Jeong *et al.*
International Journal of Arrhythmia (2024) 25:12
https://doi.org/10.1186/s42444-024-00121-w

International Journal of Arrhythmia

RESEARCH

Open Access

Clinical role of genetic testing for the Brugada syndrome overlapping with arrhythmogenic cardiomyopathy

Joo Hee Jeong[1], Hyoung Seok Lee[1], Yun Young Choi[1], Yun Gi Kim[1], Jaemin Shim[1], Jin Ha Hwang[2], Seung Gyu Yun[2], Yun Jung Cho[2], Young-Hoon Kim[1] and Jong-Il Choi[1*]

Abstract

Background Brugada syndrome (BrS) and arrhythmogenic cardiomyopathy (ACM) are inherited cardiac diseases that may predispose to ventricular arrhythmia. Although overlapping features between BrS and ACM have been demonstrated previously, it remains to be determined whether genetic testing for ACM-related genes is needed in BrS probands.

Method Based on a single-center, retrospective registry of BrS, we aimed to verify genetic profiles of BrS using a next-generation sequencing panel, and further analyzed genetic testing of ACM-related variants in Brugada phenotypes.

Results Among a total of 119 Brugada phenotypes, 114 patients (95.8%) were male and the mean age of onset was 43.6 years. Genetic variants were identified in 25 of the 42 patients who underwent genetic testing. Fifteen patients had BrS-related genotypes, including *SCN5A* in 6 patients, and non-*SCN5A* genes in 9 patients (*SCN10A, HCN4, SCN3B, and KCNE3*). Nineteen patients underwent additional genetic testing with cardiomyopathy panel, which revealed ACM-related genotypes (2 *PKP2*, 1 *DSG2*, 1 *TMEM43*, 1 *JUP*, and 1 *DSP*) present in 6 patients (31.5%). None of the patients had structural or electrocardiographic features that fulfilled the diagnostic criteria for ACM.

Conclusions In clinical setting, ACM-related genes were identified in a significant proportion of Brugada phenotypes, supporting the argument that genetic testing of ACM overlapping is needed. Follow-up imaging studies should be considered to monitor if the disease progresses to ACM.

Keywords Brugada syndrome, Arrhythmogenic cardiomyopathy, High-throughput nucleotide sequencing

Introduction

Brugada syndrome (BrS) and arrhythmogenic cardiomyopathy (ACM) are considered as distinct disease entities that predispose patients to sudden cardiac arrest (SCA) [1]. BrS is an inherited disorder of voltage-gated ion channels that cause a dysfunction of cardiac ion current. BrS predisposes to the 20% of the SCAs with structurally normal heart, which are more frequently found in East Asian population [2–4]. More than 500 pathogenic variants have been discovered to be associated with BrS [5]. Despite the diverse genetic heterogeneity of BrS, the majority of the pathogenic

*Correspondence:
Jong-Il Choi
jongilchoi@korea.ac.kr
[1] Division of Cardiology, Department of Internal Medicine, Korea University College of Medicine and Korea University Anam Hospital, Seoul, Republic of Korea
[2] Department of Laboratory Medicine, Korea University College of Medicine, Seoul, Republic of Korea

variants are found in *SCN5A* gene, which encodes for an alpha subunit of voltage-gated sodium channel (Na_V 1.5) [6, 7]. ACM is a disease of the myocardial substrate that presents as fatty fibrous replacement of the right ventricle. At the molecular level, ACM exhibits genetic alteration of cardiac desmosomes, which are located in the intercalated discs in the adhering junctions of cardiomyocytes [1, 8]. Accordingly, desmosomal genetic mutations lead to a mechanical overload of the cell–cell junctions, triggering a pathophysiological myocardial change found in ACM [9]. Furthermore, ACMs express a complex genotype–phenotype relationship, including incomplete penetrance and variable phenotype expression [8].

Despite different pathogenic mechanisms of BrS and ACM, consistent evidence indicates the presence of their overlapping features [1, 10]. Although current guidelines recommend gene testing for a single gene (*SCN5A*) in patients with BrS, overlapping features of BrS and ACM found in experimental studies raise questions on the necessity of genetic testing for ACM-related variants in BrS. This study aimed to analyze the genetic background of BrS patients and assess the significance of ACM-related genetic sequencing using a multigene panel.

Methodology

Study population

This is a single-center retrospective study based on clinical data obtained from 1998 to 2021. Data were extracted from Brugada registry of Korea University Anam Hospital, which consists of 119 patients who were clinically suspected or confirmed as BrS. Patients with Brugada phenotypes were enrolled, which includes: (1) patients who were confirmed as BrS with type 1 Brugada pattern electrocardiography (ECG, a coved-type ST-segment elevation ≥ 2 mm followed by a negative T-wave in ≥ 1 right precordial leads V_1 to V_2) either spontaneously or after a provocation with flecainide (defined as confirmed BrS) and (2) patients with type 2 or 3 Brugada pattern ECG and associated symptoms of syncope, ventricular arrhythmia, or SCA (defined as suspected BrS). Patients with all other possible causes of ST-segment elevation in the right precordial leads (known as the Brugada phenocopy) were excluded, along with the patients exhibiting evident structural heart diseases such as ACM. This study was approved by the Institutional Review Board of Korea University Anam Hospital (IRB No. 2016AN0025). Informed consent from the patients was waived due to the retrospective design and previous informed consents on genetic testing. The study complied with the principles of the Declaration of Helsinki.

Data collection and outcomes

Clinical data were collected, including demographic data such as sex, date of diagnosis, age at diagnosis, admission history, associated symptoms, history of ventricular arrhythmia, comorbidities (structural heart disease and other cardiovascular diseases), and family history of SCA or inherited arrhythmia. Clinical diagnosis of BrS was based on the diagnostic criteria of the 2013 expert consensus statement—a baseline 12-lead ECG was initially evaluated, and if the baseline ECG did not reveal Type 1 Brugada pattern, an additional 12-lead ECG (placing right precordial lead V_1-V_2 in a more cranial position of the 3rd intercostal spaces) and drug provocation tests were conducted with intravenous flecainide infusion [11]. The baseline ECG analysis included heart rate, PR interval, QRS duration, corrected QT interval, and the type of Brugada pattern (Type 1–3). Further electrocardiographic analysis was performed to screen for ACM-related features: right axis deviation, right ventricular hypertrophy with Sokolow–Lyon criteria (R wave in V_1 lead + S wave in V_5 or V_6 lead > 1.1 mV), complete or incomplete right bundle branch block, presence of epsilon wave, presence of negative T-wave at precordial or inferior leads (II, III, aVF), QRS widening of the precordial lead (duration > 110 ms in V_1–V_3), S-wave upstroke (> 55 ms in V_1–V_3), and/or the presence of paroxysmal premature ventricular complex of the right ventricular outflow tract origin [12, 13]. Transthoracic echocardiography was performed to screen for an underlying structural heart disease. Further evaluation of signal-averaged ECG, coronary angiography, cardiac magnetic resonance imaging, or electrophysiological study was done with physicians' decision, and implantable cardioverter-defibrillator was implanted if indicated. Genetic testing of available method (i.e., targeted genetic testing or next generation sequencing [NGS] panel) at the time of examination.

NGS library preparation and sequencing

Genomic DNA was extracted from blood for library preparation. Genomic DNA was fragmented as approximately 200 bp using Covaris Sonication System (Covaris, USA) and processed for Illumina sequencing by following steps; end-repair, dA-tailing, adapter ligation, and pre-polymerase chain reaction for indexed NGS library. Prepared gDNA library and capture probes were hybridized to capture target regions through the use of Celemics target enrichment kit (Celemics, Seoul, Republic of Korea). Capture probes were designed and chemically synthesized to hybridize target region. Target regions consisted of 38 and 36 genes associated with inherited arrhythmia and genetic cardiomyopathy, respectively

(Supplementary Table 1). Captured regions were then further amplified by post-polymerase chain reaction to enrich the amount of sample. The target-captured library was then sequenced on an Illumina Miseq instrument (Illumina, San Diego, CA, USA) using the read layout 2×150 bp.

Sanger sequencing for *SCN5A* mutations

We confirmed the mutations in *SCN5A* gene using polymerase chain reaction and Sanger sequencing. We designed primer sequences targeting two *SCN5A* mutations (Supplementary Table 2). The polymerase chain reaction thermal cycle was programmed for 5 min at 94 °C for initial activation step, followed by 35 cycles of 30 s at 94 °C for denaturation, 40 s at 60 °C for annealing, 1 min at 72 °C for extension, 5 min at 72 °C for final extension, and at 4 °C for hold. Sanger sequencing was conducted with polymerase chain reaction product using ABI 3500xl Genetic Analyzer (Thermo Fisher Scientific, WALTHAM MA, USA).

Sequencing data analysis

Sequence reads were aligned to the human reference genome hg19 using the Burrows-Wheeler Aligner-MEM algorithm [14]. Polymerase chain reaction-duplicated reads were marked using Picard MarkDuplicate, and base quality score recalibration was performed using a Genome Analysis Tool Kit (GATK) [15, 16]. We identified the germline mutations using GATK HaplotypeCaller with default settings and applied hard filtering to remove the false-positive variants. Hard-filtered variants were annotated using ANNOVAR and used for further analyses [17]. To remove the common germline mutations, variants with a minor allele frequency of more than 1% in the genome aggregation, Exome Aggregation Consortium, and Korean population databases were excluded. Variants were classified as pathogenic, likely pathogenic, variants of unknown significance (VUS), likely benign, or benign [18, 19]. Classification was determined based on Standards and Guidelines for the Interpretation of Sequence Variants of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology [20].

Statistical analysis

Categorical variables are described as numbers and percentages, and the chi-square test or Fisher's exact test was performed for the comparison of variables, as appropriate. Continuous variables are described as mean ± standard deviation, with Student's *t*-test and Mann–Whitney test used for the comparison of variables, as indicated. A *p*-value of < 0.05 was considered significant. All statistical analyses were performed using SPSS version 26 software (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp).

Results

Study population

The Brugada registry consists of 119 patients; 63 patients were confirmed and 56 patients were suspected of having BrS (Fig. 1). Forty-two patients were evaluated based on their genetic background. Six patients underwent targeted genetic testing for *SCN5A*, and 17 patients were tested with the inherited arrhythmia NGS panel. Nineteen patients were evaluated with cardiomyopathy NGS panel in addition to the inherited arrhythmia panel. Twenty-five patients were revealed to have genetic variants, accounting for a total of 38 variants found. Among the 25 patients, 15 had BrS-related genotypes, 6 had ACM-related genotypes, in which 3 patients had both BrS- and ACM-related genotypes. Seven patients had genetic variants that were not related to either BrS or ACM.

Clinical characteristics and outcome

Baseline characteristics of the patients are shown in Table 1. Mean age of onset was 43.6 ± 13.8 years and 114 patients (95.8%) were men. Sixty-three patients (52.9%) had confirmed BrS, with 53 patients (44.5%) presenting with spontaneous type 1 BrS on the ECG.

Patients detected with BrS genotypes ($n = 12$) and ACM genotypes ($n = 6$) were compared with their clinical characteristics; three patients with both BrS and ACM genotypes were excluded from the BrS genotype group (Supplementary Table 3). There was no statistically significant difference between the two groups in terms of demographic factors as well as left ventricular ejection fraction (49.3 ± 9.0% vs. 52.1 ± 5.6%, $p = 0.456$), and *N*-terminal pro-B-type natriuretic peptide (229.3 ± 480.9 pg/mL vs. 70.8 ± 134.4 pg/mL, $p = 0.474$).

Genetic analysis

The BrS-related genotypes are presented in Supplementary Table 4. Fifteen patients were found to have 16 BrS-related genotypes. Variations in the *SCN5A* gene were detected in six patients (40.0%) and non-*SCN5A* variants (*SCN10A, HCN4, SCN3B*, and *KCNE3*) were discovered in nine patients (60.0%). Four patients (26.6%) were found to have disease-causing variants of pathogenic or likely pathogenic BrS genotype.

Nineteen patients were further tested with cardiomyopathy NGS panel, and six patients (31.5%) revealed ACM-related variants (Fig. 1, Table 2). Various desmosomal genotypes other than *PKP2* and non-desmosomal genotypes were detected: two *PKP2*, one *DSG2*, one *TMEM43*, one *JUP*, and one *DSP* variants. Based on

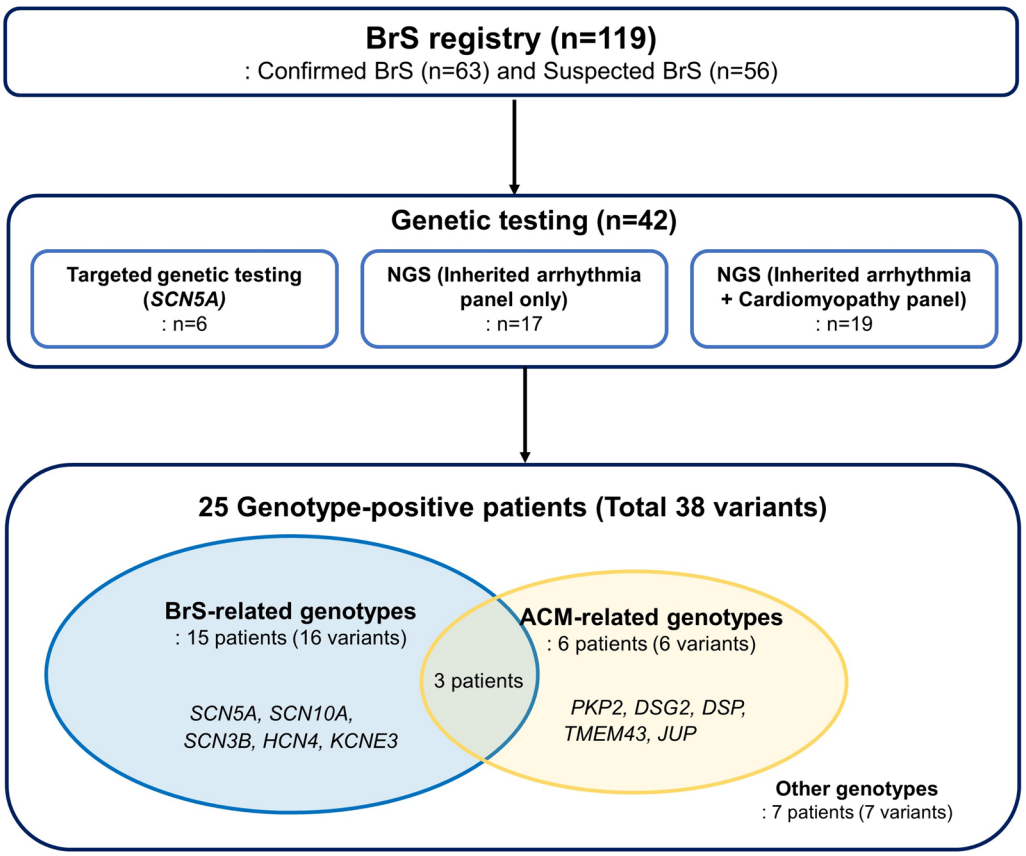

Fig. 1 Flowsheet of the study. In 119 patients with Brugada phenotypes, 42 underwent genetic testing to reveal BrS-related variants, either with targeted genetic testing or NGS panel. Among 42 patients, 19 patients underwent additional genetic testing with cardiomyopathy panel. Six out of 19 patients (31.5%) were detected with ACM-related genotypes. ACM, arrhythmogenic cardiomyopathy; BrS, Brugada syndrome; NGS, next-generation sequencing

the American College of Medical Genetics and Genomics criteria, all ACM-related genotypes were classified as VUS. None of the patients had any structural abnormalities that would fulfill the diagnostic criteria for ACM. Only one patient showed minimal sign of right ventricular abnormality on the echocardiography (Fig. 2).

Further information on each genetic variant is provided in Supplementary Table 5 and Supplementary Table 6. A substantial proportion of *SCN5A* in BrS genotypes were novel variants that were not previously recorded in population or disease databases. In addition, minor allele frequencies of both BrS- and ACM-related genotypes were found to be rare variants rather than common polymorphisms.

ECG analysis: electric features of ACM

Baseline electrocardiograms were further analyzed to assess electrocardiographic features that differentiated the ACM genotype group from patients without ACM genotypes. Electrocardiographic features of ACM were evaluated, as well as the presence of late potential on signal-averaged ECG (Table 3). A higher proportion of (1) epsilon wave, (2) right axis deviation, and (3) inferior lead T-wave inversion were observed in the ACM genotype group, but without statistical significance. In other words, the ACM genotype group exhibited fragmentary electrocardiographic signs of ACM, but none of the patients had sufficient electrocardiographic evidence to clinically confirm ACM.

Discussion

This study demonstrated the concept of BrS-ACM overlapping syndrome, suggesting the utility of ACM-related genetic testing in patients with BrS demonstrated in the previous experimental studies. The main findings of this study can be summarized as follows. First, a certain proportion (31.5%) of Brugada phenotypes were detected in patients with ACM-related genotypes. Second, various desmosomal and non-desmosomal ACM-associated genotypes were detected in Brugada phenotypes (*PKP2, DSG2, DSP, JUP, TMEM43*). Third, Brugada phenotypes that were revealed to have ACM-related genotypes did

Table 1 Baseline characteristics

	Total (*n* = 119)
Age of onset (years)	43.6 ± 13.8
Male sex	114 (95.8%)
Confirmed BrS	63 (52.9%)
Spontaneous Type 1 BrS pattern ECG	53 (44.5%)
Family history of sudden cardiac arrest	22 (18.5%)
ICD implantation	45 (40.2%)
Symptom	
Ventricular arrhythmia or sudden cardiac arrest	40 (33.6%)
Syncope	24 (20.2%)
Non-sustained ventricular tachycardia	14 (11.7%)
Echocardiography	
Left ventricular ejection fraction (%)	54.5 ± 4.1
E/e'	6.9 ± 2.4
NT-proBNP (pg/mL)	108.1 ± 232.9
Genetic testing	
Total	42
BrS related genotype	15 (35.7%)
Drug provocation test	
Total	42
Positive drug provocation test	15 (36.5%)
Signal-averaged ECG	
Total	82
Positive late potential	53 (64.6%)
Electrophysiologic study	
Total	52
Induced ventricular arrhythmia*	25 (48.0%)

Variables are described as either numbers (percentage) or mean ± SD

BrS, Brugada syndrome; ECG, electrocardiography; ICD, implantable cardioverter-defibrillator; NT-proBNP; N-terminal pro-B-type natriuretic peptide

* Induced ventricular arrhythmia was defined as induced sustained ventricular arrhythmia during the electrophysiological study

not express an evident ACM phenotype in the imaging studies or electrocardiography, which underscores the possibility of concealed ACM. Our findings indicate that genetic testing of ACM-related genotypes may be useful in patients with Brugada phenotypes in order to reveal concealed ACM.

Overlapping between BrS and ACM

The overlapping features of BrS and ACM have been suggested in previous research [1, 21, 22]. Cerrone et al. identified a relationship between a desmosomal gene mutation and sodium channelopathy in voltage-gated sodium channel (Na_V 1.5), which leads to the BrS phenotype and ventricular arrhythmia [10]. Loss of *PKP2* expression was correlated with decreased sodium current at Na_V1.5 in patch-clamp studies of murine models, which was later expanded to include human induced pluripotent stem cell cardiomyocytes.

The current study verified the concept of overlapping syndromes of BrS and ACM in the clinical field, correlating with the genetic background of Brugada phenotypes patients. A certain proportion of the BrS patients have been identified with disease-relevant genotypes, including *SCN5A*. Substantial proportion of Brugada phenotypes (31.5%) were additionally defined as having concealed ACM at the genetic level (Fig. 3). In the ACM genotype group, those with numerous desmosomal (*DSP, DSG2, JUP*) and non-desmosomal genotypes (*TMEM43*) other than *PKP2* were found to express the BrS phenotype. All of the genotypes were definite evidence genes susceptible to ACM according to ClinGen Gene Curation Expert Panel [23]. Although a few patients showed partial electrocardiographic features of ACM, there were no significant differences in baseline 12-lead ECG between ACM genotypes and other BrS patients, which highlights the significance of molecular diagnosis with genetic sequencing.

The pathophysiology of sodium channelopathy in relation to desmosomal genetic mutation might be explained by the concept of connexome [8, 24, 25]. The connexome is a recently established concept that is defined as a functional complex of a desomosome, gap junction, and voltage-gated sodium channel [24, 25]. This functional network operates as a whole, rather than each component functioning independently. In other words, genetic mutations in any component of the connexome might lead to a dysfunction of cell adhesion, electrical coupling, or cell excitability. For instance, genetic mutation of voltage-gated sodium channels (i.e., *SCN5A*) could impair proper cell-to-cell adhesion, resulting in structural defects. Likewise, genetic alterations of the desmosome could also change the sodium current, leading to the electric phenotype of BrS. Thus, the concept of the connexome leads to the recognition of BrS and ACM as two ends of a disease spectrum that cross over, rather than considering them as separate disease entities.

Genetic testing and interpretation in ACM

With advancements in genetic sequencing techniques, the genetic background of inherited arrhythmia is getting uncovered [26, 27]. Disclosure of the genetic basis for SCA is significant in terms of personalized precision medicine, which would not only enable individualized prognosis and clinical information but also imply the possibility of individualized therapy based on the specific genetic variant. However, caution must be exercised when interpreting genetic data. Pathogenicity based on the American College of Medical Genetics and Genomics criteria provides genetic evidence for medical decision-making, but it is not directly translated as the clinical significance of a specific genotype in symptomatic patients

Table 2 Arrhythmogenic cardiomyopathy -related genotypes

No	Sex	Age	Clinical manifest	ECG	ICD implantation	Sign of structural abnormality	Gene	Mutation type	DNA change	Amino acid change	Pathogenicity
1	M	54	SCA	Type 1	Yes	No	*PKP2*	Missense	c.725C > T	p.Thr242Met	VUS
2*	M	32	SCA	Type 2	Yes	No	*PKP2*	Missense	c.1889 T > C	p.Val630Ala	VUS
3*	M	20	SCA	Type 1	Yes	Increased trabeculation of right ventricular apex	*JUP*	Missense	c.1050G > C	p.Glu350Asp	VUS
4	M	46	SCA	Type 1[†]	Yes	No	*DSG2*	Missense	c.2780C > T	p.Pro927Leu	VUS
5*	M	55	SCA	Type 2	Yes	No	*DSP*	Missense	c.3701A > G	p.Asn1234Ser	VUS
6	M	28	SCA	Type 1	Yes	No	*TMEM43*	Missense	c.1111 T > C	p.Tyr371His	VUS

ECG, electrocardiography; ICD, implantable cardioverter-defibrillator; SCA, sudden cardiac arrest; VUS, variants of uncertain significance

* Three patients were detected with both Brugada syndrome and arrhythmogenic cardiomyopathy genotypes

[†] Drug(flecainide)-induced Brugada Type 1 pattern ECG

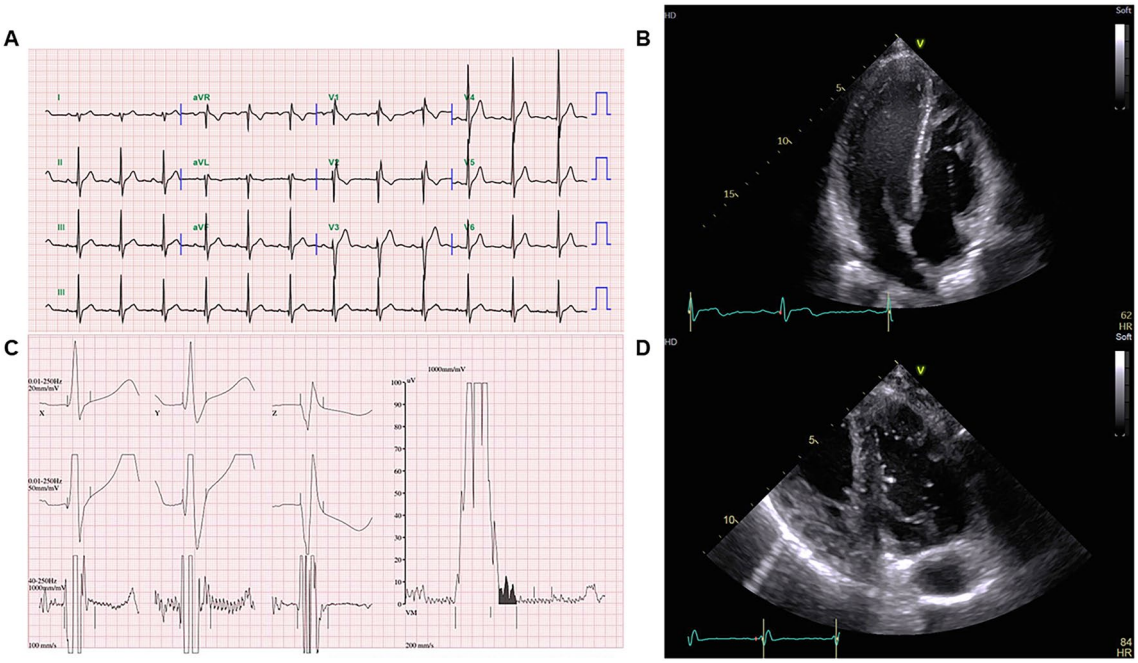

Fig. 2 Case of Brugada syndrome proband with arrhythmogenic cardiomyopathy genotype. A 20 year-old male patient was referred to clinic with recent history of ventricular fibrillation, resuscitated after 2 cycles of cardiopulmonary resuscitation. Baseline 12-lead ECG (placing right precordial lead V_1-V_2 in a more cranial position of the 3rd intercostal spaces) revealed Type 1 Brugada pattern (**A**), and signal-averaged ECG resulted positive late potential (**C**). Follow-up echocardiography revealed increased trabeculation of RV apex at apical 4-chamber view (**B**), and RV focused view (**D**). Genetic testing revealed likely pathogenic variant of *SCN10A* (No.7 at Supplementary Table 4), and missense variant with uncertain significance of *JUP* (No.3 at Table 2). ECG, electrocardiography; RV, right ventricle

with manifested arrhythmia. Genotype–phenotype correlation is required for genotype-positive individuals in the clinical field. However, establishing genotype–phenotype correlation might also be difficult if (1) the genetic variant is a novel variant that has not been recognized in previous databases; (2) gene sequencing covers a broad range of phenotypes (large panel, exome sequencing); (3) one genetic variant is associated with various phenotypes (i.e., *TTN, SCN5A*); (4) the phenotype is in the subclinical phase, requiring additional clinical evaluation to correlate with genotypes [20]. Accordingly, there are significant difficulties in the identification and interpretation of the concealed ACM. The ACM-related genotypes in this study included novel and/or rare variants and the genotypes were associated with various phenotypes other than ACM. Importantly, none of the patients showed evident clinical features (structural or electrical evidence) of ACM, which makes genotype–phenotype correlation in this case difficult. All ACM-related genotypes were classified as VUS. Although VUS is not equivalent to 'benign,' further studies are needed to confirm is effect on the phenotype.

Concealed ACM in Brugada phenotypes

The difficulty of interpreting genetic information in concealed ACM magnifies the need for in-depth genetic evaluation of familial genetic screening (cascade screening) and functional studies. Cascade screening might provide additional evidence for the genetic inheritance and supply supportive data for diagnosis [12, 28]. Functional studies include an electrophysiologic study of cardiomyocytes by the patch-clamp method, which can be conducted on animal models or human cardiomyocytes using patient-specific human induced pluripotent stem cells [29, 30]. Previous functional studies of ACM genotypes have focused on *PKP2* and there are only a few studies on other desmosomal genes or non-desmosomal genes associated with sodium channel current [31, 32]. Further experimental studies on other desmosomal genes or non-desmosomal genes may provide an in-depth understanding of the pathophysiology of channelopathies in ACM genotypes.

The disease course of ACM exhibits a preclinical phase that does not express apparent structural abnormality of the right ventricle [8]. That is, a disease-associated

Table 3 Comparison of electrocardiographic findings in ACM genotypes and others

	ACM genotype ($n=6$)	Others ($n=111$)	*p*-value
Heart rate (beats per min)	73.5 ± 16.3	70.1 ± 12.8	0.693
PR interval (msec)	171.6 ± 16.2	168.3 ± 21.1	0.481
QRS duration (msec)	108.6 ± 12.2	108.1 ± 15.6	0.747
Corrected QT interval (msec)	406.1 ± 16.0	422.1 ± 30.9	0.149
Signs of ACM			
Epsilon wave	1 (16.7%)	1 (0.9%)	0.100
Right axis deviation	2 (33.3%)	11 (9.9%)	0.133
RVH (Sokolow-Lyon criteria)	1 (16.7%)	14 (12.6%)	0.569
Right bundle branch block	1 (16.7%)	11 (9.9%)	0.485
Incomplete right bundle branch block	2 (33.3%)	33 (29.7%)	1.000
Precordial T wave inversion			
V1	3 (50.0%)	63 (56.8%)	1.000
V2	2 (33.3%)	21 (18.9%)	0.336
V3	1 (16.7%)	9 (8.1%)	0.422
Inferior T wave inversion	1 (16.7%)	5 (4.5%)	0.276
Precordial QRS widening (v1-3)	1 (16.7%)	18 (16.2%)	1.000
Prolonged S-wave upstroke (v1-3)	0 (0.0%)	9 (8.1%)	1.000
Paroxysmal RVOT VT/PVC	0 (0.0%)	4 (3.6%)	1.000
Signal-averaged electrocardiography			
Total	6	48	
Positive	5 (83.3%)	48 (63.2%)	0.416

The baseline 12-lead electrocardiographic findings of patients with Brugada syndrome are described above. Two patients were excluded because of their medical records, as the 12-lead electrocardiography was outdated

ACM, arrhythmogenic cardiomyopathy; PVC, premature ventricular complex; RVH, right ventricular hypertrophy; RVOT, right ventricular outflow tract; VT, ventricular tachycardia

phenotype might not be present, or only an electrical sign might be present in the preclinical phase. Moreover, due to incomplete penetrance and variable expressivity of phenotype, individuals who carry ACM-related genotypes might not manifest signs of the disease [33]. However, lethal ventricular arrhythmia could precede structural changes in ACM genotypes [31]. In addition, when the condition progresses to the clinical phase with right ventricular dysfunction, curative treatment for ACM might be difficult owing to advanced stage of the disease, which emphasizes the importance of early diagnosis. Nevertheless, clinical diagnosis of ACM in the pre-clinical phase is difficult because of non-specific and non-structural symptoms. With the acknowledgement of the overlapping features of BrS and ACM, constant imaging follow-up of BrS patients with ACM genotypes might make early diagnosis of ACM more feasible, providing clinical information about disease course or prognosis and allow further familial screening.

Limitations

This study had several limitations. First, presence of an ACM-related variant does not directly translate into presence of a clinically relevant disease. ACM-related variants found in our study were classified as VUS, which needs further clarification for their deleterious effects on the phenotype. Moreover, desmosomal genes of ACM are also detected in healthy controls (known as 'background noise'), which is more frequent in non-Caucasians [34]. Nevertheless, significant prevalence of ACM-related variants in Brugada phenotypes as well as their low frequency in general population should be acknowledged, and functional study of these variants is needed to clarify their clinical significance. Second, this was a retrospective analysis performed at a single clinical center. After the establishment of NGS in clinical setting, patients in the Brugada registry were recommended to undergo genetic testing. However, about half of the patients in the Brugada registry were those who had been diagnosed in the remote past that lack thorough evaluation of their genetic background. In addition, patients were screened with a variety of genetic testing (from Sanger's method to NGS panel), which reflected change in the clinical practice over period. Only minority of Brugada phenotypes ($n=19$) were evaluated with cardiomyopathy NGS

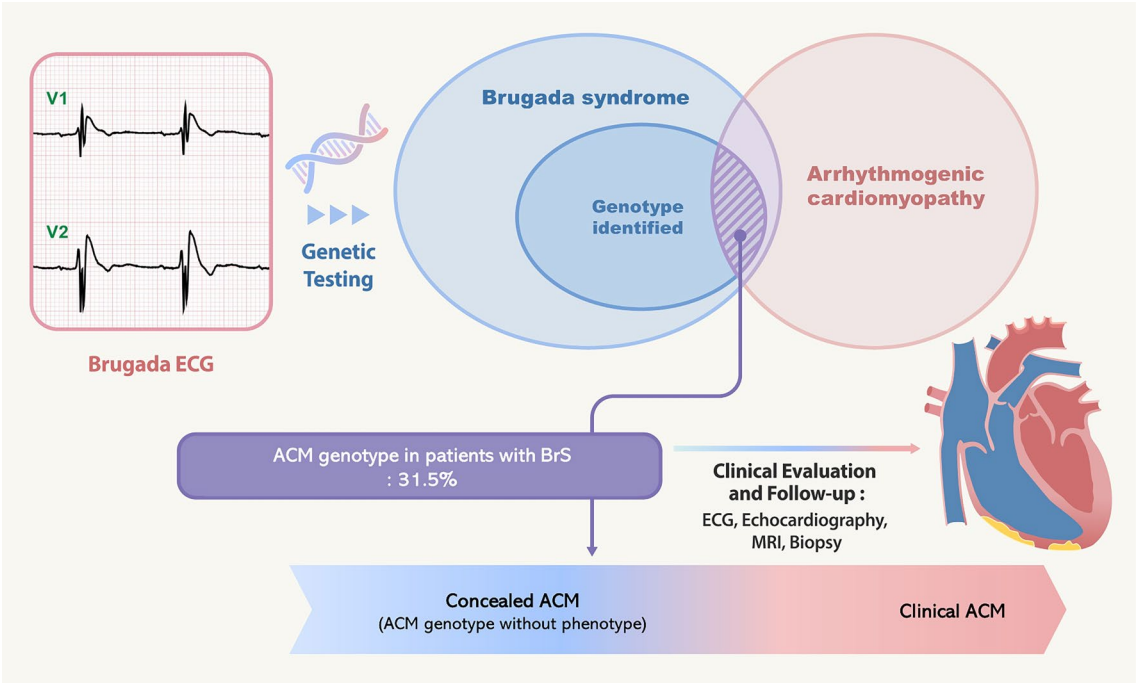

Fig. 3 Concealed arrhythmogenic cardiomyopathy with Brugada syndrome phenotype. There are overlapping characteristics of BrS with ACM at the clinical level—it is narrowed down to *concealed ACM* (purple shadow) in patients that exhibit a phenotype of BrS and genotype of ACM. In this study, 6 out of 19 (31.5%) patients with BrS phenotype were revealed to have ACM genotype, which may be in concealed phase of ACM. In these patients, further clinical evaluation and follow-up is needed to undercover possibility of disease progression as clinical ACM. ACM, arrhythmogenic cardiomyopathy; BrS, Brugada syndrome; ECG, electrocardiography; MRI, magnetic resonance imaging

panels, which limits the generalizability of the findings. The actual prevalence of concealed ACM in patients with BrS should be studied in-depth. Since genetic testing has been more easily accessible with lower cost, further genetic testing with NGS panel including ACM-related genes may confirm our findings. Third, this study was not restricted to confirmed BrS patients who fulfilled the diagnostic criteria, but also included a rather broad range of patients with Brugada pattern ECG. Patients who were clinically suspected of having BrS without satisfying the diagnostic criteria were included, because we believed that the current diagnostic criteria for BrS might underestimate the number of actual BrS patients, considering the dynamic variability of ECG patterns, depending on the vagal tone, age, hormonal differences, etc. [2]. More thorough evaluation and follow-up is needed to differentiate true BrS and Brugada-like pattern ECG with other causes of coved-type ST elevation in the right precordial leads. Lastly, although ACM refers to the final common pathway including arrhythmogenic right ventricular cardiomyopathy and arrhythmogenic left ventricular cardiomyopathy, our cohort was focused on arrhythmic right ventricular cardiomyopathy-related genotypes and phenotypes [12]. Regarding the changing concept of ACM as a broad spectrum of myocardial disorder with arrhythmic phenotypes, genetic testing and evaluation should further extend to the left ventricular cardiomyopathy.

Conclusion

A significant proportion of patients with BrS were genetically disclosed to have ACM-related genotypes and various ACM-related genotypes were identified. Genetic testing of ACM-related genotypes in BrS patients may be useful to unveil the possibility of concealed ACM. Long-term follow-up with imaging studies for Brugada phenotypes with ACM genotypes are needed to monitor possible disease progression to ACM.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42444-024-00121-w.

Additional file 1.

Acknowledgements
None.

Author contributions
JIC had full access to all data in this study and takes responsibility for data integrity and analytical accuracy. The concept and design of the study were developed by JIC. Data analysis and interpretation were performed by JHJ and

JIC. The manuscript was drafted by JHJ, SGY, YJC, JHH, and JIC. Data collection and statistical analysis were performed by JHJ, YGK, YYC, HSL, JS, JIC, and YHK.

Funding
This work was supported by supported by a grant from the Korean Heart Rhythm Society (KHRS 2023–1 to J-I.C) and a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT, Ministry of Science and ICT) (No. 2021R1A2C2011325 to J-I.C). The funders had no role in data collection, analysis, or interpretation; trial design; patient recruitment; or any other aspect pertinent to the study.

Availability of data and materials
The data underlying this article are available in the article. The raw data underlying this article cannot be shared publicly due to privacy reasons and legal regulations of Republic of Korea.

Declarations

Ethics approval and consent to participate
The Institutional Review Board of Korea University Medicine Anam Hospital approved this study (IRB No. 2016AN0025). Written informed consent was obtained for genetic testing in every patient, and further consent for the analysis was waived due to retrospective nature of the study.

Competing interests
The authors have no conflicts of interest to disclose.

Received: 13 May 2024 Accepted: 2 July 2024
Published online: 12 July 2024

References

1. Hoogendijk MG. Diagnostic dilemmas: overlapping features of brugada syndrome and arrhythmogenic right ventricular cardiomyopathy. Front Physiol. 2012;3:144.
2. Brugada J, Campuzano O, Arbelo E, Sarquella-Brugada G, Brugada R. Present Status of Brugada Syndrome: JACC State-of-the-Art Review. J Am Coll Cardiol. 2018;72:1046–59.
3. Roh SY, Choi JI, Kim MS, Cho EY, Kim YG, Lee KN, et al. Incidence and etiology of sudden cardiac arrest in Koreans: A cohort from the national health insurance service database. PLoS ONE. 2020;15: e0242799.
4. Leung KSK, Huang H, Chung CT, Radford D, Lakhani I, Li CKH, et al. Historical perspective and recent progress in cardiac ion channelopathies research and clinical practice in Hong Kong. Int J Arrhythmia. 2023;24.
5. Sarquella-Brugada G, Campuzano O, Arbelo E, Brugada J, Brugada R. Brugada syndrome: clinical and genetic findings. Genet Med. 2016;18:3–12.
6. Kim YG, Oh SK, Choi HY, Choi JI. Inherited arrhythmia syndrome predisposing to sudden cardiac death. Korean J Intern Med. 2021;36:527–38.
7. Jeong JH, Oh SK, Kim YG, Choi YY, Lee HS, Shim J, et al. Clinical and genetic features of Korean inherited arrhythmia probands. Korean Circ J. 2023;53:693–707.
8. Patel V, Asatryan B, Siripanthong B, Munroe PB, Tiku-Owens A, Lopes LR, et al. State of the art review on genetics and precision medicine in Arrhythmogenic cardiomyopathy. Int J Mol Sci. 2020;21:6615.
9. Delmar M, McKenna WJ. The cardiac desmosome and arrhythmogenic cardiomyopathies: from gene to disease. Circ Res. 2010;107:700–14.
10. Cerrone M, Lin X, Zhang M, Agullo-Pascual E, Pfenniger A, Chkourko Gusky H, et al. Missense mutations in plakophilin-2 cause sodium current deficit and associate with a Brugada syndrome phenotype. Circulation. 2014;129:1092–103.
11. Priori SG, Wilde AA, Horie M, Cho Y, Behr ER, Berul C, et al. Executive summary: HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes. Europace. 2013;15:1389–406.
12. Towbin JA, McKenna WJ, Abrams DJ, Ackerman MJ, Calkins H, Darrieux FCC, et al. 2019 HRS expert consensus statement on evaluation, risk stratification, and management of arrhythmogenic cardiomyopathy: executive summary. Heart Rhythm. 2019;16:e373–407.
13. Brosnan MJ, Te Riele A, Bosman LP, Hoorntje ET, van den Berg MP, Hauer RNW, et al. Electrocardiographic features differentiating arrhythmogenic right ventricular cardiomyopathy from an Athlete's heart. JACC Clin Electrophysiol. 2018;4:1613–25.
14. Li H, Durbin R. Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics. 2009;25:1754–60.
15. Institute B. Picard toolkit. Broad Institute, GitHub repository. 2019; http://broadinstitute.github.io/picard.
16. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010;20:1297–303.
17. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010;38: e164.
18. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alfoldi J, Wang Q, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature. 2020;581:434–43.
19. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016;536:285–91.
20. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–24.
21. Corrado D, Zorzi A, Cerrone M, Rigato I, Mongillo M, Bauce B, et al. Relationship between arrhythmogenic right ventricular cardiomyopathy and Brugada syndrome: new insights from molecular biology and clinical implications. Circ Arrhythm Electrophysiol. 2016;9: e003631.
22. Kataoka S, Serizawa N, Kitamura K, Suzuki A, Suzuki T, Shiga T, et al. An overlap of Brugada syndrome and arrhythmogenic right ventricular cardiomyopathy/dysplasia. J Arrhythm. 2016;32:70–3.
23. James CA, Jongbloed JDH, Hershberger RE, Morales A, Judge DP, Syrris P, et al. International evidence based reappraisal of genes associated with arrhythmogenic right ventricular cardiomyopathy using the clinical genome resource framework. Circ Genom Precis Med. 2021;14: e003273.
24. Agullo-Pascual E, Cerrone M, Delmar M. Arrhythmogenic cardiomyopathy and Brugada syndrome: diseases of the connexome. FEBS Lett. 2014;588:1322–30.
25. Vermij SH, Abriel H, van Veen TA. Refining the molecular organization of the cardiac intercalated disc. Cardiovasc Res. 2017;113:259–75.
26. Jeong JH, Kim YG, Oh SK, Lee HS, Choi YY, Min K, et al. Identification of concealed cardiomyopathy using next-generation sequencing-based genetic testing in Korean patients initially diagnosed with idiopathic ventricular fibrillation. Europace. 2023;25:euad313.
27. Kim MJ, Kim YR, Lee KH, Yoon N, Park HW. Value of next-generation sequencing in inherited arrhythmia syndromes. Int J Arrhythmia. 2023;24.
28. Ghafouri P, Taaghi S, Keykhavani A, Bozorgi A, diz AA. Cascade screening can be life-saving: a family with multiple cases of brugada syndrome and sudden cardiac death. Int J Arrhythmia. 2023;24.
29. Liang P, Sallam K, Wu H, Li Y, Itzhaki I, Garg P, et al. Patient-specific and genome-edited induced pluripotent stem cell-derived cardiomyocytes elucidate single-cell phenotype of Brugada syndrome. J Am Coll Cardiol. 2016;68:2086–96.
30. Kim C, Wong J, Wen J, Wang S, Wang C, Spiering S, et al. Studying arrhythmogenic right ventricular dysplasia with patient-specific iPSCs. Nature. 2013;494:105–10.
31. Gomes J, Finlay M, Ahmed AK, Ciaccio EJ, Asimaki A, Saffitz JE, et al. Electrophysiological abnormalities precede overt structural changes in arrhythmogenic right ventricular cardiomyopathy due to mutations in desmoplakin-a combined murine and human study. Eur Heart J. 2012;33:1942–53.
32. Rizzo S, Lodder EM, Verkerk AO, Wolswinkel R, Beekman L, Pilichou K, et al. Intercalated disc abnormalities, reduced Na(+) current density, and conduction slowing in desmoglein-2 mutant mice prior to cardiomyopathic changes. Cardiovasc Res. 2012;95:409–18.
33. James CA, Calkins H. Arrhythmogenic right ventricular cardiomyopathy: progress toward personalized management. Annu Rev Med. 2019;70:1–18.
34. Kapplinger JD, Landstrom AP, Salisbury BA, Callis TE, Pollevick GD, Tester DJ, et al. Distinguishing arrhythmogenic right ventricular

cardiomyopathy/dysplasia-associated mutations from background genetic noise. J Am Coll Cardiol. 2011;57:2317–27.

Publisher's Note