Journal of the American College of Cardiology Vol. 60, No. 15, 2012

Published by Elsevier Inc.
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2012.04.037

Spectrum and Prevalence of Mutations Involving BrS1- Through BrS12-Susceptibility Genes in a Cohort of Unrelated Patients Referred for Brugada Syndrome Genetic Testing

Implications for Genetic Testing

Lia Crotti, MD, PHD,*†‡ Cherisse A. Marcou, BA,§ David J. Tester, BS,§ Silvia Castelletti, MD,*† John R. Giudicessi, BA,§‖ Margherita Torchio, BS,† Argelia Medeiros-Domingo, MD, PHD,§ Savastano Simone, MD,† Melissa L. Will, BS,§ Federica Dagradi, MD,*† Peter J. Schwartz, MD,*†¶#** Michael J. Ackerman, MD, PHD§

Pavia, Italy; Neuherberg, Germany; Rochester, Minnesota; Cape Town and Stellenbosch, South Africa; and Riyadh, Saudi Arabia

Objectives The aim of this study was to provide the spectrum and prevalence of mutations in the 12 Brugada syndrome (BrS)–susceptibility genes discovered to date in a single large cohort of unrelated BrS patients.

Background BrS is a potentially lethal heritable arrhythmia syndrome diagnosed electrocardiographically by coved-type ST-segment elevation in the right precordial leads (V_1 to V_3; type 1 Brugada electrocardiographic [ECG] pattern) and the presence of a personal/family history of cardiac events.

Methods Using polymerase chain reaction, denaturing high-performance liquid chromatography, and DNA sequencing, comprehensive mutational analysis of BrS1- through BrS12-susceptibility genes was performed in 129 unrelated patients with possible/probable BrS (46 with clinically diagnosed BrS [ECG pattern plus personal/family history of a cardiac event] and 83 with a type 1 BrS ECG pattern only).

Results Overall, 27 patients (21%) had a putative pathogenic mutation, absent in 1,400 Caucasian reference alleles, including 21 patients with an *SCN5A* mutation, 2 with a *CACNB2B* mutation, and 1 each with a *KCNJ8* mutation, a *KCND3* mutation, an *SCN1Bb* mutation, and an *HCN4* mutation. The overall mutation yield was 23% in the type 1 BrS ECG pattern-only patients versus 17% in the clinically diagnosed BrS patients and was significantly greater among young men <20 years of age with clinically diagnosed BrS and among patients who had a prolonged PQ interval.

Conclusions We identified putative pathogenic mutations in ~20% of our BrS cohort, with BrS genes 2 through 12 accounting for <5%. Importantly, the yield was similar between patients with only a type 1 BrS ECG pattern and those with clinically established BrS. The yield approaches 40% for *SCN5A*-mediated BrS (BrS1) when the PQ interval exceeds 200 ms. Calcium channel–mediated BrS is extremely unlikely in the absence of a short QT interval.
(J Am Coll Cardiol 2012;60:1410–8) © 2012 by the American College of Cardiology Foundation

Brugada syndrome (BrS) is a rare heritable arrhythmia syndrome associated with an increased risk of sudden cardiac death (SCD) secondary to re-entrant polymorphic ventricular tachycardia and ventricular fibrillation (1). The diagnosis of BrS is based on the presence of coved-type ST-segment elevation in the right precordial leads (V_1 to V_3) on surface electrocardiography, referred to as a type 1 Brugada ECG

See page 1419

From the *Department of Lung, Blood, and Heart, Section of Cardiology, University of Pavia, Pavia, Italy; †Department of Cardiology, La Fondazione IRCCS Policlinico San Matteo di Pavia, Pavia, Italy; ‡Institute of Human Genetics, Helmholtz Zentrum Munich, Neuherberg, Germany; §Departments of Medicine (Division of Cardiovascular Diseases), Pediatrics (Division of Pediatric Cardiology), and Molecular Pharmacology and Experimental Therapeutics, Windland Smith Rice Sudden Death Genomics Laboratory, Mayo Clinic, Rochester, Minnesota; ‖Mayo Medical School, Mayo Clinic, Rochester, Minnesota; ¶Cardiovascular Genetics Laboratory, Hatter Institute for Cardiovascular Research, Department of Medicine, University of Cape Town, Cape Town, South Africa; #Department of Medicine, Stellenbosch University, Stellenbosch, South Africa; and the **Department of Family and Community Medicine, College of Medicine, King Saud University, Riyadh, Saudi Arabia.

pattern, in the absence of structural heart disease or pharmacological agents known to cause a Brugada-like ECG pattern (www.brugadadrugs.org). Additionally, to make a proper diagnosis, the diagnostic ECG pattern should be present together with either personal symptoms, family history of premature SCD, or at least 1 additional relative with a positive type 1 Brugada ECG pattern (2). Due to the transient and dynamic nature of the Brugada ECG pattern, administration of sodium channel blockers (e.g., ajmaline, flecainide, procainamide) are used to unmask the type 1 ECG pattern (2). In general, BrS is understood as a disorder that affects young male adults with an arrhythmogenic manifestation first occurring in the fourth decade of life with sudden death usually occurring during sleep (3,4). However, BrS may also manifest in the young and during infancy, and, when familial, BrS is inherited as an autosomal dominant trait; however, more than half may be sporadic in nature (5).

Over the past 20 years, 12 BrS-susceptibility genes (BrS1 through BrS12) have been identified. Loss-of-function mutations in the *SCN5A*-encoded α-subunit of the cardiac sodium channel (Nav1.5) represents the most common genetic substrate for BrS (annotated as type 1 BrS or BrS1), accounting for 15% to 30% of the disorder (5–7). In addition to sodium channel dysfunction, mutations involving the L-type calcium channel alpha1 (α1), beta2 (β2), and alpha2delta ($\alpha 2\delta$) subunits encoded by *CACNA1C* (8), *CACNB2B* (8) and *CACNA2D1* (9), respectively, may cause 10% to 15% of BrS (9).

Over the past 5 years, 8 of the 12 BrS-susceptibility genes have been identified in which mutations result in: 1) a reduction of the cardiac sodium channel (I_{Na}) current (*GPD1L* [10], *SCN1B* [11], including the alternatively spliced exon 3A, *SCN1Bb* [12], *SCN3B* [13], and *MOG1* [14]); 2) an increase in the transient outward potassium (I_{to}) current (*KCNE3* [15], and *KCND3* [16]); 3) an increase in the I_{KATP} current (*KCNJ8*) (17); or 4) a reduction of the pacemaker (I_f) current (*HCN4*) (18).

Although the relative contribution of *SCN5A* to BrS has been well characterized by several investigating laboratories (19), the molecular genetic contribution of the 12 BrS-susceptibility genes has not been systematically analyzed in a single large cohort of unrelated BrS patients.

In 2011, 2 expert consensus documents were published on the use of genetic testing in the clinical evaluation of cardiac channelopathies and cardiomyopathies (20,21). Although the Heart Rhythm Society (HRS)/European Heart Rhythm Association (EHRA) Expert Consensus Statement (21) recommended genetic testing for patients with a clinical diagnosis of BrS, the Canadian Cardiovascular Society (CCS)/Canadian Heart Rhythm Society (CHRS) joint position paper (20) also advised genetic testing even in the setting of an isolated type 1 Brugada ECG pattern.

In the present study, we provide the spectrum and prevalence of BrS1 through BrS12 associated gene mutations discovered in a large cohort of BrS patients. Furthermore, through the assessment of the genetic testing yield for 46 patients with clinically diagnosed BrS and for 83 unrelated patients with only a type 1 Brugada ECG pattern, we provide, for the first time, concrete data to further guide genetic testing recommendations for these 2 patient populations.

Abbreviations and Acronyms

BrS = Brugada syndrome
CCS = Canadian Cardiovascular Society
CHRS = Canadian Heart Rhythm Society
ECG = electrocardiographic
EHRA = European Heart Rhythm Association
HRS = Heart Rhythm Society
SCD = sudden cardiac death

Methods

Study population. The study population consisted of 46 unrelated patients with clinically diagnosed BrS and 83 unrelated patients having either a spontaneous or drug-induced type 1 Brugada ECG pattern as their sole finding who were referred to either the Windland Smith Rice Sudden Death Genomics Laboratory at the Mayo Clinic, Rochester, Minnesota, or to the Molecular Cardiology Laboratory, La Fondazione IRCCS Policlinico San Matteo di Pavia, Pavia, Italy, for genetic testing. A clinical diagnosis of BrS was made using the strict criteria provided in the Consensus Conference Document (2). Briefly, a clinical diagnosis of BrS was assigned to an individual presenting with a diagnostic type 1 Brugada ECG pattern (coved-type ST-segment elevation in the right precordial V_1 to V_3 leads) either spontaneously and/or after intravenous injection of a sodium channel blocking agent (ajmaline, flecainide, or procainamide) and either a personal or family history of arrhythmic syncope, cardiac arrest, or SCD. Patients with an acquired cause of a type 1 ECG pattern were excluded. Patients reporting palpitations, atypical chest pain, and/or a history of syncope with clinical characteristics strongly suggestive of vasovagal syncope were considered asymptomatic. This study was approved by both the Mayo Foundation Institutional Review Board and the Medical Ethical Committee of La Fondazione IRCCS Policlinico San Matteo di Pavia. Informed consent was obtained from all patients.

ECG analysis. The 12-lead electrocardiograms were recorded at baseline at a paper speed of 25 mm/s. The P-wave duration and PR, QRS, and QT intervals were measured manually from basal electrocardiograms, and the QTc interval was calculated according to Bazett's formula. The

Dr. Ackerman is a consultant for Biotronik, Boston Scientific, Medtronic, St. Jude Medical, Inc., and Transgenomic. Intellectual property derived from Dr. Ackerman's research program resulted in license agreements in 2004 between Mayo Clinic Health Solutions (formerly Mayo Medical Ventures) and PGxHealth (formerly Genaissance Pharmaceuticals, now recently acquired by Transgenomic). All other authors have reported that they have no relationships relevant to the contents of this paper to disclose. This work was supported by the Mayo Clinic Windland Smith Rice Comprehensive Sudden Cardiac Death Program. Mr. Giudicessi was supported by a National Heart, Lung, and Blood Institute Kirschstein NRSA Individual Predoctoral MD/PhD Fellowship (F30-HL106993). The first 2 authors contributed equally to this work.

Manuscript received March 5, 2012; accepted April 4, 2012.

presence of a spontaneous type 1 Brugada ECG pattern was evaluated both on basal electrocardiograms and 12-lead 24-h Holter recordings.

Mutational analysis. After receiving informed consent, a comprehensive open reading frame/splice site mutational analysis of all amino acid coding exons and intron borders of the 12 BrS-susceptibility genes (*SCN5A*, *GPD1L*, *CACNA1C*, *CACNB2B*, *SCN1B* [including the alternatively spliced exon 3A; *SCN1Bb*], *SCN3B*, *KCNE3*, *KCNJ8*, *KCND3*, *CACNA2D1*, *MOG1*, and *HCN4*) was performed using polymerase chain reaction, denaturing high-performance liquid chromatography, and DNA sequencing, as previously described (22). Polymerase chain reaction primer sequences and polymerase chain reaction/denaturing high-performance liquid chromatography conditions are provided in Online Tables 1 to 13.

To be considered a putative pathogenic mutation, the genetic variant had to be: 1) a nonsynonymous variant; and 2) absent in at least 700 ethnically matched controls (≥1,400 reference alleles) plus all available online databases, including the 1000 Human Genome Project database (23). Control genomic DNA was obtained from the European Collection of Cell Cultures (HPA Culture Collections, Salisbury, United Kingdom), the Human Genetic Cell Repository sponsored by the National Institute of General Medical Sciences and the Coriell Institute for Medical Research (Camden, New Jersey), and the Blood Transfusional Center at La Fondazione IRCCS Policlinico San Matteo di Pavia (Pavia, Italy).

Mutations were annotated using the single-letter nomenclature in which F892I, for example, denotes a nonsynonymous single nucleotide substitution producing a missense mutation, whereby the wild-type amino acid phenylalanine (F) has been replaced by an isoleucine (I) at amino acid 892.

Statistical analysis. Comparisons of groups identified on the basis of the clinical characteristics and genotype were performed by univariate analysis. The Student *t* test was used for continuous variables. The Fisher exact test was used for categorical variables and odds ratios for unadjusted data, and their 95% confidence intervals were calculated. Continuous variables are presented as mean ± SD. A 2-sided p value <0.05 was considered statistically significant. GraphPad Prism 3.02 was used for statistical analysis.

Results

Study population. Table 1 summarizes the clinical demographics of the 129 unrelated patients referred for laboratory-based BrS genetic testing. The majority of patients were male (n = 104, 81%) and of Caucasian descent (n = 122, 95%) with a mean age at diagnosis of 43 ± 14 years. Sixteen patients (12%) experienced cardiac events, including 6 (4.6%) with documented aborted cardiac arrest/SCD. A type 1 Brugada ECG pattern (Fig. 1) was present spontaneously in 61 patients (47%), was induced only with a sodium channel blocker in 61 patients (47%) and appeared during fever in the remaining 7 patients. Among those with a spontaneous type 1 ECG pattern, the diagnostic electrocardiogram was not evident in 18% at the time of the

Table 1 Demographics of Unrelated Patients Referred for BrS Genetic Testing

	Overall	Patients With Clinically Diagnosed* BrS	Patients With Type 1 Brugada ECG Pattern Only
Patient demographics			
No. of probands	129	46	83
Age at diagnosis, yrs	43 ± 14	43 ± 15	42 ± 14
Range	8–81	10–81	8–74
Males	104 (81)	28 (61)	76 (92)
Females	25 (19)	18 (39)	7 (8)
Average QTc interval, ms	409 ± 27	410 ± 27	409 ± 27
Average PQ interval, ms	172 ± 29	177 ± 28	170 ± 30
Symptomatic patients	16 (12)	16 (35)	0 (0)
Family history of cardiac events/ unexplained sudden death	37 (29)	37 (80)	0 (0)
Type 1 ST-segment elevation at baseline	61 (47)	30 (65)	31 (37)
Type 1 ST-segment elevation with sodium blockade	68 (53)	16 (35)	52 (63)
Mutation detection yield			
Total yield	27 (21)	8 (17)	19 (23)
Males	20 (19)	8 (29)	12 (16)
Females	7 (28)	0 (0)	7 (100)
SCN5A positive	21 (16)	6 (13)	15 (18)
PQ interval, ms	191 ± 31	199 ± 32	188 ± 31

Values are n, mean ± SD, or n (%). *Clinically diagnosed BrS includes patients with a type 1 Brugada ECG pattern (coved-type ST-segment elevation in the right precordial V_1 to V_3 leads) present either spontaneously and/or after intravenous injection of a sodium channel blocking agent (ajmaline, flecainide, or procainamide) with either a personal and/or family history of arrhythmic syncope, cardiac arrest, or sudden cardiac death.

BrS = Brugada syndrome; ECG = electrocardiographic.

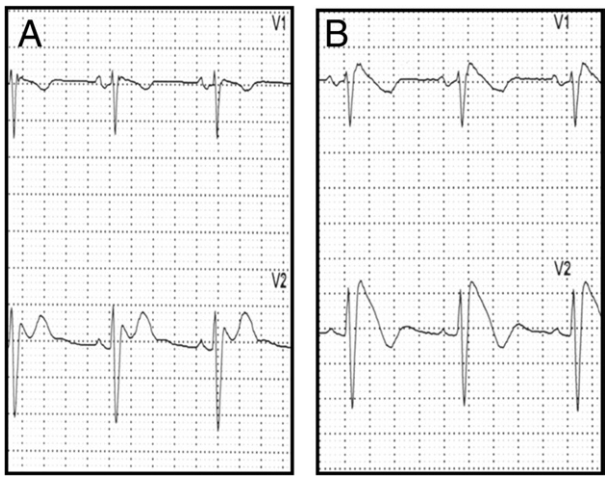

Figure 1 **Diagnostic Brugada Syndrome Electrocardiogram**

Representative electrocardiographic (ECG) traces of a type 1 Brugada ECG pattern not present in basal conditions **(A)**, but appearing during Holter recording **(B)** in a 56-year-old man.

diagnosis but only during follow-up visits (follow-up, 24 ± 14 months). A strict clinical diagnosis of BrS was assigned to 46 patients (28 male patients, average age at diagnosis of 43 ± 15 years, 30 with a spontaneous type 1 ECG pattern, 14 with a drug-induced type 1 ECG pattern, and 2 with a spontaneous ECG pattern during fever; 16 were symptomatic, and 37 had a positive family history of cardiac events). Eighty-three asymptomatic patients (77 male patients, average age, 42 ± 14 years) with no family history were referred for genetic testing solely due to the patient's idiopathic type 1 Brugada ECG pattern that occurred either spontaneously (31 cases) or after intravenous injection of a sodium channel blocker (n = 47) or during fever (n = 5).

Mutational analysis. PUTATIVE PATHOGENIC MUTATIONS. Overall, 27 putative pathogenic mutations (21 *SCN5A*, 2 *CACNB2B*, 1 *KCNJ8*, 1 *KCND3*, 1 *SCN1Bb*, and 1 *HCN4*) were identified in 27 of 129 unrelated BrS patients (21%; 20 male, 7 female) (Table 2). Each mutation was absent in not only ≥1,400 ethnicity-matched reference alleles but also in all publicly available databases including the 1000 Human Genome Project (23).

Of the 21 *SCN5A* mutations, 8 were "radical" mutations (5 nonsense and 2 insertion/deletion frameshift mutations, and 1 splice site mutation) 12 were missense mutations localizing to the N-terminus (n = 1), the transmembrane spanning region (n = 1, IS1 through IS4; n = 3, IIS1 through S4; n = 3, IIIS1 through S4; or n = 1, IVS1 through S4), the linker domain (n = 2, DIII through DIV), or the C-terminus (n = 1), and 1 was an in-frame deletion (Fig. 2). However, it must be recognized that nearly 2% of healthy Caucasians and 4% of seemingly healthy non-Caucasians also host missense *SCN5A* variants, leading to a potential conundrum in the interpretation of the genetic test results (24).

Importantly, none of the Nav1.5 missense mutations resided in low probability of pathogenicity regions of the channel (i.e., DI through DII or DII through DIII linker regions), where the vast majority of rare variants identified in healthy control populations reside (25). Instead, 5 of 12 (42%) *SCN5A* missense mutations resided in the critical pore-forming or S4 voltage-sensing regions of Nav1.5. Putative pathogenic mutations in all other genes (*CACNB2B*, *KCNJ8*, *KCND3*, *SCN1B*, and *HCN4*) were missense mutations.

Although 21 patients (16.3%) hosted *SCN5A* mutations overall, only 6 patients (4.6%) were identified with a mutation in 1 of the 11 other BrS-susceptibility genes (Table 2). Two patients (1.5%) were identified with a mutation in an auxiliary L-type calcium channel subunit. An asymptomatic 60-year-old Caucasian man, presenting with a spontaneous type 1 ECG pattern, a QTc interval of 428 ms, and a positive family history was identified as having E499D-CACNB2B and an asymptomatic 58-year-old Caucasian woman with a spontaneous type 1 ECG pattern, a QTc interval of 447 ms, and a negative family history was identified with V340I-CACNB2B. The remaining 4 patients had 1 of the following mutations: KCNJ8-S422L, KCND3-G600R, SCN1Bb-Q204R, and HCN4-S841L (Table 2). The 2 patients carrying KCNJ8-S422L and KCND3-G600R were described previously, and both mutations conferred a marked gain-of-function to their respective potassium channel (16,17). No mutations were identified in *KCNE3*, *SCN3B*, *GPD1L*, or *MOG1*.

RARE GENETIC VARIANTS. In addition to these aforementioned putative BrS-associated mutations, 11 uncommon nonsynonymous genetic variants (present in published and internal controls with a measurable frequency >0.01% but <1%) were identified in 13 additional patients (Table 3). Seven of the 11 rare variants (S216L-SCN5A [26], R1512W-SCN5A [27], R214Q-SCN1Bb [28], S160T-CACNB2B [9] [identified in 2 patients], S709N-CACNA2D1 [9] [2 patients], S755T-CACNA2D1 [29], and L450F-KCND3 [16]) have been characterized functionally as electrophysiologically abnormal and/or associated with BrS or other genetically transmitted arrhythmogenic diseases linked to BrS (i.e., short QT syndrome and early repolarization syndrome), suggesting that these genetic variants may contribute to the development of BrS. However, despite their previous implication in these various disease states, we chose to be ultraconservative and did not consider these variants as disease-causing mutations due to their presence in ostensibly healthy controls and therefore excluded them from our overall yield and genotype–phenotype correlations.

Influence of phenotype on the mutation detection yield. To better understand potential phenotypic effects on the yield of mutational analysis, we further divided our 129-patient cohort into 8 specific phenotypic categories: 1) asymptomatic, no family history, spontaneous ECG pattern only (n = 31); 2) asymptomatic, no family history, drug-induced ECG pattern only (n = 52); 3) asymptomatic,

JACC Vol. 60, No. 15, 2012
October 9, 2012:1410–8

Table 2 Summary of Brugada Syndrome-Associated Mutations

Patient #	Gene	Exon	Nucleotide Change	Mutation	Mutation Type	Location	Age, yrs	Sex	Symptomatic (Yes/No)	Family History (Yes/No)
Major BrS genotype SCN5A										
1	*SCN5A*	2	80G>A	R27H	Missense	N-terminal	8	M	No	No
2	*SCN5A*	2	127C>T	R43X*	Nonsense	N-terminal	19	M	No	Yes
3	*SCN5A*	4	477 T>A	Y159X*	Nonsense	DI–S2	40	M	No	No
4	*SCN5A*	16	2466G>T	W822C*	Missense	DI–S4	24	M	Yes	No
5	*SCN5A*	16	2632C>T	R878C	Missense	DII–S5/S6	34	M	No	No
6	*SCN5A*	16	2674T>A	F892I*	Missense	DII–S5/S6	30	F	No	No
7	*SCN5A*	17	3175C>T	Q1059X*	Nonsense	DII–DIII	38	M	No	No
8	*SCN5A*	17	3175C>T	Q1059X*	Nonsense	DII–DIII	16	M	Yes	No
9	*SCN5A*	18	3352C>T	Q1118X	Nonsense	DII–DIII	51	M	No	No
10	*SCN5A*	21	3673G>A	E1225K	Missense	DIII–S1	46	F	No	No
11	*SCN5A*	21	3673G>A	E1225K	Missense	DIII–S1	43	M	No	No
12	*SCN5A*	21	3806A>G	N1269S*	Missense	DIII–S2/S3	53	M	No	Unknown
13	*SCN5A*	23	4140C>G	N1380K*	Missense	DIII–S5/S6	31	M	Yes	No
14	*SCN5A*	Intron24	4299+1 G>T	G1433sp	Splice	DIII–S5/S6	10	M	No	Yes
15	*SCN5A*	26	4501C>G	L1501V	Missense	DIII–DIV	55	M	No	No
16	*SCN5A*	28	4849-4851delTTC	F1617del	In-frame del	DIV–S3/S4	49	F	No	No
17	*SCN5A*	28	4952-4953insT	L1650+137X*	Frame shift	DIV–S4/S5	16	F	No	No
18	*SCN5A*	28	5150T>C	L1717P*	Missense	DIV–S5/S6	44	M	No	No
19	*SCN5A*	28	5227 G>A	G1743R	Missense	DIV–S5/S6	14	M	Yes	Yes
20	*SCN5A*	28	5324delT	N1774+11X*	Frame shift	DIV–S6	34	M	No	No
21	*SCN5A*	28	5494C>G	Q1832E	Missense	C-terminal	28	M	No	No
Minor BrS genotypes										
22	*CACNB2B*	10	1018G>A	V340I*	Missense	GK domain	58	F	No	No
23	*CACNB2B*	13	1497G>C	E499D*	Missense	C-terminal	60	M	No	Yes
24	*HCN4*	8	2522C>T	S841L	Missense	Cytoplasmic	64	F	No	No
25	*KCND3*	7	1798G>A	G600R	Missense	Cytoplasmic	22	M	No	Yes
26	*KCNJ8*	2	1265C>T	S422L*	Missense	Cytoplasmic	30	M	No	No
27	*SCN1βb*	3A	611A>G	Q204R*	Missense	Cytoplasmic	41	F	No	No

*Novel mutation for this cohort.
BrS = Brugada syndrome.

positive family history, spontaneous ECG pattern (n = 17); 4) asymptomatic, positive family history, drug-induced ECG pattern only (n = 13); 5) symptomatic, no family history, spontaneous ECG (n = 6); 6) symptomatic, no family history, drug-induced ECG only (n = 3); 7) symptomatic, positive family history, spontaneous ECG pattern (n = 7); and 8) symptomatic, positive family history, drug-induced ECG pattern only (n = 0). Although the overall mutation discovery yield was 21%, this yield ranged from a low of 0% for those patients with symptoms, no family history, and a drug-induced ECG pattern (0 of 3 patients) to as high as 50% for those patients with symptoms, no family history, and a spontaneous ECG pattern (3 of 6 patients). However, none of these differences in yield achieved statistical significance due to the small sample sizes of each subcategory (Fig. 3). Notably, there was no difference in mutation detection yield between those patients with solely a spontaneous or drug-induced type 1 Brugada ECG pattern (19 of 83 patients, 23%) compared with those who fully satisfied the current clinical definition of BrS (8 of 46 patients, 17%, p = 0.51) (Table 1).

An interesting effect of age on the mutation detection yield was observed, especially among male patients. Overall, the 20 mutation-positive males were younger (age, 33 ± 15 years) compared with the 84 mutation-negative male patients (age, 43 ± 12 years) (p = 0.001). However, there was no real difference in average age between mutation-positive (age, 43 ± 16 years) and mutation-negative female patients (age, 51 ± 17 years) (p = 0.34). The overall yield was significantly greater among BrS patients younger than 20 years of age (6 of 8 patients, 75%) compared with patients between 20 and 40 years of age (10 of 46 patients, 22%) and those older than 40 years of age (11 of 75 patients, 15%) (p = 0.0003) (Fig. 4). This significant difference was even more striking when comparing male patients only (<20 years of age [5 of 6 patients], 83%; 20 to 40 years of age [9 of 42 patients], 21%; >40 years of age [6 of 56 patients], 11%) (p < 0.0001). The female patient group was too small to draw any meaningful conclusions (<20 years of age [1 of 2 patients], 50%; 20 to 40 years of age [1 of 4 patients], 24%; >40 years of age [5 of 19 patients], 26%). Interestingly, however, this age effect on the mutation detection yield was only evident among the 46 clinically certain BrS patients (<20 years of age [4 of 4 patients], 100%; 20 to 40 years of age [3 of 13 patients], 23%; >40 years of age, [1 of 29 patients], 3%) (p < 0.0001) compared with the 83

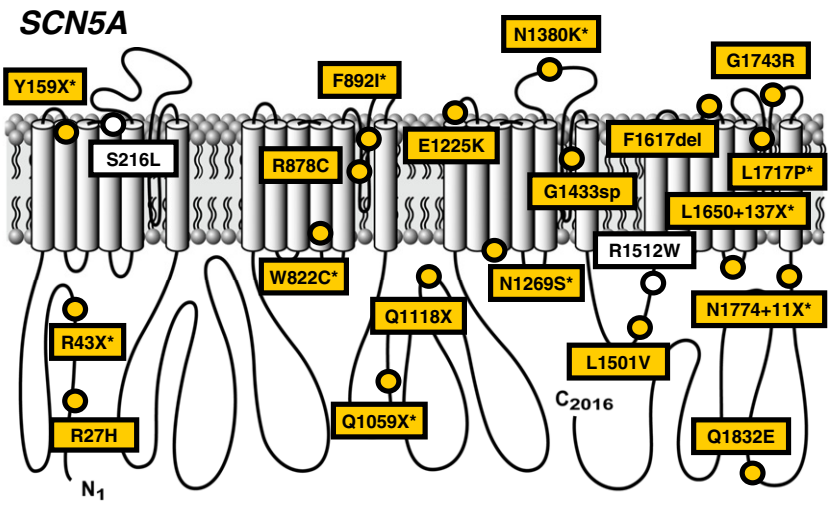

Figure 2 **Channel Topology of the Nav1.5 Pore-Forming Alpha Subunit Encoded by *SCN5A* and the Location of BrS-Associated Mutations**

Putative pathogenic *SCN5A* BrS-associated mutations, absent in at least 700 Caucasian controls, identified in this study are indicated by **yellow circles**, and nonsynonymous functional polymorphisms are indicated by **open circles**. *Mutation novel to this cohort. BrS = Brugada syndrome.

referral patients with only a type 1 Brugada ECG pattern (<20 years of age [2 of 4 patients], 50%; 20 to 40 years of age [7 of 33 patients], 21%; >40 years of age [10 of 46 patients], 22%) (p = 0.42).

Genotype–phenotype correlations. When comparing *SCN5A* mutation–positive patients with those patients who were *SCN5A* negative, the PQ interval was significantly longer (191 ± 31 ms vs. 169 ± 28 ms, p = 0.0015). In fact, an *SCN5A* mutation was identified in 39% of those with a PQ interval ≥200 ms compared with only 8% of those with a PQ interval <200 ms (odds ratio: 7; 95% CI: 3 to 20; p < 0.0001). For the subset of patients with an isolated, idiopathic type 1 ECG pattern only, 38% of the patients with a PQ interval >200 ms were *SCN5A* positive compared with 11% with a PQ interval <200 ms (OR: 8; 95% CI: 1.5 to 16; p = 0.006). Due to the rarity of mutations identified in BrS2 through BrS12 genes, we are unable to provide genotype–phenotype correlations for these specific BrS genotypes. However, none of the patients in this cohort had a QTc interval <350 ms, which might explain the total absence of *CACNA1C*-mediated BrS.

Discussion

In 2011, 2 consensus documents were published on the diagnostic, prognostic, and therapeutic impact of genetic testing in the clinical evaluation of cardiac channelopathies and cardiomyopathies (20,21). For BrS, both documents recommended genetic testing for any patient for whom there is a clinical suspicion of BrS and emphasized the importance of genetic testing of the index case in relation to overall family screening. Although the HRS/EHRA expert

Table 3 **Summary of Additional Rare Genetic Variants Identified in BrS Probands**

Gene	Exon	Nucleotide Change	Mutation	No. of BrS Patients With the Genetic Variant (N = 129)	Frequency in Controls (Caucasians)	Results Summary From Published Cellular Electrophysiology Functional Studies	Associated Disease
SCN5A	6	647C>T	S216L	1	4/1,300 (18)	I_{NA} loss of function (26)	BrS
SCN5A	26	4534C>T	R1512W	1	1/1,300 (18)	Slow inactivation recovery of I_{NA} (27)	BrS
CACNA1C	17	2449C>T	P817S	1	4/476		
CACNA1C	42	5150C>G	A1717G	1	0/796		
CACNA1C	46	5918G>A	R1973Q	1	1/489		
CACNB2B	6	479G>C	S160T	2	3/507		ERS
CACNA2D1	26	2126G>A	S709N	2	3/466		BrS
CACNA2D1	28	2264G>C	S755T	1	2/798	I_{Ca} loss of function (29)	SQTS
KCNE3	1	248G>A	R83H	1	2/300		
KCND3	3	1348C>T	L450F	1	1/800	I_{to} gain of function (16)	BrS
SCNIBb	3A	641G>A	R214Q	1	4/807	I_{NA} loss of function (28)	SIDS

BrS = Brugada syndrome; ERS = early repolarization syndrome; SQTS = short QT syndrome; SIDS = sudden infant death syndrome.

JACC Vol. 60, No. 15, 2012
October 9, 2012:1410–8

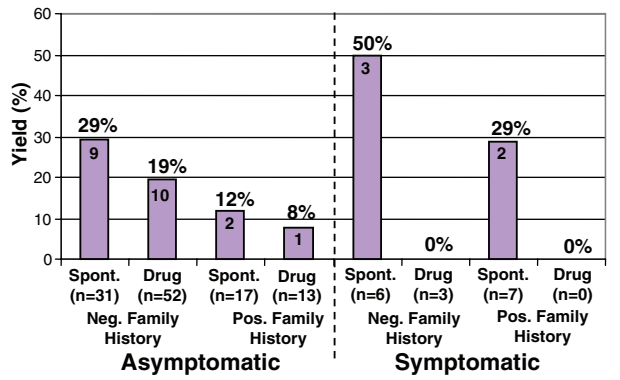

Figure 3 **Influence of Phenotype on the Mutation Detection Yield**

Depicted is a bar graph comparing the percentage of yield of the 8 specific phenotypic categories showing the phenotypic effects on overall mutation yield of our cohort. The number in each column is the number of patients with a mutation, and the percentage of yield is given at the top of each column. Neg. = negative; Pos. = positive; Spont. = spontaneous.

consensus statement (21) indicated that either a comprehensive or (BrS1) *SCN5A*-targeted genetic screen "may be useful," the CCS/ CHRS joint position paper (20) advised limiting BrS genetic testing to only *SCN5A* and to only consider the minor genes (BrS genes 2 through 12) under special circumstances. In addition, although the HRS/ EHRA group advised genetic testing for those patients who fulfill the task force criteria for a clinical diagnosis of BrS, which require an expressed type 1 Brugada ECG pattern plus 1 or more clinical variables from the patient's personal or family history (such as unexplained syncope or a family history of SCD), the CCS/CHRS group recommended genetic testing in both clinically diagnosed BrS patients and asymptomatic patients with only a type 1 Brugada ECG pattern.

Drawn from the largest cohort of unrelated patients referred for BrS genetic testing to be systematically analyzed for mutations in the 12 known BrS-susceptibility genes (as of June 1, 2011), there are several key observations that may further buttress and refine these expert opinion recommendations. First, our results show that there was no significant difference in mutation detection yield between those patients who fully satisfied the clinical definition of BrS (17%) and those patients with only a diagnostic type 1 Brugada ECG pattern (23%), suggesting that BrS genetic testing may be equally warranted for patients with solely an electrocardiographic manifestation of a type 1 Brugada ECG pattern for the main purpose of identifying probands and their family members who should take precautionary measures in certain conditions. This observation lends evidence to support the CCS/CHRS position on genetic testing of asymptomatic individuals and suggests that perhaps the HRS/EHRA recommendation (requiring both abnormal findings on an electrocardiogram and personal symptoms or family history) may be too strict. As implied in the Canadian guidelines, a positive *SCN5A* genetic test result plus a spontaneous/drug-induced type 1 Brugada ECG pattern may be sufficient for the clinical diagnosis of BrS. However, one must be mindful that the presence of a positive genetic test result is not predictive of clinical symptoms because there are pedigrees with probands that exhibit incomplete penetrance and a lifelong asymptomatic course. Nevertheless, if our observation is validated, then the genetic test might become part of the diagnostic criteria akin to the revised criteria for both arrhythmogenic right ventricular cardiomyopathy and Marfan syndrome. Furthermore, the identification of a mutation positive subject would at least enable the simple Brugada preventive measures of avoiding certain drugs and reducing the degree of hyperthermia in the setting of febrile illnesses.

Second, whether dealing with patients with either BrS or only a type 1 Brugada ECG pattern, the minor BrS-susceptibility genes are indeed minor. In other words, the sensitivity of the BrS genetic test is affected minimally by their inclusion, lending additional merit for the option of *SCN5A* (BrS1) only genetic testing rather than so-called comprehensive, multigene BrS genetic testing. This notion of targeted genetic testing for BrS was supported by both guidelines as well. In addition, this study provides useful pre-genetic test anticipatory guidance as to its pre-test probability of returning positive results. Previously, analysis of *SCN5A* among >2,000 patients, derived from 9 different cohorts of BrS patients throughout the world, indicated an 11% to 28% yield (average = 21%) for possible/probable BrS1 status (19). Consistent with that range, the overall yield in this study was 16.3% for *SCN5A*-mediated BrS (i.e., BrS1). However, the yield was far greater among young men

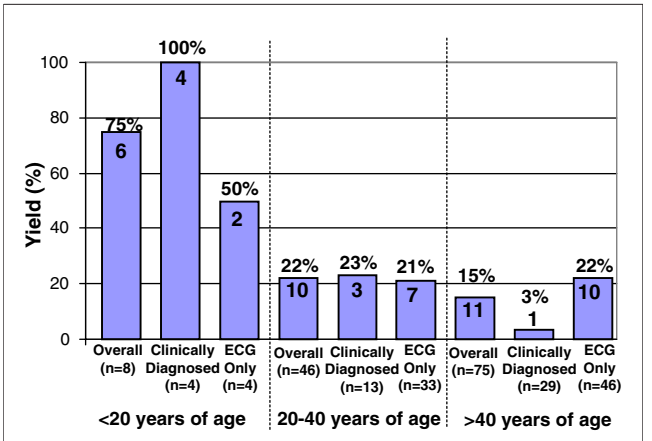

Figure 4 **Effect of Age on the Mutation Detection Yield for the Overall, Clinically Diagnosed, and Type 1 ECG Pattern-Only Cohorts**

Depicted is a bar graph showing the percentage of yield of the 3 different age groups (<20 years of age, 20 to 40 years of age, >40 years of age) for the overall, clinically diagnosed, and type 1 Brugada ECG pattern–only cohorts. The number in each column is the number of patients with a mutation, and the percentage of yield is given at the top of each column. ECG = electrocardiographic.

<20 years of age with clinically manifest BrS and among those with BrS and a prolonged PQ interval. Compared with a <10% yield for positive *SCN5A* test results for those with a PQ interval <200 ms, the yield was almost 40% when the PQ interval ≥200 ms. This is consistent with previous observations that *SCN5A*-positive BrS patients displayed prolonged HV and PQ intervals during electrophysiology study (30,31).

Finally, in the absence of a short QT interval, calcium channel–mediated BrS is extremely uncommon. Here, mutations in genes encoding the alpha1 (*CACNA1C*), beta2 (*CACNB2B*), and alpha2delta1 (*CACNA2D1*) were observed in <2% of this cohort. In contrast, perturbations involving the calcium channel macromolecular complex were implicated as the second most common genetic cause of BrS, accounting for 12% of the disease, and as much as 18% when including rare polymorphisms (9). A close examination of those seminal discoveries underscores the tight link between calcium channel–mediated disease and the clinical phenotype of BrS with a concomitant short QT interval, in which 50% of patients with BrS/short QT interval hosted a mutation in an L-type calcium channel subunit (9). Once again, this illustrates the critical importance of the phenotypic classification and the opportunity for phenotype-guided genetic testing within what is currently captured under the header of BrS. Given the increased recognition of so-called background genetic noise or potential false-positive results with respect to genetic testing for heritable arrhythmias and/or cardiomyopathic syndromes (25,32), phenotype-targeted testing within the spectrum of J-wave syndromes may minimally compromise the test's sensitivity while significantly enhancing its specificity. Just like an *SCN5A*-centric genetic test for classic BrS, especially with concomitant PQ interval prolongation, these data would suggest similar consideration for primary genetic testing of the genes that encode the calcium channel's pore-forming subunit and its auxiliary subunits rather than *SCN5A*, for the ECG phenotype of type 1 Brugada ECG pattern with concomitant short QT intervals.

Study limitations. There are 2 major limitations of this study, one dealing with the veracity of the phenotype and the other dealing with the certainty of the genotype. Although the ECG phenotype was vetted for every one of the patients in this study by at least 1 of the authors, not every patient in this study was evaluated clinically by the authors because our study population consisted of patients referred to our laboratories for genetic testing. Accordingly, for the 83 patients with only a type 1 Brugada ECG pattern, because we did not personally ask the questions to elicit the history for every patient in our cohort, we do not know that the personal and family history was indeed negative. However, this potential failure to elicit the clinical information is unlikely because 1 of the coauthors (L.C.) directly evaluated >80% of the patients in this cohort, and the same referring physicians submitted patients with detailed characterization of positive symptomatology. With respect to the genotype, the major limitation of this study pertains to the issue of mutation calling (19,24,25). None of the 15 novel mutations reported in this study cohort have been characterized functionally. Instead, strict absence from our internal set of ethnic-matched reference alleles and all available online databases, including the 1000 Human Genome Project database (23), was required. Despite this stringent bar to establish rarity (i.e., not seen in >3,000 reference alleles), it is possible that some of these variants listed as putative pathogenic mutations may be innocuous functionally. Conversely, this strict "absence from all controls" definition may have resulted in the exclusion of other variants that are nevertheless disease contributing. *S216L-SCN5A* and *R1512W-SCN5A* illustrate this possibility. In vitro expression of *S216L-SCN5A* channels was associated with a 60% reduction in peak sodium current and moderate slowing of inactivation (26). Similarly, in vitro studies of *R1512W-SCN5A* demonstrated slower inactivation and recovery from inactivation consistent with a loss-of-function phenotype anticipated for BrS1 mutations (27). However, both *S216L* and *R1512W* have been previously reported in ostensibly healthy controls. Assignment of a given variant's pathogenicity is a vexing problem and has been declared the Achilles' heel of genetic testing (21,25,33). This issue underscores the critical need to align carefully the phenotype with the appropriate genetic test panel that best balances the sensitivity/specificity issue rather than simply continuing to add the next novel disease-susceptibility gene.

Conclusions

To our knowledge, this study is the first comprehensive mutational analysis of all 12 BrS-susceptibility genes discovered to date for a single large cohort of BrS patients. *SCN5A*-mediated BrS (BrS1) is still the only common genetic substrate for BrS in general, particularly for young male BrS patients (<20 years) and those BrS patients with a PQ interval ≥ 200 ms who may have a 40% to 50% pre-test probability for a positive *SCN5A* genetic test result. In addition, our data suggest that both clinically diagnosed BrS patients and patients presenting with only a spontaneous/drug-induced type 1 Brugada ECG pattern may equally warrant *SCN5A* genetic testing. Finally, the other 11 BrS genes account for <5% of our cases, and in the absence of BrS plus a short QT interval, calcium channel–mediated BrS is very uncommon, far less than the initial 10% to 15% estimates.

Acknowledgments

The authors thank Matteo Pedrazzini, Alessandra Cuoretti, Alessandra Mugione, and Giuseppe Celano for their technical support.

Reprint requests and correspondence: Dr. Michael J. Ackerman, Mayo Clinic Windland Smith Rice Sudden Death Genomics Laboratory, Guggenheim 501, Mayo Clinic, Rochester, Minnesota 55905. E-mail: ackerman.michael@mayo.edu.

REFERENCES

1. Brugada P, Brugada J. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome. A multicenter report. J Am Coll Cardiol 1992;20:1391–6.
2. Antzelevitch C, Brugada P, Borggrefe M, et al. Brugada syndrome: report of the Second Consensus Conference. Heart Rhythm 2005;2:429–40.
3. Ruan Y, Liu N, Priori SG. Sodium channel mutations and arrhythmias. Nat Rev Cardiol 2009;6:337–48.
4. Shimizu W. Clinical impact of genetic studies in lethal inherited cardiac arrhythmias. Circ J 2008;72:1926–36.
5. Schulze-Bahr E, Eckardt L, Breithardt G, et al. Sodium channel gene (SCN5A) mutations in 44 index patients with Brugada syndrome: different incidences in familial and sporadic disease [published correction appears in Hum Mutat 2005;26:61]. Hum Mutat 2003;21:651–2.
6. Priori SG, Napolitano C, Gasparini M, et al. Clinical and genetic heterogeneity of right bundle branch block and ST-segment elevation syndrome: a prospective evaluation of 52 families. Circulation 2000;102:2509–15.
7. Priori SG, Napolitano C, Gasparini M, et al. Natural history of Brugada syndrome: insights for risk stratification and management. Circulation 2002;105:1342–7.
8. Antzelevitch C, Pollevick GD, Cordeiro JM, et al. Loss-of-function mutations in the cardiac calcium channel underlie a new clinical entity characterized by ST-segment elevation, short QT intervals, and sudden cardiac death. Circulation 2007;115:442–9.
9. Burashnikov E, Pfeiffer R, Barajas-Martinez H, et al. Mutations in the cardiac L-type calcium channel associated with inherited J-wave syndromes and sudden cardiac death. Heart Rhythm 2010;7:1872–82.
10. London B, Michalec M, Mehdi H, et al. Mutation in glycerol-3-phosphate dehydrogenase 1 like gene (GPD1-L) decreases cardiac Na+ current and causes inherited arrhythmias. Circulation 2007;116:2260–8.
11. Watanabe H, Koopmann TT, Le Scouarnec S, et al. Sodium channel beta1 subunit mutations associated with Brugada syndrome and cardiac conduction disease in humans. J Clin Invest 2008;118:2260–68.
12. Hu D, Barajas-Martinez H, Medeiros-Domingo A, et al. A novel rare variant in SCN1Bb linked to Brugada syndrome and SIDS by combined modulation of Na(v)1.5 and K(v)4.3 channel currents. Heart Rhythm 2012;9:760–9.
13. Hu D, Barajas-Martinez H, Burashnikov E, et al. A mutation in the beta3 subunit of the cardiac sodium channel associated with Brugada ECG phenotype. Circ Cardiovasc Genet 2009;2:270–8.
14. Kattygnarath D, Maugenre S, Neyroud N, et al. MOG1: a new susceptibility gene for Brugada syndrome. Circ Cardiovasc Genet 2011;4:261–8.
15. Delpón E, Cordeiro JM, Nuñez L, et al. Functional effects of KCNE3 mutation and its role in the development of Brugada syndrome 10.1161/CIRCEP.107.748103. Circ Arrhythm Electrophysiol 2008;1:209–18.
16. Giudicessi JR, Ye D, Tester DJ, et al. Transient outward current (I(to)) gain-of-function mutations in the KCND3-encoded Kv4.3 potassium channel and Brugada syndrome. Heart Rhythm 2011;8:1024–32.
17. 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.
18. Ueda K, Hirano Y, Higashiuesato Y, et al. Role of HCN4 channel in preventing ventricular arrhythmia. J Hum Genet 2009;54:115–121.
19. Kapplinger J, Tester D, Alders M, et al. An international compendium of mutations in the SCN5A-encoded cardiac sodium channel in patients referred for Brugada syndrome genetic testing. Heart Rhythm 2010;7:33–46.
20. Gollob MH, Blier L, Brugada R, et al. Recommendations for the use of genetic testing in the clinical evaluation of inherited cardiac arrhythmias associated with sudden cardiac death: Canadian Cardiovascular Society/Canadian Heart Rhythm Society joint position paper. Can J Cardiol 2011;27:232–45.
21. Ackerman MJ, Priori SG, Willems S, et al., Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies: this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). Heart Rhythm 2011;8:1308–39.
22. Ackerman MJ, Tester DJ, Jones G, Will MK, Burrow CR, Curran M. Ethnic differences in cardiac potassium channel variants: implications for genetic susceptibility to sudden cardiac death and genetic testing for congenital long QT syndrome. Mayo Clin Proc 2003;78:1479–87.
23. A map of human genome variation from population-scale sequencing. Nature 2010;467:1061–73.
24. Ackerman MJ, Splawski I, Makielski JC, et al. Spectrum and prevalence of cardiac sodium channel variants among black, white, Asian, and Hispanic individuals: implications for arrhythmogenic susceptibility and Brugada/long QT syndrome genetic testing. Heart Rhythm 2004;1:600–7.
25. Kapa S, Tester DJ, Salisbury BA, et al. Genetic testing for long-QT syndrome: distinguishing pathogenic mutations from benign variants. Circulation 2009;120:1752–60.
26. Marangoni S, Di Resta C, Rocchetti M, et al. A Brugada syndrome mutation (p.S216L) and its modulation by p.H558R polymorphism: standard and dynamic characterization. Cardiovasc Res 2011;91:606–16.
27. Deschênes I, Baroudi G, Berthet M, et al. Electrophysiological characterization of SCN5A mutations causing long QT (E1784K) and Brugada (R1512W and R1432G) syndromes. Cardiovasc Res 2000;46:55–65.
28. Hu D, Barajas-Martinez H, Burashnikov E, et al. A novel mutation in SCN1BB linked to Brugada syndrome by modulating Nav1.5 and Kv4.3. Heart Rhythm 2010;7:S320.
29. Templin C, Ghadri J-R, Rougier J-S, et al. Identification of a novel loss-of-function calcium channel gene mutation in short QT syndrome (SQTS6). Eur Heart J 2011;32:1077–88.
30. Smits JP, Eckardt L, Probst V, et al. Genotype-phenotype relationship in Brugada syndrome: electrocardiographic features differentiate SCN5A-related patients from non-SCN5A-related patients. J Am Coll Cardiol 2002;40:350–6.
31. Meregalli PG, Tan HL, Probst V, et al. Type of SCN5A mutation determines clinical severity and degree of conduction slowing in loss-of-function sodium channelopathies. Heart Rhythm 2009;6:341–8.
32. Kapplinger JD, Landstrom AP, Salisbury BA, et al. Distinguishing arrhythmogenic right ventricular cardiomyopathy/dysplasia-associated mutations from background genetic noise. J Am Coll Cardiol 2011;57:2317–27.
33. Landstrom AP, Ackerman MJ. The Achilles' heel of cardiovascular genetic testing: distinguishing pathogenic mutations from background genetic noise. Clin Pharmacol Ther 2011;90:496–99.

Key Words: Brugada syndrome ■ cardiac arrest ■ genetic testing ■ ST-segment elevation ■ ventricular arrhythmias.

APPENDIX

For supplemental tables, please see the online version of this article.