International Journal of Cardiology 274 (2019) 263–270

Contents lists available at ScienceDirect

International Journal of Cardiology

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

A founder homozygous *DSG2* variant in East Asia results in ARVC with full penetrance and heart failure phenotype

Liang Chen [1], Man Rao [1], Xiao Chen [1], Kai Chen [1], Jie Ren [1], Ningning Zhang [1], Qian Zhao [1], Wenhua Yu [1], Bin Yuan [1], Jiangping Song [*,1]

State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences (CAMS) and Peking Union Medical College (PUMC), Beijing 100037, PR China

ARTICLE INFO

Article history:
Received 12 February 2018
Received in revised form 17 June 2018
Accepted 27 June 2018
Available online 28 June 2018

Keywords:
Arrhythmogenic right ventricular cardiomyopathy
Desmoglein-2
Founder mutation
Haplotype

ABSTRACT

Background: Variants in the desmoglein-2 (*DSG2*) gene account for a significant proportion of patients with Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC). The aim of this study was to evaluate the genetic epidemiology of *DSG2* and the impact of a frequent homozygous *DSG2* variant in East Asia.
Methods: Genetic screening of 14 ARVC related genes was performed in 118 unrelated index patients using next-generation sequencing. Following that, family screening, clinical evaluation and haplotype analysis were performed among eight probands who carry the same homozygous *DSG2* variant. We also examined the histopathology and protein expression using immunofluorescence staining on the myocardial tissue of two probands undergoing heart transplant.
Results: Eighteen (15.2%) patients bear rare putatively deleterious variants in *DSG2*, among which 8 patients shared the homozygous *DSG2* p.Phe531Cys variant. Family screening demonstrated that only homozygous variant carriers exhibited definite ARVC phenotype with 100% penetrance, while heterozygous variant carriers were either unaffected or only presented mild ARVC related symptoms in 25% relatives. Left ventricular involvement and bi-ventricular failure were common among homozygous p. Phe531Cys variant patients even at early age. Haplotype analysis demonstrated p. Phe531Cys was a founder variant in East Asia population with an allele frequency of 0.12%.
Conclusions: We identified, for the first time, a homozygous founder variant of *DSG2* in East Asia, which was at surprisingly high frequency of 8.47% among Chinese ARVC patients with a full penetrance. This result suggested an urgent demand of genetic counseling for the probands and their relatives with heterozygous variant.

1. Introduction

Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) is a hereditary cardiomyopathy mainly manifests malignant arrhythmias and myocardial fibro-fatty replacement. [1, 2] It is one of the main causes for sudden cardiac death among athletes and the youth under 35-year old. [3, 4] The predominant pathogenic genes are desmosomal genes, including plakophilin-2 (*PKP2*), plakoglobin (*JUP*), desmoglein-2 (*DSG2*), desmocollin-2 (*DSC2*) and desmoplakin (*DSP*); these genes, account for ~50% ARVC patients in different cohorts [5–7]. Moreover, many non-desmosomal genes have been reported to be associated with ARVC, such as transmembrane protein 43 (*TMEM43*), desmin (*DES*), phospholamban (*PLN*), lamin A/C (*LMNA*), Catenin Alpha 3 (*CTNNA3*) and recent cadherin-2 (*CDH2*) [3, 8].

Although autosomal recessive inheritance is reported in several cases such as Naxos syndrome [9] and Carvajal syndrome [10], ARVC is mainly considered as an autosomal dominant inheritance disease. Many ARVC cohort studies reported some carriers with multiple variants including compound and digenic heterozygosity [5, 11–13]. Furthermore, those carriers with compound/digenic variant are usually at higher risk of malignant events compared to single variant carriers [5, 14]. According to our center and Dutch cohort, among the 5 desmosomal genes, *DSG2* and *DSC2* are most frequently identified to be compound in ARVC patients, which suggests that single variant is not sufficient to penetrate ARVC phenotype [15]. Furthermore, some ARVC patients with homozygous variants were also reported especially in *DSC2*, and family screen revealed that heterozygous variant carriers were healthy or only have mild symptoms. These results bring up the possibility of potential recessive inheritance pattern in these

* Corresponding author at: State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, 167A Beilishi Road, Xi Cheng District, Beijing 100037, PR China.
E-mail address: fwsongjiangping@126.com (J. Song).
[1] This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

https://doi.org/10.1016/j.ijcard.2018.06.105
0167-5273/

mutations [16–19]. Moreover, some *DSC2* homozygous variants were identified to be founder mutations such as truncated variant c.1660C > T (p.Gln554X) [17] and missense variant c.536A > G (p.Asp179Gly) [16]. Considering that both DSC2 and DSG2 proteins are extracellular desmosomal cadherins known to have overlapping functions in binding to plakoglobin and plakophilin 2, we inferred that *DSG2* might have the similar inheritance pattern with *DSC2*. Although several case reports have mentioned homozygous genetic pattern of *DSG2* in ARVC families, [20, 21] to date, no founder variant was reported yet.

In this study, we investigated the *DSG2* variants in a large Chinese ARVC index patient cohort containing 118 unrelated probands. Eleven rare (putatively) pathogenic variants were identified in 18 probands, of whom 15 have multiple variants (including compound or homozygous variants). Strikingly, eight patients shared the same missense variant DSG2 c.T1592G (p.Phe531Cys), which was verified to be a founder variant through haplotype analysis. Combining with previous reports, we observed that this variant is distributed in East Asia, with expressing ARVC phenotype in homozygotes but no disease expression or only mild phenotype in heterozygotes.

2. Methods

2.1. Study population

This study enrolled 118 (81 males) unrelated probands diagnosed with ARVC according to 2010 revised Task Force Criteria [22] from Fuwai hospital and National Center for Cardiovascular Disease in Beijing, China. This study was approved by the Ethics Committee of Fuwai Hospital. All the participants provided written informed consent before inclusion in this study.

2.2. Clinical evaluation and family screening

All index patients received clinical evaluations including medical history, 12-leads electrocardiograph (ECG), echocardiography and 24-h Holter monitoring as they were diagnosed ARVC at our hospital, and the same clinical evaluations were performed for the follow-up session. Cardiac Magnetic Resonance (CMR) imaging was performed in a subgroup of patients to evaluate ventricular structure/function and fibrofatty infiltration using 1.5 Tesla MR scanner (Magnetom Avanto, Siemens Medical Solutions, Erlangen, Germany). Collected images included cine, fat-suppressed T1 and T2-weighted Turbo Spin Echo (TSE) and late gadolinium enhancement (LGE) images. Family members of ARVC index patients were assessed by evaluating personal history, echocardiography and ECG. Any individuals suspected as potential ARVC received further examination such as 24 h Holter monitoring and CMR. Peripheral blood of these individuals was also collected for genetic testing during family assessment.

2.3. Pathology examination

The ARVC hearts from patients undergoing heart transplantation (HTx) were examined immediately after dissected from chest according to the consensus issued by Society for Cardiovascular Pathology [23] as previously described [24]. The myocardial tissues were fixed in 10% formalin, followed by dehydration, then embedded in paraffin and cut into 3 μm-thick sections (Leica RM2265, Germany) for staining and immunofluorescence. The Hematoxylin and Eosin (H&E) and Masson's trichrome staining were performed according to manufacturer's instructions.

2.4. Immunofluorescence

The distribution and expression of DSG2 (HPA012615-100, Sigma, USA) and Connexin 43 (Cx43, ab11370, abcam, UK) were detected by immunofluorescence based on standard procedures. Sarcomeric alpha actinin (ab9465, abcam, UK) was used to display cardiomyocytes in each slice, and the nucleus was stained using DAPI (ZLI-9557, ZSGB, China). Each fluorescence image was collected using laser confocal microscopy (Leica TCS SP8, Leica, Germany) and four areas were collected for each slice. Image J was used to quantify the signal intensity of positive staining.

2.5. Genetics analysis

The genomic DNA was extracted from peripheral blood according to manufacturer's instruction (Blood DNA Extraction Kit, Enriching Biotechnology, China). Probands were screened with 14 ARVC related genes including 5 desmosome genes and non-desmosomal genes including *TMEM43*, *PLN*, *CTNNA3*, *LMNA*, *DES*, transforming growth factor beta 3 (*TGFB3*), Sodium Voltage-Gated Channel Alpha Subunit 5 (*SCN5A*), Titin (*TTN*) and Ryanodine receptor 2 (*RYR2*) by captured next-generation sequencing using Illumina 2500 platform (Illumina, USA) as previous reported [24]. All variants were annotated by the following strategies. First, all rare variants with allele frequency (MAF) <0.5% in the control population of the 1000 Genomes Project (2014 Oct release, http://www.1000genomes.org), ExAC (http://exac.broadinstitute.org) and gnomAD (http://gnomad.broadinstitute.org/) were processed for pathogenicity evaluation. Secondly, frameshift, stop-gain/stop-loss and splicing variants were considered as disease-causing variants. Thirdly, rare missense variants were evaluated as (putatively) pathogenic variants when high damage effects were predicted by at least three out of five appropriate programs including Sorting Intolerant from Tolerant [SIFT], Polyphen2-HDIV, MutationTaster and MutationAssessor. [25] For TTN variants, only truncated variants remained for pathogenesis analysis. Sanger sequencing was used to validate putatively pathogenic variants and screen family members.

2.6. Haplotype analysis

To investigate whether the missense carriers have a common haplotype, four polymorphic microsatellites (D18S847, D18S963, D18S36, D18S47) and five SNPs in close proximity to DSG2 c.T1592G locus were analyzed (Human Genome Browser, http://genome.ucsc.edu/). These polymorphic markers covered 2 Mb flanking *DSG2* gene. The most possible haplotype was constructed among eight ARVC probands with homozygous DSG2 c.T1592G variant. The primers for PCR reaction was shown in Supplementary Table 6.

2.7. Statistical analysis

Continuous variables were presented as mean ± standard deviation. Categorical variables were presented as percentages. The haplotype analysis was performed as previous reported [16].

3. Results

3.1. Patients population and genetic analysis

Genetic screening was performed among 118 unrelated AC patients. More than half patients (56.8%) had positive genotype, most of which were desmosomal variant, including 22 patients (18.6%) with *PKP2*, 5 patients with *DSP* and 2 with *DSC2*. Different from most western ARVC populations, which has low proportion of *DSG2* mutation [12, 26, 27], eleven different *DSG2* variants, including one frameshift and ten missense variants, were identified among 18 probands (15.3%) in this Chinese cohort. (Supplementary Table 1). Out of these 18 patients, six carried compound heterozygous variants, nine patients were homozygotes, and only three patients carried single heterozygous variant. Among all these *DSG2* variant carriers, most patients showed no additional (putative/likely) pathogenic variant in other ARVC related genes with an exception of a missense variant (p.Arg484Cys) of *CTNNA3* identified in one patient carrying the homozygous DSG2 p.Arg292Cys variant.

Most strikingly, the missense variant *DSG2* p.Phe531Cys was identified in 12 out of 118 (10.2%) index cases (Fig. 1A), among which eight carried homozygous variant and four were compound heterozygous carriers with other variants within *DSG2*. In this study, we focused on the eight homozygotes carrying *DSG2* p.Phe531Cys, which was a very rare variant in control population of 1000G (MAF 0), ExAC (MAF 6.00e-5) and gnomAD (MAF 5.78e-5). Consanguineous marriage was not found among these families. This amino acid was evolutionarily highly conserved (Fig. 1B), and located in extracellular anchor (EA) domain (Fig. 1C). Fig. 1D and E showed two examples for family pedigree charts of homozygous *DSG2* p.Phe531Cys variant, in which all homozygous variant carriers were diagnosed as ARVC while heterozygous and genotype negatives relatives were not affected or only had slight related symptoms.

3.2. Clinical findings

We further reviewed the clinical diagnosis of eight *DSG2* p.Phe531Cys probands (5 males) based on the primary in-patient examination in our hospital. Each patient was diagnosed as ARVC according to 2010 revised Task Force Criteria (Table 1). Age of initial ARVC related symptom ranged from 14 to 34 years old (mean age 24.5 ± 5.9). Five probands started with palpitation, syncope and ventricular arrhythmias; one patient was diagnosed as ECG abnormality as well as right ventricle (RV) enlargement and dysfunction without apparent

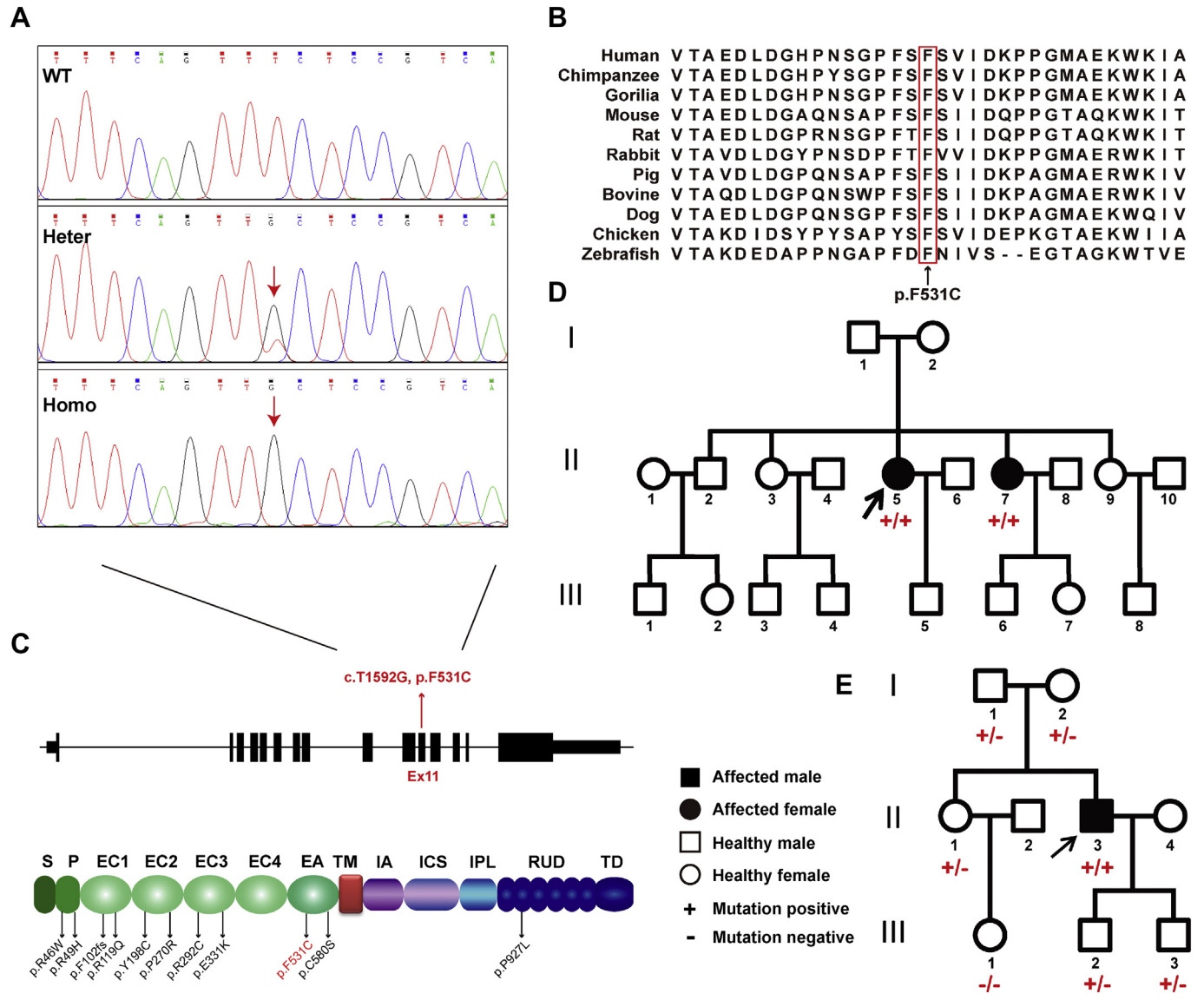


Fig. 1. Genetic analysis of DSG2 p. Phe531Cys variant. Sanger sequencing validation (A) and conservation analysis (B) of p.Phe531Cys variant; C. exons and domains diagram and the variant position annotation. D and E. Two representative family pedigrees.

symptoms; one patient presented ventricular arrhythmias and RV dominant cardiac dysfunction during perinatal period, and received heart transplant as a result of progressive biventricular heart failure; and the youngest proband had a fulminant heart failure course, requiring transplant on 6 months after first symptom. All patients have representative ECG abnormalities such as precordial T wave inversion (TWI), QRS prolongation and sometimes epsilon wave (Supplementary Fig. 1A). Two patients received implantable cardioverter defibrillator (ICD) implantation and radiofrequency catheter ablation (RFCA) for recurrent sustained ventricular tachycardia. Appropriate ICD therapy occurred in both patients after ICD implantation, but did not reappear after RFCA. One patient received ICD implantation to prevent sudden cardiac death (for primary prevention). The clinical characteristics of these patients were shown in Supplementary Tables 1 & 2.

3.3. CMR characteristics

We further analyzed the CMR results imaging from five patients. Although these patients were in different stages, the main pathological presentation was found in all those 5 patients including extensive delayed enhancement and fibrofatty tissue replacement with significant dilation in entire RV (Supplementary Fig. 1B–G). Interventricular septum also showed delayed enhancement at distal level adjacent to cardiac apex under four-chamber section (Supplementary Fig. 1D, G). Additionally, left ventricle (LV) was also affected reflected by cardiac dysfunction (LV ejection fraction<50%) and fibrofatty infiltration (shown by LGE) mainly at posterolateral free wall in four out of five patients. These results were similar to previous pathology study [28] (Supplementary Table 2). Therefore, it was noteworthy that *DSG2* p. Phe531Cys patients were prone to left ventricular dysfunction, which increased the risk for heart transplantation and pre-mature death.

3.4. Pathology examination

Two explanted hearts from the ARVC patient with *DSG2* p. Phe531Cys were examined. Consistent with the CMR imaging, both hearts exhibited classical pathological characteristics of end-stage ARVC shown as extensive fibro-fatty replacement in entire RV as well as post-lateral free wall of LV (Supplementary Fig. 1H–K). H&E staining revealed myocyte degeneration, vacuolization and inflammatory infiltration at the transition zone of fibrofatty remodeling (Supplementary Fig. 1L,M). Meanwhile,

Table 1
Clinical diagnosis of patients carrying homozygous DSG2 p. Phe531Cys variant.

Patient ID	Tissue characterization		Repolarization abnormalities		Depolarization/conduction abnormalities		Arrhythmias		Family history[a]		Ventricular alterations		Diagnosis	
	Major	Minor	Major	Minor	Major	Minor	Major	Minor	Major	Minor	Major	Minor	Major	Minor
Case-1	1			1	1			1		1	1		3	3
Case-2	1		1		1		1		1		1		6	0
Case-3	N/A[b]			1		1	1			1		1	1	4
Case-4	N/A			1		1	1				1		2	2
Case-5	N/A			1		1	1				1		2	2
Case-6	N/A		1					1	1			1	2	2
Case-7	N/A		1		1			1			1		3	1
Case-8	N/A			1		1	1			1	1		2	3

[a] Genetic mutation was not included in this criterion.
[b] Pathological examination was not performed in these patients.

ultrastructure of myocardium was also significantly disrupted, including decreased convolution index of disc, enlarged desmosome gap, irregular desmosome structure and myofiber degeneration. (Supplementary Fig. 1I) The immunofluorescence imaging showed that the expression of DSG2 was significant decreased ($p = 0.002$) in ARVC heart compared to non-diseased heart. At the same time, Cx43 was not decreased ($p = 0.528$) at the intercalated disc but exhibited ectopic expression at non-intercalated disc areas. We also observed myocardial hypertrophy and intercalated disc elongation/distortion in the myocardium with *DSG2* p. Phe531Cys variant. (Fig. 2, Supplementary Fig. 2).

3.5. Family screening

Clinical and genetic evaluations were also performed among relatives of seven probands. One patient refused for family screening in this study. Among 31 relatives, 23 were identified to be genotype positive, including 1 homozygous variant and 22 heterozygous variant carriers. In addition to one family member with homozygous variant who was diagnosed with ARVC and received ICD implantation in our hospital, 6 of 22 heterozygous family members showed mild ARVC related symptoms, including two with palpitation, two received RFCA due to ventricular arrhythmias, one with TWI and one with mild RV dilation. No heterozygous variant carriers fulfilled the definite diagnosis criteria and none of them received therapy by the present time. Furthermore the mean age of these family members with mild symptom were 51 ± 13.79 years old, suggesting an age-dependent symptom presentation in mutation carriers. No ARVC related symptom was observed in genotype-negative members. (Supplementary Table 3).

3.6. Penetrance analysis

We further reviewed and found four previous reports about this variant from Japan [29], Chinese Taiwan [30] and South [21] and East China as shown in Supplementary Table 4. Similar to our cohort, all of the probands and relatives diagnosed as ARVC from 3 out of 4 studies were homozygous variant, while heterozygous variant carriers only presented with mild symptoms; the remaining one index patient with heterozygous variant from Japan study also carried homozygous *DSP* p.Lys1581Glu variant, and no family screening was performed for this index patient. We combined the patients from previous reports and our cohort to evaluate the penetrance of homozygous and heterozygous variant carriers. The patient with digenic variant in Japan was excluded from the evaluation. Among 19 homozygous variant carriers, 32 heterozygous carriers and 28 genotype negative family members, all (100%) of the homozygotes were diagnosed as ARVC, and 25% of heterozygous variant carriers presented moderate ARVC symptoms at senior age, and no ARVC related symptoms were discovered in family members without mutation. (Fig. 3A, B).

3.7. Genetic epidemiology and haplotype analysis

All these *DSG2* p.Phe531Cys positive ARVC patients in previous reports and our cohort were from East Asia as shown in Fig. 3C, indicating that this variant might be a founder variant. To investigate whether probands carrying the DSG2 p. Phe531Cys have a common ancestor, we performed haplotype analysis using 4 polymorphic microsatellites (D18S847, D18S963, D18S36, D18S47) and 5 SNPs in vicinity to *DSG2* p. Phe531Cys locus. A shared haplotype, spanning >450 kb, was identified in both alleles of 8 probands carrying homozygous p.Phe531Cys and one allele of 4 index patients with heterozygous p.Phe531Cys variant, hence indicating that these families most likely had a common founder. (Fig. 3D) Meanwhile, we also screened these markers among 23 family members with p.Phe531Cys variant and demonstrated that all mutation carriers share the same haplotype within the allele of p. Phe531Cys variant. (Supplementary Table 5).

We further screened *DSG2* p.Phe531Cys variant in different population. Compared to a significant enrichment in Chinese ARVC cohort (8.47%), the general population allele frequency was 0.006% according to gnomAD database. This allele frequency is 0.12% in East Asia population, but none in other areas worldwide. Additionally, another database HGVD from Japan also showed a similar allele frequency (0.09%) as East Asia population of gnomAD database. We also screened the *DSG2* p.Phe531Cys variant among 400 non-ARVC cardiomyopathies in our cardiac center, and only two patients (0.25%) carried heterozygous variant, which is a similar allele frequency in East Asia population. This suggested that *DSG2* p. Phe531Cys carriers mainly exhibit the phenotype of ARVC without overlap with other cardiomyopathies. (Fig. 3E).

4. Discussion

This study reveals that *DSG2* variants are highly frequent (25%) in the largest Chinese ARVC cohort, in which most are compound mutation. Eight unrelated probands share the same *DSG2* p. Phe531Cys homozygous variant that is demonstrated to be a founder variant with an allele frequency of 0.12% in East Asia and 8.47% in Chinese ARVC cohort. This is the first reported founder variant identified among ARVC patients in East Asia, and also the first founder variant of *DSG2* worldwide. Most importantly, this homozygous mutation presents full disease penetrance with classical pathological remodeling phenotype [28] and progressive heart failure, while heterozygous mutation carriers are asymptomatic or only manifest mild symptom (25%).

DSG2 was firstly demonstrated to be associated with ARVC through genetic analysis by two independent teams from Italy and the United States in 2006 [31, 32]. The mice that carry the DSG2-N266S variant identified from ARVC patient displays significant biventricular dysfunction with myocyte degeneration and myocardial fibrosis further confirming that *DSG2* is a causative gene of ARVC [33]. Although *PKP2* is recognized

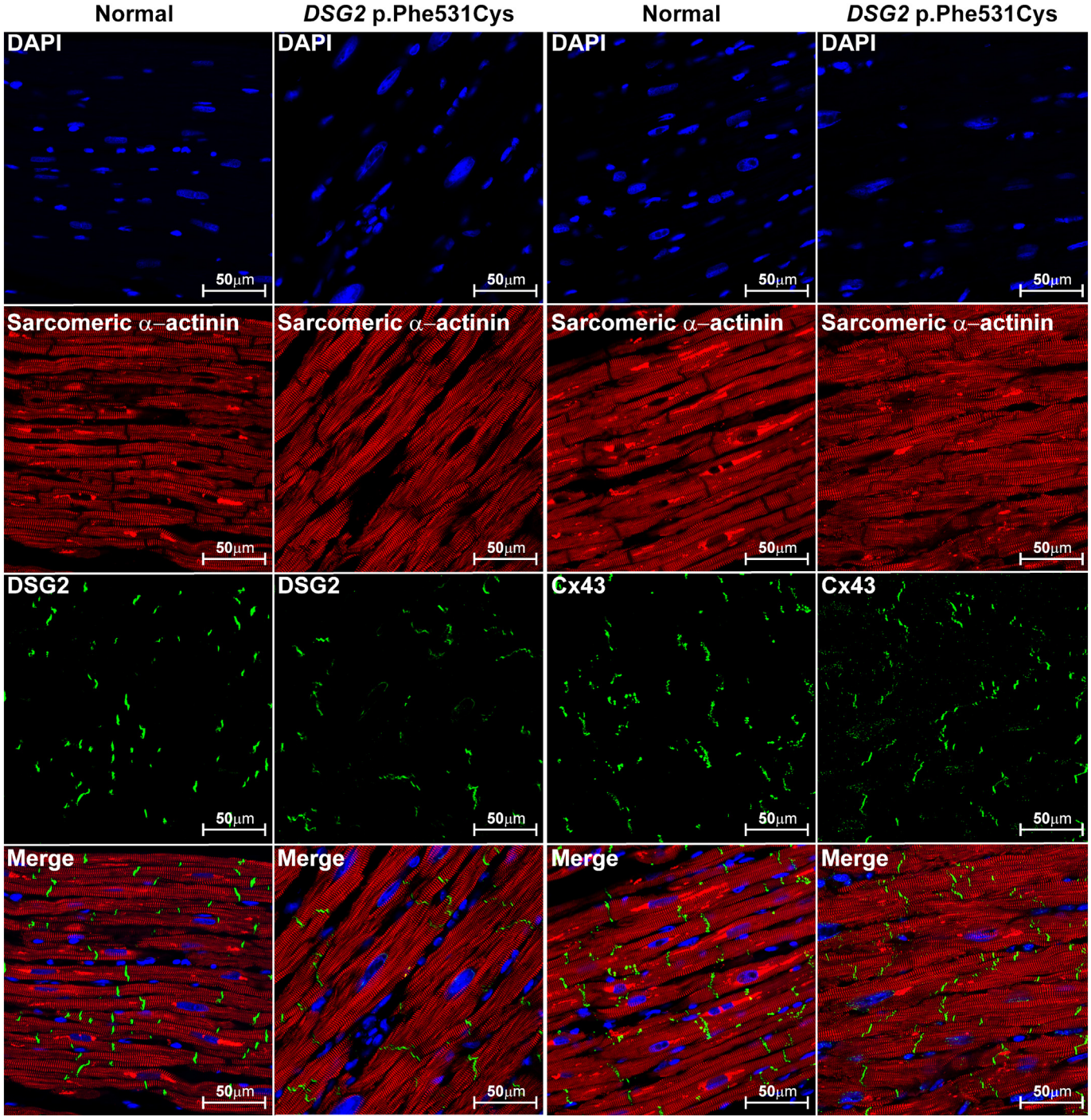
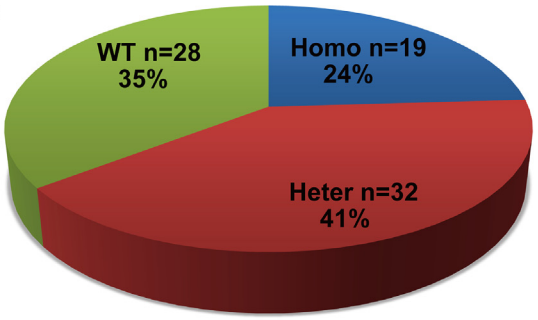


Fig. 2. The expression and distribution of DSG2 and Cx43 in DSG2 p. Phe531Cys mutant heart and non-diseased heart. DSG2, desmoglein-2; Cx43, connexin-43.

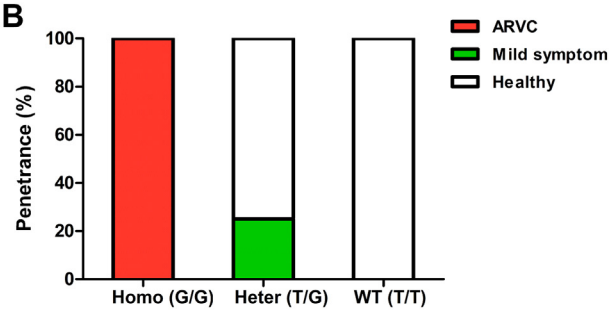
as the most common pathogenic gene in most ARVC cohorts [5, 12], *DSG2* often acts as the second common causative gene in many cohorts, especially in Chinese cohort [11], ranging from 5% to 15% [5]. Our result has demonstrated that most (83.3%) *DSG2* variants are compound mutation (including homozygous) in ARVC patients, which is similar to Dutch cohort, suggesting that single variant of *DSG2* might be not adequate to penetrance ARVC phenotype. [15] Furthermore, it was of great importance that we also excluded additional variants of other 14 ARVC related genes including the recently defined *CDH2* [8] and *SCN5A* [34] among these *DSG2* mutation carriers, except for one patient with rare variant (p.Arg484Cys) in *CTNNA3*. Recently, copy-number variations (CNVs) of desmosome genes like *PKP2* and *DSG2* were reported to be associated with pathogenesis of ARVC, especially in the genotype-negative probands [35–37]. Thus it will be more confirmative to exclude the CNVs in *DSG2* among these patients in the future.

In this study, we identified eight probands with homozygous *DSG2* p. Phe531Cys variant, which was also previously reported in other regions of East Asia [21, 29, 30]. Similar to our discovery, these previous reports also showed that only homozygous carriers presented as ARVC with heart transplant or pre-mature death, while heterozygous carriers were not affected. [21] Through screening in the open-access database, we found that *DSG2* p. Phe531Cys only occurred in East Asia population with a relative high frequency (0.12%). Haplotype analysis of these eight index patients and their relatives further demonstrated that it was a founder variant in East Asia population. According to our cohort and previous reports [21, 29, 30], we could infer the variant *DSG2*

A



B



C

D

Marker	Position	Allele	Case-1	Case-2	Case-3	Case-4	Case-5	Case-6	Case-7	Case-8	Case-10	Case-11	Case-12	Case-13
D18S847	27703526	Allele 1	217	221	217	217	213	217	217	217	213	213	213	209
	27703313	Allele 2	221	221	217	217	213	217	217	217	217	217	217	217
SNP3	28223938	Allele 1	A	A	A	A	A	A	A	A	A	A	A	A
		Allele 2	G	G	A	G	A	A	A	A	G	A	G	G
D18S963	28659026	Allele 1	138	138	138	138	138	138	138	138	138	138	138	138
	28659166	Allele 2	138	138	138	138	138	138	138	138	138	138	138	138
SNP6	29090734	Allele 1	A	A	A	A	A	A	A	A	A	A	A	A
		Allele 2	A	A	A	A	A	A	A	A	G	G	G	G
SNP7	29095434	Allele 1	C	C	C	C	C	C	C	C	C	C	C	C
		Allele 2	C	C	C	C	C	C	C	C	G	G	G	G
SNP8	29096919	Allele 1	G	G	G	G	G	G	G	G	G	G	G	G
		Allele 2	G	G	G	G	G	G	G	G	G	T	T	T
SNP4	29104698	Allele 1	C	C	C	C	C	C	C	C	C	C	C	C
		Allele 2	C	C	C	C	C	C	C	C	C	T	T	T
DSG2 c.T1592G	29116333	Allele 1	G	G	G	G	G	G	G	G	G	G	G	G
		Allele 2	G	G	G	G	G	G	G	G	T	T	T	T
D18S36	29164196	Allele 1	139	139	139	139	139	139	139	139	139	139	139	139
	29164339	Allele 2	139	143	139	139	141	139	139	139	152	139	145	137
D18S47	29781230	Allele 1	201	197	197	197	197	197	201	197	197	201	197	197
	29781429	Allele 2	201	201	203	197	197	197	201	197	197	211	203	205

E

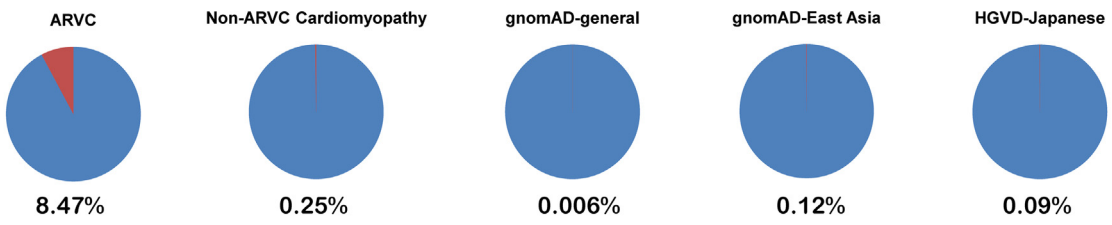


Fig. 3. Penetrance and genetic epidemiology analysis of DSG2 p. Phe531Cys variant. (A) The summary of mutation carriers in combination with previous case reports and our cohort. (B) Penetrance of homozygous, heterozygous and non-carriers. (C) Distribution of the families carrying p.Phe531Cys variant. (D) Genetic analysis revealed eight homozygous mutation probands shared a haplotype spanning >450 kb (Case means the homozygous p. Phe531Cys probands, and Con means the ARVC patients without the p. Phe531Cys variant). (E) Allele frequency of DSG2 p. Phe531Cys in different population and cohort.

p. Phe531Cys specifically distributed in East Asia, including North China, South China, Taiwan and Japan. According to large-scale genetic haplotype analysis, Chinese Han, Taiwanese, Korean and Japanese were identified in the maximum-likelihood based cluster because they shared haplotypes, which suggested that individuals from these geographic regions derived from a single migration and/or gene admixture occurred in history [38]. Furthermore, Y-chromosome and mitochondrial DNA variation study of Chinese Han population revealed demic diffusions from north to south China during Western Jin Dynasty (265–316 CE) and Tang Dynasty (1127–1279 CE) [39]; North Chinese Han was also considered to be originated from the same ancestors as Japanese and Korean [40]. Taiwan also mainly consisted of population from Chinese Han because of emigration especially in the 20th century. This could explain the wide distribution of *DSG2* p. Phe531Cys in East Asia. Most importantly, *DSG2* p. Phe531Cys is a prominently high frequent (8.47%) variant among Chinese ARVC patients with a full penetrance, which suggests an urgent demand of genetic counseling for the index patients and their relatives carrying heterozygous mutation.

It is a gradually growing concern about the correlation between the genotype and pathological characteristics in hereditary cardiomyopathy in recent years [28, 41]. Our *DSG2* p. Phe531Cys positive patients shared the similar clinical myocardial remodeling presentation that was shown as fibro-fatty replacement of entire RV as well as post-lateral free wall of LV at progressive stage. In addition, we also screened for *DSG2* p. Phe531Cys among 400 non-ARVC cardiomyopathies and found no enrichment of this variant. This suggests that this genotype was specific to ARVC without overlapping with other primary cardiomyopathies. Although these patients have the similar pathological features of ARVC, the clinical presentation varied among the patients. The patient in serious condition had a fulminant heart failure course, requiring transplant in 6 months after first symptom, while some patients showed late-onset symptom at >50 years old. This indicated that some other modulator genes or environmental factors might promote the pathogenicity and penetrance of *DSG2* p. Phe531Cys [42]. It was noteworthy that most ARVC patients carrying homozygous *DSG2* p. Phe531Cys variant suffered biventricular heart failure, which was shown in our cohort as well as previous case report [21]. Ventricular arrhythmias used to be considered as the major adverse prognosis of ARVC with great concerns, but recent study revealed that heart failure was also common and under-recognized in ARVC patients. [43] Thus, our observations also suggested that more concern or interventions for progressed heart failure should be dedicated to the ARVC patients with homozygous *DSG2* p. Phe531Cys variant.

Several molecular mechanisms have been reported for different variants of *DSG2*. The mutations at N-terminal of DSG2 caused the deficiency of adhesive EC1-EC2 domains, resulting in dissociation of cell-to-cell connection, reduction of desmosome units and abnormal distribution of Cx43, and consequentially induced electrical conduction abnormality [44]. Mutations at C-terminal of DSG2 could cause C-terminal unique region (DSG unique region [DUR]) dysfunction, leading to dysfunction of intercellular adhesion and loss of DSG2 tail-tail interactions, which could destroyed the stabilization of DSG2 at the cell surface and thus caused rapid endocytosis in cardiac muscle cells. [45] Different from previous reports, the p. Phe531Cys variant is located in EA domain that mediates the surface levels of this protein. Our histopathology study showed that DSG2 expression was decreased in *DSG2* p. Phe531Cys positive myocardium, accompanied by maldistribution of Cx43 as well as elongation and distortion of intercalated disc. However, more approaches will be needed to illustrate the mechanical dysfunction and molecular mechanism of *DSG2* p. Phe531Cys variant in the pathogenesis of ARVC.

5. Conclusions

We identified for the first time a homozygous founder variant (p. Phe531Cys) of *DSG2* in East Asia, which was highly prevalent among Chinese ARVC patients with full penetrance for homozygous carriers; this result suggested an urgent demand of genetic counseling for the index patients and their relatives carrying heterozygous variant.

Conflicts of interest

The authors report no relationships that could be construed as a conflict of interest.

Acknowledgment

This study was supported by CAMS Innovation Fund for Medical Sciences (2016-I2M-1-015), the National Natural Science Foundation of China (81670376), and PUMC Youth Fund and the Fundamental Research Funds for the Central Universities (to J-P Song). We thank Ms. Yawen Grace Tang from University of Alabama at Birmingham for language editing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2018.06.105.

References

[1] D. Corrado, M.S. Link, H. Calkins, Arrhythmogenic right ventricular cardiomyopathy, N. Engl. J. Med. 376 (2017) 61–72.
[2] C. Basso, G. Thiene, D. Corrado, A. Angelini, A. Nava, M. Valente, Arrhythmogenic right ventricular cardiomyopathy. Dysplasia, dystrophy, or myocarditis? Circulation 94 (1996) 983–991.
[3] D. Corrado, C. Basso, D.P. Judge, Arrhythmogenic cardiomyopathy, Circ. Res. 121 (2017) 784–802.
[4] G. Thiene, A. Nava, D. Corrado, L. Rossi, N. Pennelli, Right ventricular cardiomyopathy and sudden death in young people, N. Engl. J. Med. 318 (1988) 129–133.
[5] A. Bhonsale, J.A. Groeneweg, C.A. James, et al., Impact of genotype on clinical course in arrhythmogenic right ventricular dysplasia/cardiomyopathy-associated mutation carriers, Eur. Heart J. 36 (2015) 847–855.
[6] M.G. Cox, P.A. van der Zwaag, C. van der Werf, et al., Arrhythmogenic right ventricular dysplasia/cardiomyopathy: pathogenic desmosome mutations in index-patients predict outcome of family screening: Dutch arrhythmogenic right ventricular dysplasia/cardiomyopathy genotype-phenotype follow-up study, Circulation 123 (2011) 2690–2700.
[7] C. Basso, D. Corrado, F.I. Marcus, A. Nava, G. Thiene, Arrhythmogenic right ventricular cardiomyopathy, Lancet 373 (2009) 1289–1300.
[8] B.M. Mayosi, M. Fish, G. Shaboodien, et al., Identification of cadherin 2 (CDH2) mutations in Arrhythmogenic right ventricular cardiomyopathy, Circ. Cardiovasc. Genet. 10 (2017).
[9] G. McKoy, N. Protonotarios, A. Crosby, et al., Identification of a deletion in plakoglobin in arrhythmogenic right ventricular cardiomyopathy with palmoplantar keratoderma and woolly hair (Naxos disease), Lancet 355 (2000) 2119–2124.
[10] E.E. Norgett, S.J. Hatsell, L. Carvajal-Huerta, et al., Recessive mutation in desmoplakin disrupts desmoplakin-intermediate filament interactions and causes dilated cardiomyopathy, woolly hair and keratoderma, Hum. Mol. Genet. 9 (2000) 2761–2766.
[11] J. Bao, J. Wang, Y. Yao, et al., Correlation of ventricular arrhythmias with genotype in arrhythmogenic right ventricular cardiomyopathy, Circ. Cardiovasc. Genet. 6 (2013) 552–556.
[12] J.A. Groeneweg, A. Bhonsale, C.A. James, et al., Clinical presentation, long-term follow-up, and outcomes of 1001 Arrhythmogenic right ventricular dysplasia/cardiomyopathy patients and family members, Circ. Cardiovasc. Genet. 8 (2015) 437–446.
[13] T. Xu, Z. Yang, M. Vatta, et al., Compound and digenic heterozygosity contributes to arrhythmogenic right ventricular cardiomyopathy, J. Am. Coll. Cardiol. 55 (2010) 587–597.
[14] I. Rigato, B. Bauce, A. Rampazzo, et al., Compound and digenic heterozygosity predicts lifetime arrhythmic outcome and sudden cardiac death in desmosomal gene-related arrhythmogenic right ventricular cardiomyopathy, Circ. Cardiovasc. Genet. 6 (2013) 533–542.
[15] Z.A. Bhuiyan, J.D. Jongbloed, J. van der Smagt, et al., Desmoglein-2 and desmocollin-2 mutations in dutch arrhythmogenic right ventricular dysplasia/cardiomypathy patients: results from a multicenter study, Circ. Cardiovasc. Genet. 2 (2009) 418–427.
[16] A. Lorenzon, K. Pilichou, I. Rigato, et al., Homozygous Desmocollin-2 mutations and Arrhythmogenic cardiomyopathy, Am. J. Cardiol. 116 (2015) 1245–1251.
[17] B. Gerull, F. Kirchner, J.X. Chong, et al., Homozygous founder mutation in desmocollin-2 (DSC2) causes arrhythmogenic cardiomyopathy in the Hutterite population, Circ. Cardiovasc. Genet. 6 (2013) 327–336.
[18] M.A. Simpson, S. Mansour, D. Ahnood, et al., Homozygous mutation of desmocollin-2 in arrhythmogenic right ventricular cardiomyopathy with mild palmoplantar keratoderma and woolly hair, Cardiology 113 (2009) 28–34.
[19] B. Al-Sabeq, A.D. Krahn, S. Conacher, G.J. Klein, Z. Laksman, Arrhythmogenic right ventricular cardiomyopathy with recessive inheritance related to a new homozygous desmocollin-2 mutation, Can. J. Cardiol. 30 (2014) 696 e1–3.

[20] S. Qadri, O. Anttonen, J. Viikila, et al., Case reports of two pedigrees with recessive arrhythmogenic right ventricular cardiomyopathy associated with homozygous Thr335Ala variant in DSG2, BMC Med. Genet. 18 (2017) 86.
[21] Y. Lin, Q. Zhang, Z.A. Zhong, et al., Whole genome sequence identified a rare homozygous pathogenic mutation of the DSG2 Gene in a familial Arrhythmogenic cardiomyopathy involving both ventricles, Cardiology 138 (2017) 41–54.
[22] F.I. Marcus, W.J. McKenna, D. Sherrill, et al., Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia: proposed modification of the task force criteria, Circulation 121 (2010) 1533–1541.
[23] J.R. Stone, C. Basso, U.T. Baandrup, et al., Recommendations for processing cardiovascular surgical pathology specimens: a consensus statement from the standards and definitions Committee of the Society for cardiovascular pathology and the Association for European Cardiovascular Pathology, Cardiovasc. Pathol. 21 (2012) 2–16.
[24] X. Chen, L. Chen, Z. Chen, X. Chen, J. Song, Remodelling of myocardial intercalated disc protein connexin 43 causes increased susceptibility to malignant arrhythmias in ARVC/D patients, Forensic Sci. Int. 275 (2017) 14–22.
[25] C. Dong, P. Wei, X. Jian, et al., Comparison and integration of deleteriousness prediction methods for nonsynonymous SNVs in whole exome sequencing studies, Hum. Mol. Genet. 24 (2015) 2125–2137.
[26] V. Fressart, G. Duthoit, E. Donal, et al., Desmosomal gene analysis in arrhythmogenic right ventricular dysplasia/cardiomyopathy: spectrum of mutations and clinical impact in practice, Europace 12 (2010) 861–868.
[27] A.M. Lahtinen, E. Lehtonen, A. Marjamaa, et al., Population-prevalent desmosomal mutations predisposing to arrhythmogenic right ventricular cardiomyopathy, Heart Rhythm. 8 (2011) 1214–1221.
[28] S. Sepehrkhouy, J. Gho, R. van Es, et al., Distinct fibrosis pattern in desmosomal and phospholamban mutation carriers in hereditary cardiomyopathies, Heart Rhythm. 14 (2017) 1024–1032.
[29] S. Ohno, I. Nagaoka, M. Fukuyama, et al., Age-dependent clinical and genetic characteristics in Japanese patients with arrhythmogenic right ventricular cardiomyopathy/dysplasia, Circ. J. 77 (2013) 1534–1542.
[30] C.C. Yu, C.H. Yu, C.H. Hsueh, et al., Arrhythmogenic right ventricular dysplasia: clinical characteristics and identification of novel desmosome gene mutations, J. Formos. Med. Assoc. 107 (2008) 548–558.
[31] M.M. Awad, D. Dalal, E. Cho, et al., DSG2 mutations contribute to arrhythmogenic right ventricular dysplasia/cardiomyopathy, Am. J. Hum. Genet. 79 (2006) 136–142.
[32] K. Pilichou, A. Nava, C. Basso, et al., Mutations in desmoglein-2 gene are associated with arrhythmogenic right ventricular cardiomyopathy, Circulation 113 (2006) 1171–1179.
[33] K. Pilichou, C.A. Remme, C. Basso, et al., Myocyte necrosis underlies progressive myocardial dystrophy in mouse dsg2-related arrhythmogenic right ventricular cardiomyopathy, J. Exp. Med. 206 (2009) 1787–1802.
[34] A.S. Te Riele, E. Agullo-Pascual, C.A. James, et al., Multilevel analyses of SCN5A mutations in arrhythmogenic right ventricular dysplasia/cardiomyopathy suggest non-canonical mechanisms for disease pathogenesis, Cardiovasc. Res. 113 (2017) 102–111.
[35] K. Sonoda, S. Ohno, S. Otuki, et al., Quantitative analysis of PKP2 and neighbouring genes in a patient with arrhythmogenic right ventricular cardiomyopathy caused by heterozygous PKP2 deletion, Europace 19 (2017) 644–650.
[36] I.E. Li Mura, B. Bauce, A. Nava, et al., Identification of a PKP2 gene deletion in a family with arrhythmogenic right ventricular cardiomyopathy, Eur. J. Hum. Genet. 21 (2013) 1226–1231.
[37] K. Pilichou, E. Lazzarini, I. Rigato, et al., Large genomic rearrangements of Desmosomal genes in Italian Arrhythmogenic cardiomyopathy patients, Circ. Arrhythm. Electrophysiol. 10 (2017).
[38] Consortium HP-AS, Abdulla MA, Ahmed I et al. mapping human genetic diversity in Asia, Science 326 (2009) 1541–1545.
[39] B. Wen, H. Li, D. Lu, et al., Genetic evidence supports demic diffusion of Han culture, Nature 431 (2004) 302–305.
[40] L. Jin, B. Su, Natives or immigrants: modern human origin in east Asia, Nat. Rev. Genet. 1 (2000) 126–133.
[41] C. Basso, G. Thiene, Are we ready for a "tissue identity card" of inherited cardiomyopathies? Heart Rhythm. 14 (2017) 1033–1034.
[42] E.M. McNally, D.Y. Barefield, M.J. Puckelwartz, The genetic landscape of cardiomyopathy and its role in heart failure, Cell Metab. 21 (2015) 174–182.
[43] N.A. Gilotra, A. Bhonsale, C.A. James, et al., Heart failure is common and under-recognized in patients with Arrhythmogenic right ventricular cardiomyopathy/dysplasia, Circ. Heart Fail. 10 (2017).
[44] S. Kant, B. Holthofer, T.M. Magin, C.A. Krusche, R.E. Leube, Desmoglein 2-dependent Arrhythmogenic cardiomyopathy is caused by a loss of adhesive function, Circ. Cardiovasc. Genet. 8 (2015) 553–563.
[45] J. Chen, O.E. Nekrasova, D.M. Patel, et al., The C-terminal unique region of desmoglein 2 inhibits its internalization via tail-tail interactions, J. Cell Biol. 199 (2012) 699–711.