 points. Cell numbers on the array were counted after recordings with detaching them from the arrays with Trypsin-EDTA. These procedures were repeated 4 times for each line. AQ14 AQ15

In Vivo Evaluation of Overexpressed *MYH6* in Zebrafish

Transgenic zebrafish (cmlc2:GFP, *Danio rerio*) embryos were used to functionally characterize the zebrafish *myh6* and human *MYH6* variant. *MYH6* ATG-blocking morpholino antisense oligonucleotide (myh6 ATG-MO) was designed to target *myh6* (Table I in the Data Supplement).[27] Myh6 ATG-MO (0.5–1 ng/embryo) was injected alone or coinjected with WT or delE933 *MYH6* cRNA (0.4 ng/embryo) at the 1- to 2-cell stage. After the injection, embryos were maintained in embryo water at 28°C and staged according to age and morphological criteria.[28] Cardiac phenotypes were screened using

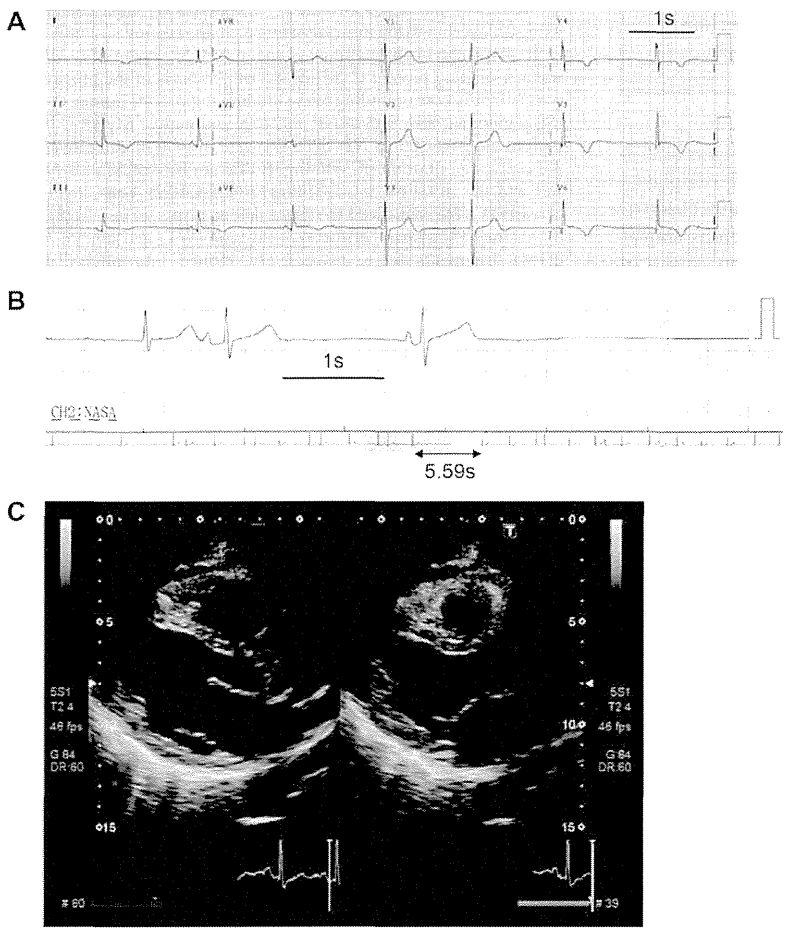

Figure 1. ECG and echocardiography of the sick sinus syndrome (SSS) proband. **A**, ECG recordings of the proband (age 62 years) displayed sinus bradycardia (42 beats per minute) with unusual P wave axis and junctional escape beat (last beat in V4-V6). T waves in I-III, aVF, and V4-6 were inverted. **B**, Holter ECG showed sinus arrest with a maxium RR interval of 5.59 s. **C**, Echocardiography revealed mild dilatation of left ventricle (LV) and right atrium without obvious evidence for cardiomyopathy, congenital heart disease, or cardiac dysfunction. LV internal diameter, 57 mm; LV posterior and interventricular wall thickness, each 6 mm; LV ejection fraction, 63%.

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fluorescent microscopy at 48 hour-post-fertilization (hpf). Heart rate and rhythm were recorded. Videos obtained from the embryos were analyzed using Image J (National Institutes of Health) to determine the heart rate and the duration of cardiac pauses.

Statistical Analyses

Results are presented as means±SE otherwise stated, and statistical comparisons were made by using 1-way analysis of variance followed by Bonferroni adjustment to estimate the significance of differences between the mean values of all pairwise. Statistical significance was assumed for $P<0.05$.

Results

Case Presentation

Genetic screening of *MYH6* mutations in 9 probands with familial SSS identified a novel mutation in a 62-year-old Japanese woman. She attended the hospital because of several episodes of presyncope with which she had been afflicted for 5 years. Her 12-lead ECG showed sinus bradycardia (heart rate 42 beats per minute) with unusual P wave axis and junctional
F1 escape beat (Figure 1A), and Holter ECG revealed sinus arrest with maximum RR interval of 5.59 s (Figure 1B). She had no history of other arrhythmias, including atrial fibrillation. Echocardiography revealed mild dilatation of the left ventricle (LV) and right atrium, but there were no obvious signs of cardiomyopathy, congenital heart disease, or cardiac dysfunction (LV internal diameter in diastole, 57 mm; LV posterior wall in diastole, 6 mm; interventricular septal wall in diastole, 6 mm; and LV ejection fraction, 63%; Figure 1C). A pacemaker was implanted after the diagnosis of SSS. Her deceased mother also had a pacemaker implanted because of SSS during the 7th decade of her life.

Identification of the Novel *MYH6* Mutation delE933

The novel mutation identified in the proband was an in-frame 3-bp deletion, c.2797_2799delGAG, located in exon 22 of *MYH6*. This was predicted to delete one residue within the glutamic acid triplet at aa.931–933 of α-MHC (delE933;
F2 Figure 2A). This triplet is located in the S2 segment of α-MHC, a crucial structure required for binding to MyBP-C and for regional phosphorylation of MyBP-C,[6] thereby facilitating a flexible link between thin and thick filaments. The S2 hinge region is highly conserved among α-MHC from different species, as well as between other MHC isoforms (Figure 2B).

The proband has no siblings or offspring, and DNA was not available from her deceased mother. The delE933 mutation was not identified in 800 *MYH6* alleles from healthy Japanese controls or in the public genetic variation databases of dbSNP, 1000 Genomes, Exome Variant Server, and HGVD. The common variation A1101V was not found in the proband, whereas 3 out of 8 other probands in our cohort were heterozygous for A1101V. The rare *MYH6* variation R721W (c.C2161T), associated with SSS in Icelanders,[20] was not found in our familial SSS cohort. No other disease-related mutations were identified in *SCN5A*, *HCN4*, *SCN3B*, *KCNJ3*, *KCNJ5*, or *GJA5* in our familial SSS cohort. Polymorphisms identified in *MYH6* are listed in Table IV in the Data Supplement.

delE933-*MYH6* Mutation Disrupts Sarcomere Structures

The S2 segment is a coiled-coil domain of α-MHC composed of a motif of heptad repeats of amino acids.[6,29,30] SWISS-MODEL simulation predicted that the delE933 mutation would cause local disruption of the coiled-coil structure (Figure 3A). Immunoprecipitation studies using a recombi- F3
nant MyBP-C C1-C2 protein and WT and delE933α-MHC S2 region proteins expressed in HeLa cells showed that the binding ability of α-MHC with MyBP-C was substantially enhanced by the delE933 mutation (Figure 3B).

Because structural damage of sarcomere have been reported in association with *MYH6* mutations responsible for atrial

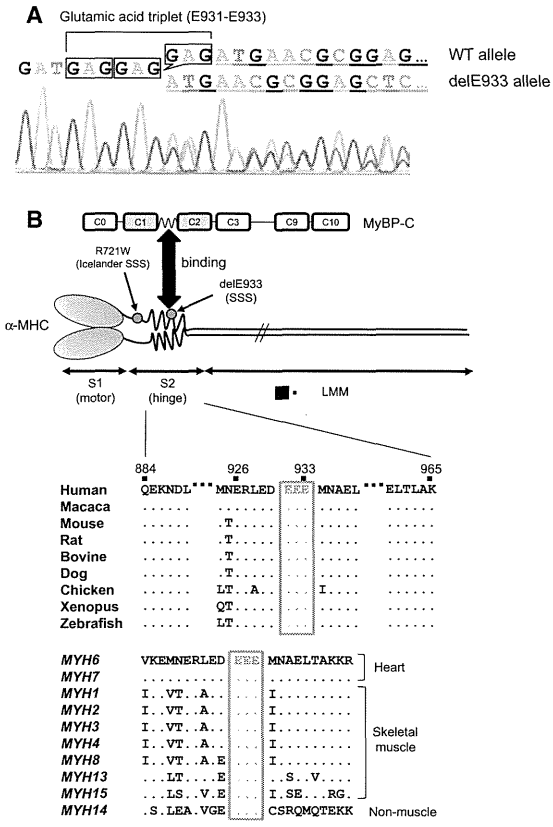

Figure 2. Genetic and protein information of the *MYH6* mutations. **A**, An electropherogram of exon 22 of *MYH6* of the proband. Boxes indicate the codons of triplicate glutamic acids E931-E933 of the wild-type (WT) allele and an in-frame deletion of GAG resulting in delE933. **B**, Protein structures of α-myosin heavy chain (α-MHC) and its binding partner myosin-binding protein C (MyBP-C). α-MHC consists of S1 motor, S2 hinge, and light meromyosin (LMM) regions. The S2 hinge region interacts with the region of MyBP-C between the 1st (C1) and 2nd globular structure (C2). Locations of the 2 *MYH6* mutations, a rare variant R721W identified in Icelanders[20] and delE933 (this study), are shown with red dots. Protein sequence alignment shows that the MyBP-C binding site (residues 884–965) are highly conserved among α-MHCs from different species, and the glutamic acid triplet is perfectly conserved among different species and different MHC isoforms of cardiac (*MYH6*, *MYH7*), skeletal muscle (*MYH1*, *MYH2*, *MYH3*, *MYH4*, *MYH8*, *MYH13*, *MYH15*), and a nonmuscle type (*MYH14*). SSS indicates sick sinus syndrome.

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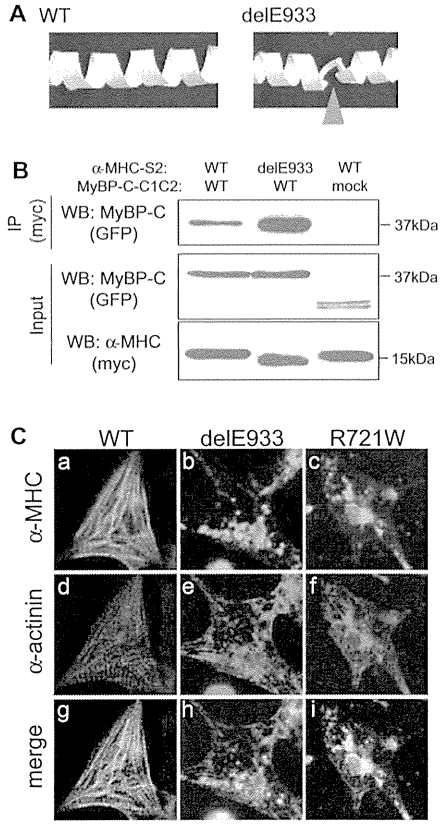

Figure 3. In silico prediction and in vitro functional evaluation of delE933-*MYH6*. **A**, Ribbon representation of 3-dimensional structure of the S2 region in human α-myosin heavy chain (α-MHC) predicted and visualized by SWISS-MODEL and RasTop, respectively. The coiled-coil structure is partially disrupted at the truncated amino acid E933 (arrowhead). **B**, Co-immunoprecipitation study of the S2 region of α-MHC and C1C2 region of cardiac myosin-binding protein C (MyBP-C). The S2 fragment of delE933 shows increased binding to the C1C2 fragment of MyBP-C. A nonspecific double band was often observed on the input of a mock pEGFP-C1 plasmid (third column). **C**, Fluorescence images of neonatal rat ventricular cardiomyocytes transiently expressing wild-type (WT), delE933, or R721W *MYH6* fused to green fluorescent protein (GFP). WT α-MHC shows a striated pattern of GFP together with the proper striated sarcomeric pattern of α-actinin (**a** and **d**). α-MHC with mutations of R721W and delE933 show brightly fluorescent speckles without well-organized sarcomere structure (**b** and **c**). The α-actinin images show a misaligned and disrupted pattern of myofibrils (**e** and **f**), indicating sarcomere disintegration. Scale bar, 10 μm.

septal defect,[7] we next explored whether the *MYH6* variation R721W, as well as delE933, disrupted integrity of sarcomere structures. To investigate the functional consequences of *MYH6* mutations on the atrial sarcomere structure, we used a heterologous expression system in cultured rat cardiomyocytes in which the predominant ventricle MHC isoform is α-MHC.[31] Neonatal rat ventricular cardiomyocytes were transiently transfected with a GFP-tagged *MYH6* WT, delE933, or R721W plasmids. Confocal microscopy analysis revealed comparable GFP intensities after transfection of all 3 *MYH6* plasmids (Figure 3C, a–c), indicating that the expression levels and stability of heterologously expressed α-MHC proteins were similar. Endogenous sarcomeric α-actinin expression at the Z-disc indicated the sarcomere integrity of transfected myocardial cells (Figure 3C, d–f). Cells expressing WT-*MYH6* displayed a striated staining pattern, indicating that heterologous α-MHC was correctly integrated into the sarcomere. However, both *MYH6* mutants, delE933 and R721W, exhibited a substantially disrupted α-actinin staining pattern and perinuclear aggregation of α-MHC, suggesting that structural damage to the sarcomere had occurred in cells expressing *MYH6* variants predisposing to sinus node dysfunction.

Atrial HL-1 Cells Stably Expressing the delE933-*MYH6* Showed Impaired Electric Propagation

A recent genome-wide association study showed that the Iceland-specific *MYH6* variant was significantly associated with atrial fibrillation,[20] we hypothesized that the functional defects caused by mutated *MYH6* may affect action potential propagation in the atrium surrounding the sinus node, leading to SSS manifestation. We cultured the mouse atrial cardiomyocyte cell line HL-1 stably expressing WT or mutant *MYH6* on 64-well electrode arrays and analyzed electric propagation velocities (Figure 4A). The propagation velocity was unchanged between WT-*MYH6* and control mock-transfected cells, but cells expressing delE933-*MYH6* exhibited a significantly slower propagation velocity (control, 3.6±0.6 mm/s; WT, 3.8±1.2 mm/s; delE933, 2.9±0.8 mm/s; n=252 for each line, $P<0.001$ for WT versus delE933; Figure 4B). Cell numbers of each array were comparable ($P=0.49$; Table V in the Data Supplement). These data suggest that mutant *MYH6* delE933 impairs cell-to-cell action potential propagation in the atrial myocardium. F4

delE933-*MYH6* Failed to Rescue the Heart Rate Reduction in Zebrafish With Morpholino *myh6* Knockdown

To determine whether the human *MYH6* orthologue *myh6* could influence heart rate control in zebrafish, we performed targeted *myh6* knockdown experiments with ATG-MO. Zebrafish cardiac phenotypes, including heart rate and cardiac rhythm, were assessed at 48 hpf. The *myh6* morphants exhibited atrial dilatation (Figure 4C), which is consistent with a previous report using decreased functional *myh6* transcript.[27] *Myh6* morphants also showed a significantly slower heart rate than uninjected embryos (*myh6*-MO, 137.7±2.2 beats per minute, n=28; uninjected 150.2±1.6 beats per minute, n=25; $P<0.001$; Figure 4D). Cardiac asystole was not observed in uninjected embryos or morphants. As shown in Figure 4D, coinjection of WT human *MYH6* cRNA rescued the bradycardia (148.7±1.4 beats per minute, n=26; versus *myh6*-MO $P<0.001$), suggesting that the human *MYH6* compensated for the loss of the zebrafish orthologue. By contrast, human *MYH6* carrying the delE933 mutation failed to rescue the bradycardia (142.3±2.5 beats per minute, n=24). Human *MYH6* RNA was detected by reverse transcription-polymerase chain reaction in embryos at 24 and 48 hours after injection (Figure in the Data Supplement), suggesting that the delE933 mutation of *MYH6* is responsible for sinus node dysfunction.

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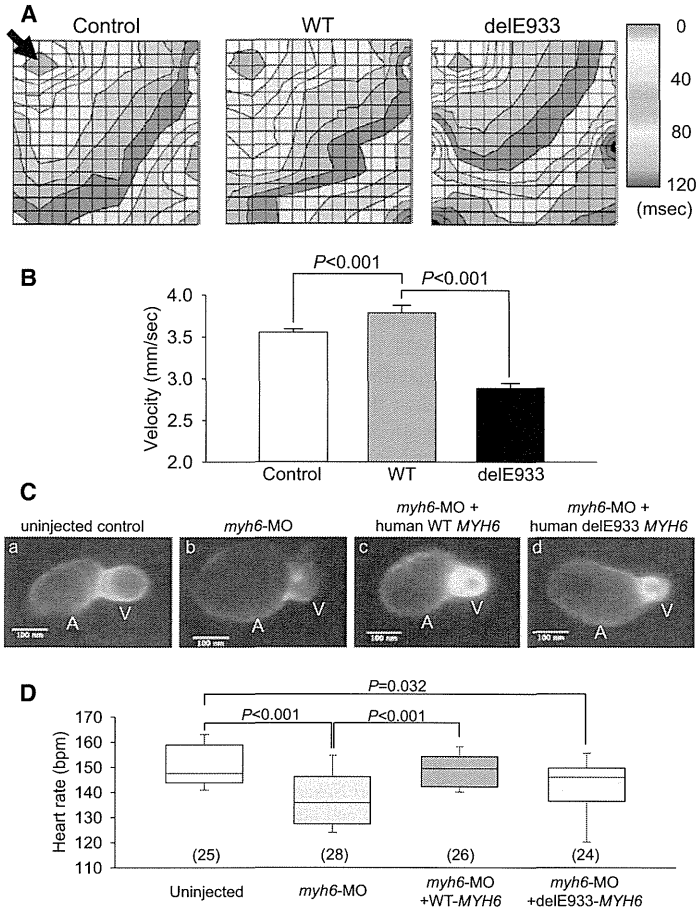

Figure 4. Electrophysiological phenotypes of delE933-*MYH6*. **A**, Representative activation isochronal maps of HL-1 cells stably expressing wild-type (WT) or delE933 *MYH6* and control HL-1 cells. An electric provocation with 10 μA was input on the pointed electrode (arrow). **B**, Averaged conduction velocity calculated from the time elapsed for the impulse to reach all remaining electrodes (n=252 for each recording). **C**, Representative fluorescent diastolic images of embryonic zebrafish hearts at 48 hpf: uninjected control (**a**), *myh6*-MO only (b), *myh6*-MO coinjected with human WT *MYH6* cRNA (c), and *myh6*-MO coinjected with human delE933 *MYH6* cRNA (d). The atria of the *myh6* MO morphant in the presence or absence of coinjected human *MYH6* cRNAs (b-d) were slightly dilated compared with the uninjected control (**a**). The ventricle and cardiac looping pattern of the morphants, with or without *MYH6* cRNAs, were similar to that of the control. Scale bars, 100 μm **D**, Heart rate recordings from zebrafish (**a–d**). Data are shown as box and whisker plots with minimum, maximum, median, 25th, and 75th quartiles bars. Number in parentheses represents the number of zebrafish in each group.

Discussion

A growing body of evidence from genome-wide association studies has demonstrated an association of *MYH6* with sinus node function.[17–20] A common nonsynonymous single-nucleotide polymorphism of *MYH6* (A1101V) was previously shown to be a genetic modifier for resting heart rate and PR interval,[18] and this was further replicated in a large meta-analysis, including subjects of European ancestry from both the United States and Europe.[17,19] A combination of A1101V with other loci controlling heart rate further reduced the risk of SSS and pacemaker implantation, implicating a heritable quantitative trait.[17] By contrast, the rare *MYH6* variation R721W, unique to Icelanders, predisposes individuals to SSS and pacemaker implantation.[20] These studies clearly demonstrate that *MYH6* is a genetic modifier of sinus node function, but the mechanisms of this have been unclear, and it was uncertain whether *MYH6* could be a causative gene of familial SSS.

In this study, we identified a novel *MYH6* mutation, delE933, in one SSS individual among 9 probands of our familial SSS cohort.[22] We found that the mutant delE933-*MYH6* slowed down action potential propagation when heterologously expressed in the atrial myocardial cell line HL-1 (Figure 4A). Moreover, knockdown of endogenous *MYH6* leading to reduced heart rate in zebrafish could be compensated for by the coexpression of WT-*MYH6* but not by delE933-*MYH6* (Figure 4B). To our knowledge, this is the first experimental evidence demonstrating that *MYH6* variations can influence heart rate and action potential propagation. However, limited information is available to delineate the functional link between sarcomere components and sinus node function, and it remains unknown whether α-MHC directly affects pacemaker function or whether its actions are mediated though undefined mechanisms.

The delE933 mutation is located in the coiled-coil structure of the α-MHC S2 region, a binding motif for MyBP-C, and so is predicted to alter the tertiary structure and the cross-linking affinity between 2 sarcomere components (Figure 2A and 2B). Although the final consequences of such structural and functional modifications are unknown, a previous study that the MyBP-C mutation E334K, responsible for hypertrophic cardiomyopathy, impaired the ubiquitin-proteasome system, leading to an accumulation of cardiac ion channels at the sarcomere and electrophysiological dysfunction.[32] Increased protein levels were also observed for several other cardiac ion channels, including Kv1.5, Nav1.5, HCN4, Cav3.2, Cav1.2, SERCA, RYR2, and NCX1, which play major roles in controlling normal pacemaker function and atrial conductivity. Based on these findings, we speculate that an abnormal association AQ16

between delE933-α-MHC and MyBP-C might modulate the expression of cardiac ion channels that affect pacemaker function, which in turn would lead to the development of SSS.

In the present study, overexpression of the SSS-susceptible α-MHC mutants of R721W and delE933 in neonatal rat ventricular cardiomyocytes impaired sarcomere structures. However, it is unknown whether *MYH6* mutations preferentially impair the sinus node function or whether they might eventually cause further extensive electric damage manifesting as atrial fibrillation. Because the patient did not undergo electrophysiological studies or cardiac biopsy, further information regarding the spatial distribution and heterogeneity of pathological damages and electrophysiological abnormalities in the atrium is not available. Nevertheless, the T wave inversion of ECG and mild dilatation of the right atrium and LV (Figure 1A and 1B) are in accordance with the observation that targeted *myh6* knockdown in zebrafish induced the atrial dilatation associated with negative chronotropic effects, indicating that *MYH6* mutations may directly cause substantial damage and electric disorder to the myocardium in the atrium. This idea is further supported by the finding that heterozygous zebrafish expressing the *MYH6* mutation N695K (*MYH6*$^{hu1423/+}$) exhibited loss of atrial contractility, with residual beating restricted to the region near the atrioventricular junction and sinus venosus.[21] These observations strongly suggest that the final consequences of *MYH6* mutations in humans might also exhibit considerable heterogeneity with respect to the structural and electrophysiological properties of the atrium and sinus node.[33,34] Furthermore, these structural abnormalities of atrial sarcomere may extend to more severe conduction dysfunctions, such as atrial fibrillation or contractile failure, depending on the functional severity caused by each mutation. The slower conduction velocity observed in the HL-1 cells stably expressing delE933-*MYH6* in the present study suggests the possible involvement of *MYH6* in conduction dysfunction. This idea is supported by a recent genome-wide association study in which correlation studies of the *MYH6* variant R721W exhibited a significantly higher association with atrial fibrillation both before (odds ratio, 2.39; P=0.00010) and after (odds ratio, 2.03; P=0.015) exclusion of known SSS cases.[20] It is of note that the R719W of the ventricular β-MHC gene *MYH7*, homologous to the R721W-*MYH6,* is responsible for a malignant hypertrophic cardiomyopathy frequently associated with conduction abnormalities,[35] suggesting that α-MHC and β-MHC may share some pathophysiological mechanisms affecting cardiac action potential propagation.

SSS commonly occurs in older individual in the absence of accompanying heart diseases but comprises a variety of electrophysiological abnormalities in sinus node impulse formation and propagation. Although less common, SSS also shows familial inheritance, and implicated causative genes include those encoding cardiac ion channels, such as *SCN5A*. We recently found that familial SSS probands carrying *SCN5A* mutations showed a significantly earlier disease onset and a strong male predominance, whereas nonfamilial SSS had a disease onset of over 70 years for both sexes, which were affected equally.[22] The affected members of the SSS family in the present study were both women, aged over 60 years, suggesting that familial SSS with *MYH6* mutations might constitute an SSS subgroup distinct from that caused by *SCN5A* mutations. This may suggest the existence of a new disease entity of inherited arrhythmias attributable to mutations in genes encoding sarcomere proteins other than cardiac ion channel or ion channel-associated genes.

Limitation of the Study

Lack of information about the genotype–phenotype cosegregation of delE933-*MYH6* is the major limitation of this study from the standpoint of human genetics. Although bioinformatics evaluations, as well as in vitro and in vivo studies, have suggested pathophysiological significance of the rare *MYH6* variation delE933, it still does not exclude the possibility that the proband manifested SSS attributable to factors, such as aging rather than the *MYH6* mutation. To demonstrate the causality between SSS and *MYH6*, more extensive genetic screenings in patients with familial SSS to find novel *MYH6* mutations are required. Furthermore, it remains to be elucidated how the impaired sarcomere structures and conduction velocity elicited by delE933-*MYH6* ultimately result in the sinus node dysfunction. Electrophysiological studies using induced pluripotent stem cell–derived cardiomyocytes from *MYH6* mutation carriers, as well as basic evaluations of *MYH6* using genetically engineered animals, are also warranted.

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Disclosures

None.

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Public access defibrillation improved the outcome after out-of-hospital cardiac arrest in school-age children: a nationwide, population-based, Utstein registry study in Japan

Yoshihide Mitani[1]*, Kunio Ohta[2], Noriko Yodoya[1], Shoichiro Otsuki[1], Hiroyuki Ohashi[1], Hirofumi Sawada[1], Masami Nagashima[3], Naokata Sumitomo[4], and Yoshihiro Komada[1]

[1]Department of Pediatrics, Mie University Graduate School of Medicine, 2-174 Edobashi, Tsu City, Mie Prefecture 514-8507, Japan; [2]Department of Pediatrics, Kanazawa University Graduate School of Medicine, 13-1 Takaramachi, Kanazawa City, Ishikawa Prefecture 920-8641, Japan; [3]Department of Pediatric Cardiology, Aichi Children's Health and Medical Center, 1-2 Osakada, Morioka-Machi, Obu City, Aichi Prefecture 474-8710, Japan; and [4]Department of Pediatrics, Nihon University Itabashi Hospital, 30-1 Otaniguchiuemachi, Itabashi Ward, Tokyo 173-8610, Japan

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Aims The purpose of this study was to determine whether implementation of public access defibrillation (PAD) improves the outcome after out-of-hospital cardiac arrest (OHCA) in school-age children at national level.

Methods and results We conducted a prospective, nationwide, population-based Japanese Utstein registry study of consecutive OHCA cases in elementary and middle school children (7–15 years of age) who had a bystander-witnessed arrest of presumed cardiac origin during 2005–09 and received pre-hospital resuscitation by emergency responders. The primary endpoint was a favourable neurological outcome 1 month after an arrest. Among 230 eligible patients enrolled, 128 had ventricular fibrillation (VF) as an initial rhythm. Among these 128 patients, 29 (23%) children received a first shock by a bystander. Among these 29 patients, the proportion of the favourable neurological outcome after OHCA was 55%. During the study period, the proportion of patients initially shocked by a bystander among eligible patients increased from 2 to 21% ($P = 0.002$ for trend). The proportion of patients with a favourable neurological outcome after OHCA increased from 12 to 36% overall ($P = 0.006$). The collapse to defibrillation time was shorter in bystander-initiated defibrillation when compared with defibrillation by emergency responders (3.3 ± 3.7 vs. 12.9 ± 5.8 min, $P < 0.001$), and was independently associated with a favourable neurological outcome after OHCA [$P = 0.03$, odds ratio (OR) per 1 min increase, 0.90 (95% confidence interval 0.82–0.99)]. A non-family member's witness was independently associated with VF as the initial rhythm [$P < 0.001$, OR 4.03 (2.08–7.80)].

Conclusion Implementation of PAD improved the outcome after OHCA in school-age children at national level in Japan.

Keywords Cardiopulmonary resuscitation • Sudden unexplained death • School health • Public access defibrillation • School-age children

Introduction

Sudden cardiac death in elementary and middle school children is a rare but tragic event, which has tremendous impact on the family, school, communities, and health-care providers, and which may be relevant to cardiopulmonary resuscitation (CPR)/automated external defibrillator (AED) programmes in the public environment surrounding these children.[1,2] Recently, implementation of public

* Corresponding author. Tel: +81 59 231 5024; fax: +81 59 231 5213, Email: ymitani@clin.medic.mie-u.ac.jp

What's new?

- This is the first population-based study, which specifically addressed the impact of public access defibrillation on the outcome of out-of-hospital cardiac arrests (OHCAs) in elementary and middle school children.
- Among 230 eligible patients, 128 (56%) had ventricular fibrillation (VF) as an initial rhythm. Among these 128 patients, 29 (23%) children received a first shock by a bystander. Among these 29 patients, the proportion of the favourable neurological outcome after OHCA was 55%.
- During the study period 2005–09, the proportion of patients initially shocked by a bystander among eligible patients increased from 2 to 21%. The proportion of patients with a favourable neurological outcome after OHCA increased from 12 to 36% overall.
- A non-family member's witness was independently associated with VF as the initial rhythm. The collapse to defibrillation time was independently associated with a favourable neurological outcome, the survival at 1 month, and the prehospital return of spontaneous circulation after OHCA.

access defibrillation (PAD) improved outcomes among adults after out-of-hospital cardiac arrest (OHCA) in public locations, by reducing the time interval from the patient's collapse to defibrillation.[3–6] However, the impact of PAD on the outcome after OHCA in such school-age children was unclear. This question is challenging two-fold. First, paediatric patients of different ages have diverse aetiologies of OHCA; relatively poor survival has been reported in this heterogeneous group of patients.[7,8] The reported incidence of ventricular fibrillation (VF) as an initial rhythm in paediatric OHCA is lower than that reported in adults, and the effectiveness of early defibrillation programmes even for paediatric patients in VF arrest has been questioned.[7,8] Secondly, although school-age children are reported to spend a large part of their active daytime in public locations,[9] it is uncertain whether PAD programme, if any, would be effective for ordinary children in the children's public environment, including schools.[2,9–11] Recently, VF was found to be present in a higher percentage of high school-age athletes with sudden arrest, and recent small series have noted improved survival when early defibrillation with CPR was provided for such patients in high schools.[11–13] However, the limited deployment of AED devices in elementary and middle schools, and other public locations, a small sample size of OHCA in this age population in local studies, and the lack of an appropriate reporting system of OHCA, may have hampered any investigations involving the epidemiological basis of the benefit of PAD for OHCA in such school-age children.[11,12,14,15]

In Japan in July 2004, the Ministry of Health, Labour and Welfare approved AED use by citizens. By 2009, the number of AED devices in public places increased to 203 924 (106.6/100 000 population).[16,17] Of note, up to 28.9% of public access AED devices in Japan were placed in schools; by 2009, AEDs were placed in 72% of elementary schools and 89.8% of middle schools.[18,19] In January 2005, the Fire and Disaster Management Agency of Japan launched a prospective, nationwide, population-based, Utstein-style registry involving consecutive OHCA victims in all the age groups.[20] A recent study, using the Utstein registry database, demonstrated that there was a temporal increase in public access AED application and improved outcomes after OHCA in adults at the national level.[20] However, the impact of the national PAD programme on outcomes of OHCA in elementary and middle school children has not been reported. We therefore investigated whether PAD may have an impact on the outcome after OHCA in such school-age children at the national level, by using the Japanese Utstein registry database.[20,21]

Methods

Study design

The All-Japan registry of the Fire and Disaster Management Agency of Japan is a prospective, nationwide, population-based registry of OHCA, which is based on the standardized Utstein style, as reported in detail previously.[20,21] Briefly, this cohort enrolled all consecutive patients who suffered OHCA all over Japan, and were treated by emergency medical service (EMS) personnel and transported to hospitals. Specific enrolment process was described in Supplementary material online, Supplementary methods.[20,21] Among these patients, who had OHCA during January 2005–December 2009, we identified eligible patients who were 7–15 years of age, because we would include school-age students in compulsory education, which corresponds to the elementary and middle schools in Japan: high school students were thereby excluded. We identified those school-age victims with bystander-witnessed OHCA of presumed cardiac origin occurring during the entire day. Cardiac arrest was defined as the cessation of cardiac mechanical activity as confirmed by the absence of signs of circulation.[22–24] The arrest was presumed to be of cardiac origin unless it was caused by non-cardiac (respiratory disease, malignant tumours, and central nervous system disorders), external (trauma, hanging, drowning, drug overdose, and asphyxia) , or any other non-cardiac factors.[22–24] The data form was filled out by the EMS personnel in co-operation with the physicians in charge of the patients, and the data were integrated into the registry system on the database server. The working group for All-Japan Utstein registry designed the study protocol; collected and managed the data; and the authors analysed the data and wrote the manuscript. The protocol for analyses was approved by the Ethics Committee of Mie University Graduate School of Medicine.

Study setting

Emergency medical service and training system in Japan was previously reported in detail.[20,21] Briefly, Japan has an area of ~378 000 km^2, and its population was 127 million, including 3 666 839 male and 3 496 405 female 7–12-year-old children (elementary school students), and 1 871 134 male and 1 780 230 female 13–15-year-old children (middle school students) in 2005.[25] Placement of AEDs in public locations was driven by either public or private initiatives.[17] The cumulative number of public access AEDs, excluding those in medical facilities and EMS institutions, as estimated from sales of AEDs, increased from 9906 to 203 924 during the 5-year study period (see Supplementary material online, *Table*).[16] A total of 96.5% of public access AEDs are located in public locations (28.9% in schools, 20.6% in workplaces, 8.8% in nursing homes, 5.7% in sports facilities, 4.8% in cultural facilities, 2.6% in public transportation facilities, and 25.1% in other public locations), 1.4% in residential areas, and 2.1% in others.[18] From 2007 to 2009, the

percentage of elementary or middle schools equipped with at least one AED device increased from 18.1 to 72.9% in elementary schools and from 38.3 to 89.8% in middle schools (see Supplementary material online, *Table*).[19] School teachers and other staff were trained in CPR programmes by EMS providers or other instructors, under the guidance of local school boards, in which paediatric and adult PADs were generally recommended for children at 7 years of age and older, respectively, in accordance with the Japanese CPR guidelines.[26] In Japan, ~1.4–1.5 million citizens per year participated in the CPR/AED training programmes, generally provided by local fire departments.[27]

Data collection

Procedure of data collection was described previously.[20,21] Briefly, registry data were prospectively collected in accordance with the Utstein-style reporting guidelines for OHCA, which is a standardized form (uniform definitions, terminology, and recommended data sets) for clinical investigators to report human resuscitation studies.[20,21,23,24] Specific data sets and data collecting process were described in Supplementary material online, Supplementary methods.[20,21,23,24] In the present Japanese Utstein reporting system, a patient initially shocked by a bystander was defined as one in which a public access AED was used and the shock was delivered; if the public access AED was applied but the shock was not delivered, the patient was not included in this category.[20,21] In this analysis, an OHCA witnessed by non-family members was presumed to be an event in a public location, because of a lack of data with respect to specific locations in the registry; when a bystander delivered shocks with an AED, the initial rhythm of the patient was regarded as VF, including pulseless ventricular tachycardia.

Endpoints

The primary endpoint was survival at 1 month with minimal neurological impairment, which was defined as a Glasgow–Pittsburg cerebral performance category of 1 (good performance) or 2 (moderate disability).[23,24] Secondary endpoints were survival at 1 month and return of spontaneous circulation (ROSC) before arrival at the hospital.

Statistical analysis

The age-stratified annual incidence of OHCA was calculated with the use of 2005 census data.[25] Continuous variables between two groups were assessed by the unpaired *t*-test. Trends in categorical and continuous variables were analysed with the use of univariate regression models and linear tests, respectively, in overall and subgroups of eligible patients, determined by the relation of bystanders to the victims (family or non-family member). The planned subgroup analysis was intended to determine the impact of PAD on trends in outcome parameters of arrest in presumed public locations (non-family member-witnessed arrests) in comparison with the non-public location arrest (family member-witnessed arrests). Univariate and multivariable logistic regression analyses were performed to assess the factors associated with VF as the initial rhythm, and outcome parameters. Adjusted and unadjusted odds ratios with their 95% confidence intervals and *P* values were reported. Potential confounding factors adjusted for VF as the initial rhythm included the calendar year, the age, gender, the relation of the bystander to the patient (family or non-family member), the type of CPR initiated by a bystander (compression-only or conventional CPR), and the time from the witnessed collapse to the EMS arrival, in accordance with previous reports.[20,21,28] Potential confounding factors for outcome parameters included VF as an initial rhythm, bystander's AED use at the first shock, and the time from the witnessed collapse to the first shock, in addition to the potential confounders for VF, in accordance with previous reports.[20,21,28] All statistical analyses were performed with the use of the SPSS statistical package, version 16.0J (SPSS). Data were reported as mean ± standard deviation. All tests were two-tailed, and *P* values of <0.05 were considered to indicate statistical significance.

Results

Among 2072 OHCA children, 522 were of presumed cardiac origin; 230 of 522 arrests were witnessed by bystanders (*Figure 1*). Among a total of 230 eligible patients, 128 (56%) children had VF as the initial rhythm. Among these 128 patients, 29 (23%) children received a first shock by bystanders using a public access AED before the arrival of EMS personnel and 96 (75%) children received a first shock by EMS personnel (32 with a monophasic and 64 with a biphasic defibrillator). In addition, among 102 patients without VF as the initial rhythm, none received bystander's defibrillation, but 13 (13%) received a shock by EMS personnel following CPR. Among 128 children with VF as the initial rhythm, 53 (41%) survived with a favourable neurological outcome, 67 (52%) survived 1 month after the arrest, and 55 (43%) had pre-hospital ROSC. Among the subset of 29 school-age children with OHCA who received initial AED shock by bystanders, 16 (55%) survived with favourable neurologic outcome, 19 (66%) survived 1 month after the arrest and 19 (66%) had prehospital ROSC. Among 102 patients without VF as the initial rhythm, 8 (8%) survived with favourable neurological outcome, 15 (15%) survived 1 month after the arrest and 11 (11%) had pre-hospital ROSC. The time interval from collapse to the initiation of CPR was shorter in bystander-initiated CPR than EMS-initiated CPR (3.2 ± 4.9 vs. 8.9 ± 6.8 min, $P < 0.001$). The interval from collapse to the initiation of AED use was shorter in bystander-initiated AED use than EMS-initiated one (3.3 ± 3.7 vs. 12.9 ± 5.8 min, $P < 0.001$). Clinical and outcome parameters in the overall, family and non-family member witnessed arrests were reported in *Table 1*. The population-based age-stratified incidence of bystander-witnessed OHCA of presumed cardiac origin in children was constant during the study period (see Supplementary material online, *Table*).

Trends in clinical and outcome parameters

During the study period (*Table 2*), the proportion of patients initially shocked by a bystander's AED among total patients increased from 2% in 2005 to 21% in 2009 ($P = 0.002$). Such a temporal increase was observed in non-family member-witnessed arrests, from 4% in 2005 to 37% in 2009 ($P = 0.001$), but not in family member-witnessed arrests. The collapse to AED time tended to become shorter only in non-family member-witnessed arrests, from 11.1 min in 2005 to 8.3 min in 2009 ($P = 0.07$). The proportion of any other categorical and continuous variables investigated in either subgroup of patients did not change significantly (see Supplementary material online, *Appendix 1*). As the outcome parameters (*Figure 2*), the proportion of patients with a favourable neurological outcome among total patients increased from 12% in 2005 to 36% in 2009 ($P = 0.006$). Such a temporal improvement

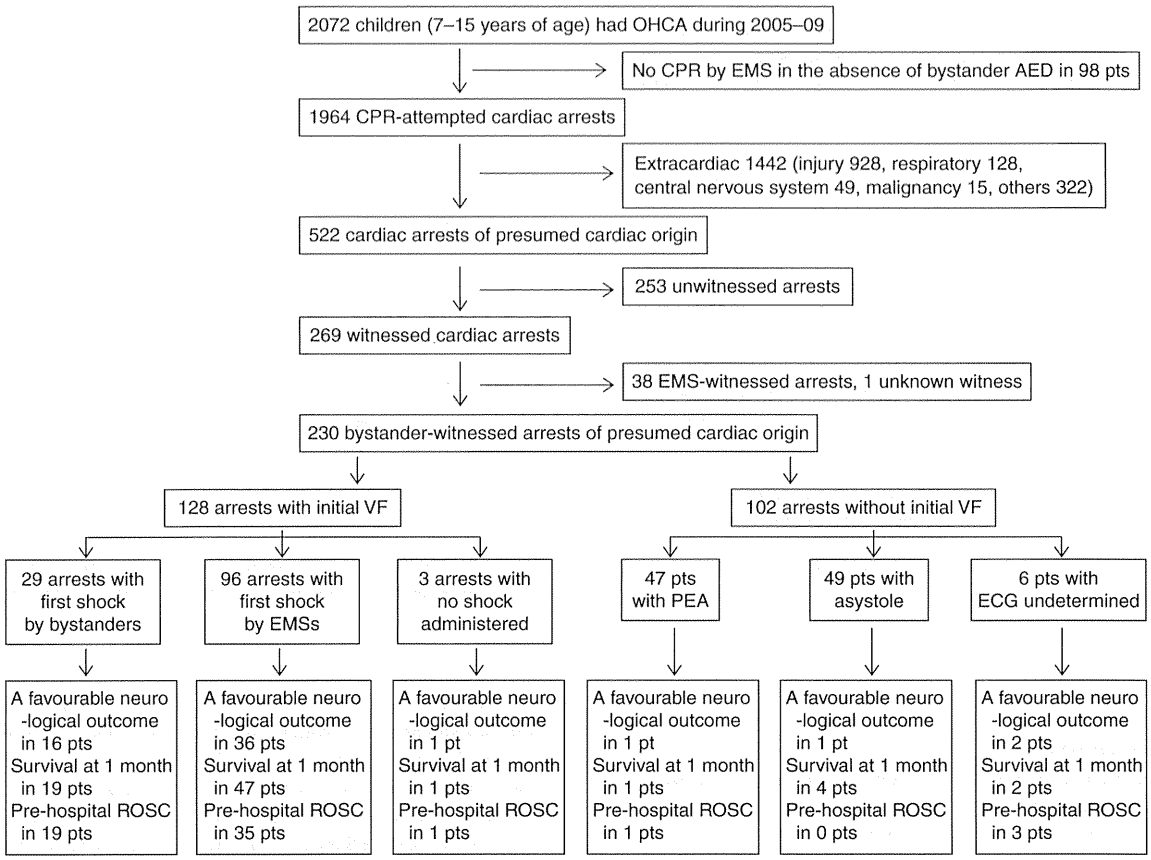

Figure 1 Study profile. OHCA, out-of-hospital cardiac arrest; CPR, cardiopulmonary resuscitation; EMS, emergency medical service; AED, automated external defibrillator; VF, ventricular fibrillation; PEA, pulseless electrical activity; ECG, electrocardiography; ROSC, return of spontaneous circulation; pts, patients.

was observed only in non-family member-witnessed arrests, from 9% in 2005 to 53% in 2009 ($P = 0.001$). The proportion of survival at 1 month after OHCA ($P = 0.008$) and ROSC before arrival at the hospital ($P = 0.046$) increased only in non-family member-witnessed arrests, from 17 and 17% in 2005 to 53 and 42% in 2009, respectively. Trends in specific values in all the clinical and outcome parameters investigated in overall and subgroups of patients were reported (see Supplementary material online, *Appendix 1*).

Multivariable analysis

In multivariable analysis (*Table 3*), a non-family member's witness [$P < 0.001$, adjusted odds ratio (OR) 4.03 (2.08–7.80)] was independently associated with the presence of VF as the initial rhythm. The collapse to AED time, either by a bystander or an emergency responder [$P = 0.03$, OR per 1 min increase, 0.90 (0.82–0.99)], and female gender [$P = 0.008$, 3.20 (1.35–7.56)] were independently associated with a favourable neurological outcome. The collapse to AED time was the only variable independently associated with the survival at 1 month [$P = 0.045$, 0.92 (0.85–0.99)] and pre-hospital ROSC [$P = 0.001$, 0.82 (0.73–0.92)]. Results of univariate analysis were reported in the see Supplementary material online, *Appendix 2*.

Discussion

Although the epidemiological data related to the impact of disseminating PAD programmes on OHCA in elementary and middle school children were limited,[7,8] the present Utstein registry study would supply evidence supporting that implementation of PAD programmes increases the likelihood of early defibrillation by bystanders, and improves the outcome after OHCA in such school-age children. These findings may underscore the benefit of PAD in the prevention of sudden cardiac death in school-age children.

Table 1 Clinical and outcome parameters

Parameters	Total (n = 230)	Family witnessed (101)	Non-family witnessed (129)
Age, years of age	12.2 ± 2.5	11.3 ± 2.7	12.8 ± 2.1
Male gender, *n* (%)	145 (63)	63 (62)	82 (64)
Ventricular fibrillation, *n* (%)	128 (56)	37 (37)	91 (71)
CPR initiated by bystanders, *n* (%)	161 (70)	55 (55)	106 (82)
Conventional CPR, *n* (%)	102 (64)	30 (55)	72 (69)
Collapse to CPR time (min)	4.9 ± 6.1	5.3 ± 6.3	4.6 ± 6.0
Shock initiated, *n* (%)			
by bystanders	29 (13)	2 (2)	27 (21)
by EMS	109 (47)	38 (38)	71 (55)
Collapse to AED time (min)	10.9 ± 6.7	13.1 ± 7.0	10.0 ± 6.4
Collapse to EMS arrival (min)	34.6 ± 17.2	35.0 ± 16.5	34.3 ± 17.7
Favourable neurological outcome, *n* (%)	63 (27)	15 (15)	48 (37)
Survival at 1month, *n* (%)	84 (37)	22 (22)	62 (48)
Prehospital ROSC, *n* (%)	66 (29)	20 (20)	46 (36)

Favourable neurological outcome denotes cerebral performance category 1 or 2 at 1 month.
Conventional CPR indicated chest compression with rescue breathing, as a type of bystander-initiated CPR. Percentages were calculated on the basis of the available data in overall or each subgroup of arrests (family or nonfamily witnessed). Plus–minus values are means ± SD.
CPR, cardiopulmonary resuscitation; EMS, emergency medical service; AED, automated external defibrillator; ROSC, return of spontaneous circulation.

Table 2 Trends in clinical parameters

Variables	2005	2006	2007	2008	2009	*P* value for trend
Type of bystanders, (*n*)						
Total	41	46	51	48	44	
Family member	18	18	21	19	25	0.27
Non-family member	23	28	30	29	19	
CPR initiated by bystanders, *n* (%)						
Total	26 (63)	33 (72)	36 (72)	37 (77)	29 (66)	0.65
Family member	8 (44)	9 (50)	12 (60)	12 (63)	14 (56)	0.35
Non-family member	18 (78)	24 (86)	24 (80)	25 (86)	15 (79)	0.90
Shock initiated by bystanders, *n* (%)						
Total	1 (2)	1 (2)	10 (20)	8 (17)	9 (21)	0.002
Family member	0 (0)	0 (0)	0 (0)	0 (0)	2 (8)	0.99
Non-family member	1 (4)	1 (4)	10 (33)	8 (28)	7 (37)	0.001
Shock initiated by EMS, *n* (%)						
Total	18 (44)	24 (52)	26 (51)	19 (40)	22 (50)	0.94
Family member	6 (33)	3 (17)	10 (48)	7 (37)	12 (48)	0.14
Non-family member	12 (52)	21 (75)	16 (53)	12 (41)	10 (53)	0.22
Collapse to AED time (min)						
Total	11.8 ± 4.8	12.5 ± 5.4	10.4 ± 7.2	10.2 ± 6.2	10.3 ± 8.4	0.22
Family member	13.5 ± 7.2	14.3 ± 4.6	13.3 ± 4.4	12.7 ± 3.0	12.6 ± 10.3	0.71
Non-family member	11.1 ± 3.2	12.2 ± 5.5	9.2 ± 7.8	9.4 ± 6.8	8.3 ± 5.9	0.07

Percentages were calculated on the basis of the available data in overall or each subgroup of arrests (family or non-family witnessed) in the respective year. Plus-minus values are means ± SD.
CPR, cardiopulmonary resuscitation; EMS, emergency medical service; AED, automated external defibrillator.

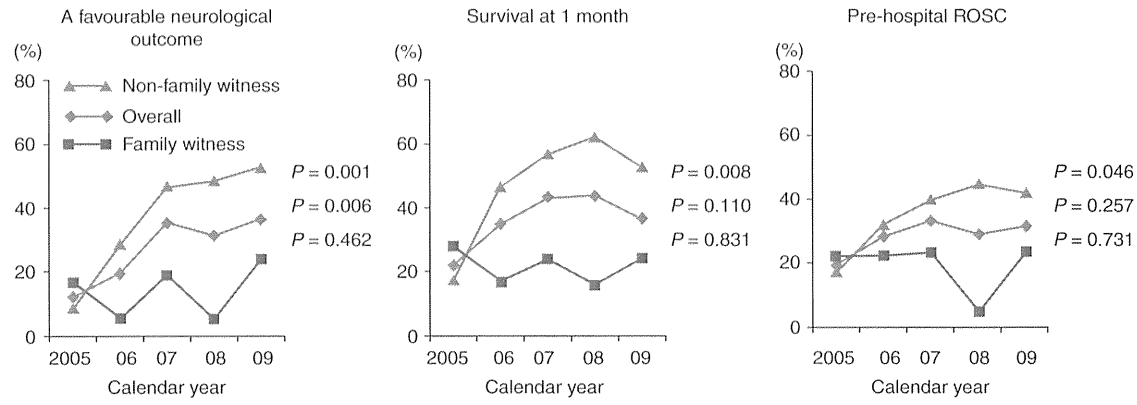

Figure 2 Trends in outcome parameters in arrests, by the relationship of a bystander to the victim. P values are for trend; ROSC, return of spontaneous circulation.

Table 3 Multivariable analyses of factors associated with ventricular fibrillation as the initial rhythm and outcome parameters

Variable	Ventricular fibrillation	Favourable neurological outcome	Survival at 1 month	Pre-hospital ROSC
	Adjusted OR (95% CI)	Adjusted OR (95% CI)	Adjusted OR (95% CI)	Adjusted OR (95% CI)
Year (per 1-year increase)	1.09 (0.87–1.37)	1.19 (0.87–1.63)	0.97 (0.73–1.30)	0.80 (0.58–1.10)
P value	0.46	0.29	0.86	0.17
Age≥13 years	1.83 (0.96–3.48)	1.79 (0.76–4.20)	1.60 (0.72–3.54)	1.60 (0.67–3.80)
P value	0.07	0.18	0.25	0.29
Female gender	0.76 (0.39–1.47)	3.20 (1.35–7.56)	1.80 (0.79–4.10)	2.17 (0.91–5.19)
P value	0.41	0.008	0.16	0.08
Non-family witnessed	4.03 (2.08–7.80)	1.53 (0.58–4.03)	1.68 (0.70–4.02)	0.86 (0.32–2.29)
P value	<0.001	0.39	0.27	0.76
CPR				
Not bystander-initiated	reference	reference	reference	reference
Bystander-initiated				
Conventional	0.76 (0.35–1.65)	1.01 (0.34–3.00)	1.24 (0.45–3.43)	0.90 (0.29–2.78)
P value	0.49	0.98	0.68	0.86
Compression only	0.79 (0.34–1.83)	1.55 (0.51–4.71)	1.08 (0.38–3.06)	1.77 (0.58–5.46)
P value	0.58	0.44	0.88	0.32
Collapse–EMS time (per 1 min increase)	0.99 (0.97–1.37)	1.00 (0.98–1.03)	1.00 (0.98–1.03)	1.01 (0.99–1.04)
P value	0.30	0.74	0.89	0.33
Ventricular fibrillation as the initial rhythm		2.03 (0.43–9.46)	1.30 (0.34–4.91)	0.76 (0.18–3.20)
P value		0.37	0.70	0.71
Bystander's AED		0.49 (0.12–2.02)	0.59 (0.15–2.23)	0.61 (0.14–2.76)
P value		0.32	0.43	0.53
Collapse–AED time (per 1-min increase)		0.90 (0.82–0.99)	0.92 (0.85–0.99)	0.82 (0.73–0.92)
P value		0.03	0.045	0.001

Favourable neurological outcome denotes cerebral performance category 1 or 2 at 1 month.
OR, odds ratio; ROSC, return of spontaneous circulation; EMS, emergency medical service; CPR, cardiopulmonary resuscitation; AED, automated external defibrillator.

Impact of public access defibrillation on out-of-hospital cardiac arrest in school-age children

Between 2005 and 2009 in Japan, there was a remarkable increase in the availability of AED in public spaces surrounding school children, including schools.[16–19] During this period, there was an increase in the proportion of OHCA in which the victim was initially shocked by a bystander, and this was temporally associated with an improvement in the neurological outcome in children with OHCA. In subgroup analyses, (i) temporal trends in these parameters were evident in non-family member-witnessed arrests, but not in family member-witnessed arrests, (ii) similar trends in secondary outcome parameters were observed in non-family member-witnessed arrests, and (iii) trends in other clinical parameters were not affected in either subgroup of patients during the same period. Therefore, trends in relevant variables, together with multivariable analysis data, consistently support that introduction of PAD programmes would increase the likelihood of early defibrillation by bystanders, and improve the outcomes of school-age children after public location arrest. Such an impact of PAD on OHCA in school-age children is consistent with that reported in adults.[20] In an adult study (≥18 years of age) by using the same Japanese Utstein registry data during 2005–07, 32% of patients with bystander-witnessed OHCA of presumed cardiac origin with initial rhythm of VF who received bystander AED shock delivery had a favourable neurological outcome.[20] In the present study during the corresponding years 2005–07 (data not shown), 58% (7/12) of children who received bystander-initiated shock had a favourable neurological outcome. In other adult studies, the survival rate of OHCA patients initially shocked by a bystander was ~60%.[3,4,6,13] Thus, the survival to 1 month with good neurological outcome of school-age children who experience witnessed OHCA with bystander CPR and AED shock delivery appears to equal or surpass that reported in adults. The more favourable outcome in this paediatric population may result from the higher rate of bystander CPR, and the shorter collapse to CPR and collapse to AED shock delivery intervals than those observed in adults with OHCA during the same period in Japan. This may be explained in part by factors in the school environment, such as constant visual observation of the children and focused training of teachers and staff.

Frequency of ventricular fibrillation as the initial rhythm in out-of-hospital cardiac arrest in school-age children

The frequency of VF in OHCA in children has been debated for a decade, and has been negatively influenced by the young age (<1 year of age), and traumatic and respiratory aetiologies.[7,8,14,15] In the present study, as high as 56% of bystander-witnessed arrests of presumed cardiac origin in school-age children were associated with VF. This is consistent with the results in local studies (in King county of USA, and in a province of the Netherlands), in which the frequency of VF has been positively associated with the advanced age (≥8 years of age), witnessed arrest, and cardiac aetiology, and a half of arrest patients had an initial rhythm of VF among adolescents aged 13–18 years with witnessed arrest.[11,29] In our study, we could further demonstrate that the non-family member-witnessed arrest was independently associated with VF as the initial rhythm, which is consistent with the results in an adult study.[28] The relatively low proportion of initial VF in adolescent OHCA in ROC study may be related to the difference of witness status, aetiology, and the reporting system.[14] The present study suggests that the relatively high proportion of initial VF in bystander-witnessed OHCAs of presumed cardiac origin in public locations in school-age children may confer an epidemiological basis for early defibrillation in this age population.

Limitations

Several limitations could be acknowledged in this study, in addition to those, as described previously.[20,21] First, the proportion of OHCA patients in schools among total eligible samples is unknown, because of the lack of data with respect to school as a specific location in the registry. Secondly, there might be unmeasured confounding factors (i.e. quality of bystander's CPR) that might influence the association between bystander's defibrillation and outcomes. Thirdly, information on in-hospital treatment (ie, hypothermia) is unavailable, which might affect survival after OHCA. Fourthly, it is unknown whether the present information can be generalized to other communities with different emergency response programmes at schools and other public locations surrounding children,[18,19] or different EMS systems.[20] Fifthly, the present investigation is not a cost-effectiveness analysis, although a previous study of cardiac arrests in high schools indicated that PAD may be cost-effective in schools.[12] Sixthly, specific data on the scope of the budgetary barriers and logistic issues (i.e. the locations of AED placement, training schedule for teachers) in implementing and refining AED/CPR programmes at the national level in Japan is unavailable.[17]

Conclusions

Although the impact of PAD has been largely elusive in overall children of different ages after etiologically diverse OHCAs in their public environmnent,[7,8] the present study would supply evidence which could dissect an epidemiological basis of the benefit of PAD in school-age children after bystander-witnessed OHCA of presumed cardiac origin. We believe that these findings are relevant to medical emergency response and CPR/AED programmes in the public environment surrounding school-age children.[1,2]

Supplementary material

Supplementary material is available at *Europace* online.

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Conflict of interest: none declared.

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QT Is Longer in Drug-Free Patients with Schizophrenia Compared with Age-Matched Healthy Subjects

Kumiko Fujii[1], Yuji Ozeki[1,2]*, Hiroaki Okayasu[1], Yumiko Takano[1], Takahiro Shinozaki[1], Hiroaki Hori[2], Masami Orui[3], Minoru Horie[4], Hiroshi Kunugi[2], Kazutaka Shimoda[1]

1 Department of Psychiatry, Dokkyo Medical University School of Medicine, Tochigi, Japan, **2** Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan, **3** Department of Health Care, Dokkyo Medical University School of Medicine, Tochigi, Japan, **4** Department of Cardiovascular and Respiratory Medicine, Shiga University School of Medical Science, Shiga, Japan

Abstract

The potassium voltage-gated channel KCNH2 is a well-known gene in which mutations induce familial QT interval prolongation. KCNH2 is suggested to be a risk gene for schizophrenia. Additionally, the disturbance of autonomic control, which affects the QT interval, is known in schizophrenia. Therefore, we speculate that schizophrenic patients have characteristic features in terms of the QT interval in addition to the effect of antipsychotic medication. The QT interval of patients with schizophrenia not receiving antipsychotics (n = 85) was compared with that of patients with schizophrenia receiving relatively large doses of antipsychotics (n = 85) and healthy volunteers (n = 85). The QT interval was corrected using four methods (Bazett, Fridericia, Framingham or Hodges method). In ANCOVA with age and heart rate as covariates, patients not receiving antipsychotic treatment had longer QT intervals than did the healthy volunteers, but antipsychotics prolonged the QT interval regardless of the correction method used ($P<0.01$). Schizophrenic patients with and without medication had a significantly higher mean heart rate than did the healthy volunteers, with no obvious sex-related differences in the QT interval. The QT interval prolongation may be manifestation of a certain biological feature of schizophrenia.

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* E-mail: ozeki@dokkyomed.ac.jp

Introduction

The KCNH2 channel (which is also known by the name of herg), a member of the subfamily of voltage-gated K+ channels, is responsible for a delayed rectifier potassium current of myocardial cells, which is a major component of cardiac repolarization [1]. The KCNH2 channel gene variant causes a congenital form of long QT syndrome or predisposes an individual to acquiring long QT syndrome [2–3]. In addition, antipsychotics can induce QT interval prolongation [4–5]. The blockage of the KCNH2 channel via antipsychotics has been identified as a mechanism underlying QT interval prolongation from antipsychotic administration [6]. Therefore, a decline in KCNH2 channel function is assumed to be a mechanism of QT interval prolongation. In addition, several recent association studies have cited isoforms/polymorphisms of the KCNH2 gene as a risk factor for schizophrenia [6–9]. Moreover, the QT interval is affected by autonomic control [10], and studies of the autonomic system in schizophrenia have detected a number of abnormalities [11]. Therefore, we hypothesized that the prolongation of the QT interval in patients with schizophrenia might not only be caused by antipsychotic administration but also be characteristic of the disease.

Accordingly, to reveal the characteristics of QT intervals of schizophrenia excluding the effect of antipsychotic medications, we compared the QT interval of patients with schizophrenia not receiving antipsychotics with that of patients with schizophrenia receiving antipsychotics and healthy volunteers.

Materials and Methods

Subjects

Between 1996 and 2008, 111 drug-free schizophrenic patients were recruited at the National Institute of Neurology and Psychiatry Hospital, Japan. These participants were all non-adherent patients or naïve of antipsychotics. Whether a patient was drug free was judged based on the doctor's description in the medical record. According to medical records, no antipsychotic medication was prescribed to "drug-free" patients within several months before ECG recording. No patient was administered depot antipsychotics. Of the 111 patients, 23 with hypokalemia ($K<3.5$), two with a complete right bundle branch block, and one with a postoperative atrial septal defect were excluded from the study. No participant suffered from arrhythmia or other conduction disorders of the heart. Accordingly, data from 85 drug-free patients were analyzed. For the antipsychotic administration group, we selected 85 age-matched patients with schizophrenia (examined during the same time period as the first group) who were receiving pharmacotherapy and had no QT prolongation factors (e.g., hypokalemia, hypothyroidism, and/or ischemic heart disease) other than antipsychotic medication. To reveal the QT prolon-