Journal of Arrhythmia 29 (2013) 261–264

Contents lists available at ScienceDirect

Journal of Arrhythmia

journal homepage: www.elsevier.com/locate/joa

Original Article

Genetic screening of *KCNJ8* in Japanese patients with J-wave syndromes or idiopathic ventricular fibrillation ☆

Qi Wang, BSc[a], Seiko Ohno, MD, PhD[a], Koichi Kato, MD[a], Megumi Fukuyama, MD[a], Takeru Makiyama, MD, PhD[b], Hiromi Kimura, MD, PhD[a], Nobu Naiki, MD[a], Mihoko Kawamura, MD[a], Hideki Hayashi, MD, PhD[a], Minoru Horie, MD, PhD[a,*]

[a] Department of Cardiovascular and Respiratory Medicine, Shiga University of Medical Science, Otsu 520-2192, Japan
[b] Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan

ARTICLE INFO

Article history:
Received 29 October 2012
Received in revised form
18 December 2012
Accepted 25 January 2013
Available online 28 March 2013

Keywords:
J-wave syndrome
KCNJ8
Idiopathic ventricular fibrillation
Japanese

ABSTRACT

Background: J-point elevation has been demonstrated to be associated with ventricular fibrillation (VF) and has been proposed as a cause of the J-wave syndrome (JWS). A mutation of *KCNJ8*, S422L, was reported as a culprit gene. This study aimed to determine the prevalence of *KCNJ8* mutations in a Japanese population with JWS or idiopathic VF (IVF).
Methods: A total of 230 probands with JWS and IVF underwent genetic screening of *KCNJ8*. To analyze and compare clinical and electrocardiographic characteristics, the probands were divided into 4 groups: Brugada (Br) pattern only, early repolarization (ER) pattern only, Br and ER patterns, and true IVF.
Results: The results of the genetic analysis revealed no S422L or other *KCNJ8* mutations and indicated no significant difference between the groups.
Conclusion: The *KCNJ8* mutation showed no association with JWS or IVF among our Japanese patients.

1. Introduction

Ventricular fibrillation (VF) is the most malignant arrhythmia that causes sudden cardiac death. VF, which occurs in apparently healthy individuals without structural heart diseases, is regarded as an idiopathic or primary electrical disease. Among patients with idiopathic VF (IVF), an increased prevalence of early repolarization (ER) was observed, which was considered as a benign electrocardiographic (ECG) finding in the past [1]. Recently, the concept of J-wave syndrome (JWS), including the ER and Brugada (Br) syndromes, has emerged [2].

The mutation *KCNJ8*-S422L was first identified as the cause of ER syndrome in a 14-year-old Caucasian girl with VF. An ECG showed a prominent ER pattern in the inferolateral leads [3]. *KCNJ8* encodes an inward rectifier potassium 6.1 protein (Kir6.1), which is a subunit of cardiac ATP-sensitive K (K_{ATP}) channels. The succeeding cases of the mutation were found in five men and two women with ER or Br syndrome [4,5]. However, whether *KCNJ8* mutations are present among the Asian population remains to be known. Therefore, the present study aimed to investigate the prevalence of *KCNJ8* gene mutation in Japanese patients with JWS or IVF.

2. Method

2.1. Study population

The study population was selected from unrelated families and consisted of 230 Japanese probands with IVF, Br syndrome, or suspected Br syndrome. All the probands underwent genetic analysis between 1996 and 2011 at the Shiga University of Medical Science and Kyoto University Graduate School of Medicine. Probands with structural heart diseases or other inheritable arrhythmic disorders were excluded from the study.

2.2. DNA isolation and mutation analysis

The genetic analysis protocol was approved by the institutional ethics committees of both universities and performed under the respective institutional guidelines. All the patients provided informed

☆Grants: This work was supported, in part, by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology and from the Ministry of Health, Labor and Welfare of Japan for Clinical Research on Measures for Intractable Diseases and Translational Research Funds from the Japan Circulation Society (to MH).
Abbreviations: Br, Brugada; ECG, electrocardiogram; ER, early repolarization; ICD, implantable cardioverter-defibrillator; IVF, idiopathic VF; JWS, J-wave syndrome
* Corresponding author. Tel./fax: +81 77 548 2213.
E-mail address: horie@belle.shiga-med.ac.jp (M. Horie).

1880-4276/$ - see front matter © 2013 Japanese Heart Rhythm Society. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.joa.2013.01.013

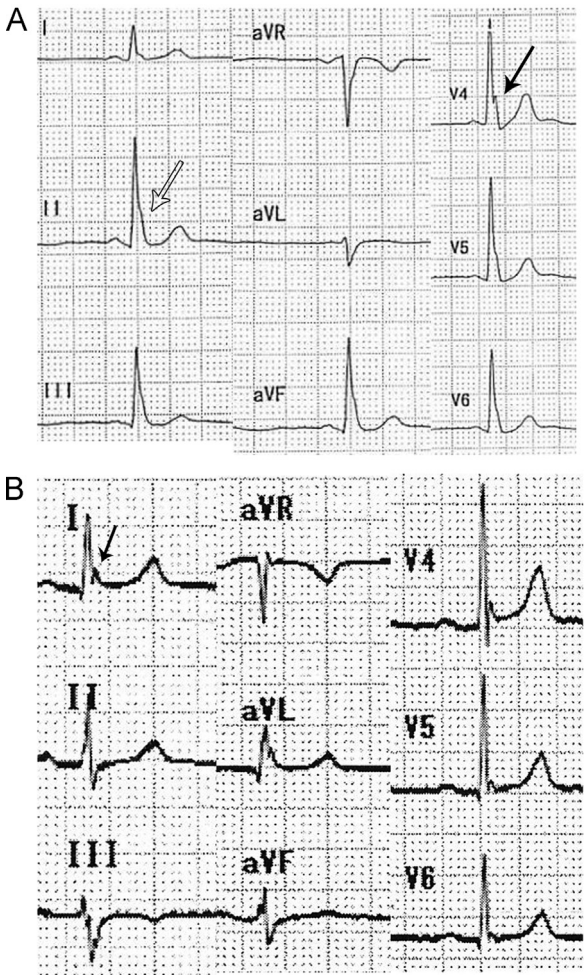

Fig. 1. Twelve-lead ECGs representing an early repolarization (ER) pattern. (A) ER pattern in the inferior and lateral leads. (B) ER pattern in the lateral leads only. Arrows indicate a slurring (opened) or notching (closed) ER morphology.

consent before undergoing the genetic analysis. Genomic DNA was isolated from leukocyte nuclei. We performed genetic screening for *KNCJ8*, *SCN5A*, *CACNA1C*, *CACNB2*, *SCN1B*, *KCNQ1*, *KCNH2*, *KCNE1-3*, and *KCNE5* using denatured high-performance liquid chromatography (WAVE System Model 3500, Transgenomic, Omaha, NE, USA) [6–8], high-resolution melting (LightCycler®480, Roche Diagnostics GmbH, Mannheim, Germany), and direct sequencing with the ABI PRISM 3130 sequencer (Applied Biosystems, Foster City, CA, USA). The cDNA sequence numbering of *KCNJ8* was based on the GenBank reference sequence (NC_000012). We excluded all the probands who had genetic test results positive for all the above-mentioned genes, except *KCNJ8*.

2.3. Clinical phenotype

We analyzed the following demographic characteristics in 230 patients: sex, age at diagnosis, history of symptoms, and indication for treatment with an implantable cardioverter-defibrillator (ICD). Patients who had a history of syncope or aborted cardiac arrest were considered symptomatic. The presence of Br [9] and ER patterns was examined using a 12-lead ECG. PR intervals, QRS durations, and QT intervals were manually measured in lead V_5. QTc intervals were calculated by correcting for heart rate using the Bazett formula. Patients in whom QTc values in resting ECG were lower than 350 $ms^{1/2}$ or higher than 470 $ms^{1/2}$ (men) and 480 $ms^{1/2}$ (women) were also excluded [10].

2.4. Data analysis

The patients with JWS were stratified into 4 groups according to the following ECG characteristics: Br pattern only [9] (Br group), ER pattern only (ER group), overlapping Br and ER patterns (Br–ER group), or IVF with neither a Br nor ER pattern (true IVF group). Patients whose ECG displayed only a Br-type ST elevation in leads V_1 through V_3 [9] were categorized under the Br-only group, regardless of whether the Br pattern was spontaneous or drug induced. We defined ER as either a slurring or notching J-point elevation of at least 0.1 mV from the baseline in at least 2 conjugate leads (Fig. 1A and B) [11]. Patients who exhibited both Br and ER patterns were assigned in the Br–ER group, whereas those with VF/ventricular tachycardia whose ECG showed no Br or ER pattern were assigned in the true IVF group.

2.5. Statistical analysis

All continuous data were expressed as mean ± standard deviation values. One-way analysis of variance with the Fisher least significant difference test and Games-Howell test was used for multiple comparisons. A $P < 0.05$ was considered statistically significant.

3. Results

3.1. Gene analysis

The gene analysis revealed that neither *KCNJ8*-S422L nor other *KCNJ8* mutations were present in our Japanese JWS and IVF cohorts. Nevertheless, we found a previously reported synonymous variant, p.I37I (c.C457T, rs112604741), in 2 probands, 1 who had VF belonged to the true IVF group and the other, who was asymptomatic, belonged to the Br–ER group. The mutation was absent in 112 healthy controls; however, the frequency of this SNP was reported to be 1.5% in heterozygotes (http://www.ncbi.nlm.nih.gov/snp).

3.2. Clinical characteristics

Table 1 presents the classification of the patients enrolled in this study according to clinical and ECG characteristics. The data of 230 probands (205, men) were included in the analysis. The patients' mean age at diagnosis was 45.1 ± 15.4 years. In terms of ECG characteristics, 135 patients had an ECG showing a Br pattern only; 15 an ER pattern only; and 53 Br and ER patterns. Only 27 patients with VF had no ECG abnormality.

The total clinical phenotypes are presented in Table 1. The symptomatic group included 72 patients who had experienced syncope and 75 who had an aborted cardiac arrest. Among the 230 probands, 88 (38%) had cardioverter-defibrillator implants. In the ECG analysis, the mean heart rate, PR, QRS, and QTc interval were 67.1 ± 13.6 bpm, 176.5 ± 25.8 ms, 94.6 ± 52.0 ms, and 404.2 ± 32.6 $ms^{1/2}$, respectively. Of the 230 patients, 188 (82%) had a Br ECG pattern (Br and Br–ER subgroups); 68 (30%), an ER pattern (ER and Br–ER subgroups); 53, (23%), Br and ER patterns; and 27 (12%), neither a Br nor ER pattern (true IVF).

Table 1 summarizes the clinical and ECG characteristics in the Br, ER, Br–ER, and true IVF groups. Male patients accounted for 89%, 87%, 96%, and 78% of the patients in the groups, respectively. The mean ages were similar in the 4 groups. Most of the ER and true IVF patients had experienced an aborted cardiac arrest compared with the Br and Br–ER patients (93% and 93% vs. 17% and 25%; $P < 0.0001$), indicating a statistically significant difference. The reason for the difference is that most of the patients in

Table 1
Comparison of the clinical and electrocardiographic (ECG) characteristics.

	Total	Br only	ER only	Br–ER	True IVF
n (%)	230	135 (59)	15 (7)	53 (23)	27 (12)
Men	205 (89)	120 (89)	13 (87)	51 (96)	21 (78)
Age (years)	45.1 ± 15.4	45.3 ± 15.5	44.2 ± 14.0	46.3 ± 14.1	42.7 ± 18.5
Symptomatic	139 (60)	66 (49)	15 (100)	31 (59)	27 (100)
Syncope	72 (31)	46 (34)	2 (13)	18 (34)	6 (22)
Aborted cardiac arrest	75 (33)	23 (17)	14 (93)	13 (25)	25 (93)
Treatment ICD	88 (38)	41 (30)	10 (67)	21 (40)	16 (60)
Heart rate (bpm)	67.1 ± 13.6	68.3 ± 14.5	63.8 ± 13.1	65.4 ± 11.7	66.4 ± 13.1
PR interval (ms)	176.5 ± 25.8	176.6 ± 24.2	167.3 ± 24.6	179.8 ± 28.9	174.6 ± 27.9
QRS duration (ms)	94.6 ± 52.0	97.8 ± 65.2	90.0 ± 17.7	88.5 ± 23.1	92.7 ± 17.5
QTc interval ($ms^{1/2}$)	404.2 ± 32.6	401.2 ± 30.9	416.5 ± 55.1	401.8 ± 29.1	417.6 ± 25.4
Men	403.1 ± 33.0	400.1 ± 31.5	417.0 ± 58.1	401.6 ± 28.3	417.0 ± 25.6
Women	412.0 ± 28.2	409.6 ± 24.9	413.2 ± 44.2	405.4 ± 63.2	419.3 ± 27.4

Br, Brugada pattern; ER, early repolarization pattern; IVF, idiopathic ventricular fibrillation.
The values are presented as *n* (%) and mean ± SD. PR, QRS and QTc were calculated in lead V_5.

the ER and true IVF groups were immediately hospitalized after the first attack, whereas most of the Br patients were receiving medication based on an ECG abnormality during the asymptomatic stage. However, no significant differences were observed in heart rate, PR interval, and QRS duration. Regarding QTc intervals, the probands in the Br subgroup had slightly shorter intervals than those in the true IVF subgroup (401.2 ± 30.9 vs. 417.6 ± 25.4 $ms^{1/2}$, $P=0.052$).

4. Discussion

We found no *KCNJ8* mutations in our 230 Japanese JWS and/or IVF probands from unrelated families. JWS and Br syndromes are regarded as primary electrical disorders causing VF that occur in individuals without structural heart disease. Antzelevitch and Yan [12] proposed the concept of JWS, in which the fundamental mechanism of the J-point elevation is caused by an increased transient outward current (I_{to}) due to a decrease in sodium or calcium current or an increase in potassium current. Irrespective of ECG subtypes such as the ER, Br, or Br–ER pattern, increased I_{to} seems to play a central role in generating life-threatening events in patients with JWS [13].

A *KCNJ8* mutation, S422L, was identified in 6 probands and 1 family member with JWS [3–5]. The probands consisted of 4 Br syndrome and 2 ER patients. Functional analyses showed that this hotspot mutation increased the activity of cardiac ATP-sensitive K current ($I_{K\text{-}ATP}$), which resulted in the AP notch accentuation in collaboration with epicardial I_{to}, and then caused a larger J-wave and/or ST-segment elevation on the patients' ECGs. In our cohort, however, we failed to identify a *KCNJ8* mutation. This finding may be related to the difference in ethnicities between Asian and Western countries.

Recently, 2 novel *KCNJ8* mutations, E322del and V346I, were reported in cases of sudden infant death syndrome [14]. In the functional analysis, both mutations displayed a decrease in $I_{K\text{-}ATP}$, which was opposite to the effect of S422L mutation on $I_{K\text{-}ATP}$ [4,5]. Therefore, JWS and sudden infant death syndrome seemed to have different underlying mechanisms, though having the same culprit gene, *KCNJ8*.

Based on our subgrouping according to ECG patterns, men were predominant in the Br, ER, or Br–ER group compared with the true IVF group (Table 1). This sex difference was also noted in previous reports [15–17] and may be partially due to different levels of I_{to} channel expression between sexes [18]. In this regard, we recently reported that 2 novel *KCNE5* mutations produced gain-of-function effects on I_{to} [19]. Because *KCNE5* is located in the X-chromosome, it is interesting that all 3 male mutation carriers were symptomatic and 2 of them displayed Br, whereas only 2 of the 8 female carriers were symptomatic, none of whom had a Br pattern.

4.1. Study limitations

The ER or Br ECG pattern is known to fluctuate temporarily. If a patient had an ER pattern, for example, among several ECG records, we stratified the patient in the ER subgroup (Table 1). However, in some cases, only 1 ECG was available; therefore, it was difficult to exclude the possibility of missing specific ECG patterns that might have been detected by multiple ECG recordings. The ER pattern has been reported to be predominant in 5–10% of general populations [20–22]. A total of 112 controls underwent genetic testing for *KCNJ8*, but their clinical data were not available because of complete anonymity. Some of them may have had an ER ECG pattern. One possible reason why we were not able to identify any *KCNJ8* mutation within our cohort is that the number of our patients ($n=230$) might have been relatively small.

5. Conclusion

We identified no *KCNJ8* gene mutation in our IVF and JWS patients. Therefore, *KCNJ8* seemed to be not a major gene responsible for the JWS or IVF cases in Japan.

Conflict of interest

None

Acknowledgments

We thank the patients for their participation in this study. We thank Arisa Ikeda for helping with the genetic analysis.

References

[1] Haïssaguerre M, Derval N, Sacher F, et al. Sudden cardiac arrest associated with early repolarization. N Engl J Med 2008;358:2016–23.
[2] Antzelevitch C, Yan GX. J wave syndromes. Heart Rhythm 2010;7:549–58.
[3] Haïssaguerre M, Chatel S, Sacher F, et al. Ventricular fibrillation with prominent early repolarization associated with a rare variant of KCNJ8/K_{ATP} channel. J Cardiovasc Electrophysiol 2009;20:93–8.

[4] Medeiros-Domingo A, Tan BH, Crotti L, et al. Gain-of-function mutation S422L in the KCNJ8-encoded cardiac $K_{(ATP)}$ channel kir6.1 as a pathogenic substrate for J-wave syndromes. Heart Rhythm 2010;7:1466–71.
[5] Barajas-Martínez H, Hu D, Ferrer T, et al. Molecular genetic and functional association of brugada and early repolarization syndromes with S422L missense mutation in KCNJ8. Heart Rhythm 2012;9:548–55.
[6] Itoh H, Shimizu W, Hayashi K, et al. Long qt syndrome with compound mutations is associated with a more severe phenotype: a Japanese multicenter study. Heart Rhythm 2010;7:1411–8.
[7] Ohno S, Zankov DP, Yoshida H, et al. N- and C-terminal KCNE1 mutations cause distinct phenotypes of long QT syndrome. Heart Rhythm 2007;4:332–40.
[8] Jongbloed R, Marcelis C, Velter C, et al. Dhplc analysis of potassium ion channel genes in congenital long QT syndrome. Hum Mutat 2002;20:382–91.
[9] Wilde AA, Antzelevitch C, Borggrefe M, et al. Proposed diagnostic criteria for the brugada syndrome: consensus report. Circulation 2002;106:2514–9.
[10] Viskin S. The QT interval: too long, too short or just right. Heart Rhythm 2009;6:711–5.
[11] Miyazaki S, Shah AJ, Haïssaguerre M. Early repolarization syndrome: a new electrical disorder associated with sudden cardiac death. Circ J 2010;74:2039–44.
[12] Antzelevitch C, Yan GX, Viskin S. Rationale for the use of the terms J-wave syndromes and early repolarization. J Am Coll Cardiol 2011;57:1587–90.
[13] Antzelevitch C. Genetic, molecular and cellular mechanisms underlying the J wave syndromes. Circ J 2012;76:1054–65.
[14] Tester DJ, Tan BH, Medeiros-Domingo A, et al. Loss-of-function mutations in the KCNJ8-encoded kir6.1 $K_{(ATP)}$ channel and sudden infant death syndrome. Circ Cardiovasc Genet 2011;4:510–5.
[15] Carrel L, Willard HF. X-inactivation profile reveals extensive variability in X-linked gene expression in females. Nature 2005;434:400–4.
[16] LYON MF. Sex chromatin and gene action in the mammalian X-chromosome. Am J Hum Genet 1962;14:135–48.
[17] Benito B, Sarkozy A, Mont L, et al. Gender differences in clinical manifestations of brugada syndrome. J Am Coll Cardiol 2008;52:1567–73.
[18] Di Diego JM, Cordeiro JM, Goodrow RJ, et al. Ionic and cellular basis for the predominance of the brugada syndrome phenotype in males. Circulation 2002;106:2004–11.
[19] Ohno S, Zankov DP, Ding WG, et al. KCNE5 (KCNE1L) variants are novel modulators of Brugada syndrome and idiopathic ventricular fibrillation. Circ Arrhythmia Electrophysiol 2011;4:352–61.
[20] Klatsky AL, Oehm R, Cooper RA, et al. The early repolarization normal variant electrocardiogram: correlates and consequences. Am J Med 2003;115:171–7.
[21] Mehta MC, Jain AC. Early repolarization on scalar electrocardiogram. Am J Med Sci 1995;309:305–11.
[22] Noseworthy PA, Tikkanen JT, Porthan K, et al. The early repolarization pattern in the general population: clinical correlates and heritability. J Am Coll Cardiol 2011;57:2284–9.