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Mutation analysis of the candidate genes *SCN1B-4B*, *FHL1*, and *LMNA* in patients with arrhythmogenic right ventricular cardiomyopathy

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ABSTRACT

Introduction: Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a genetically determined heart disease characterized by fibrofatty infiltrations in the myocardium, right and/or left ventricular involvement, and ventricular tachyarrhythmias. Although ten genes have been associated with ARVC, only about 40% of the patients have an identifiable disease-causing mutation. In the present study we aimed at investigating the involvement of the genes *SCN1B-SCN4B*, *FHL1*, and *LMNA* in the pathogenesis of ARVC.

Methods: Sixty-five unrelated patients (55 fulfilling ARVC criteria and 10 borderline cases) were screened for variants in *SCN1B-4B*, *FHL1*, and *LMNA* by direct sequencing and LightScanner melting curve analysis.

Results: A total of 28 sequence variants were identified: seven in *SCN1B*, three in *SCN2B*, two in *SCN3B*, two in *SCN4B*, four in *FHL1*, and ten in *LMNA*. Three of the variants were novel. One of the variants was non-synonymous. No disease-causing mutations were identified.

Conclusions: In our limited sized cohort the six studied candidate genes were not associated with ARVC.

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1. Introduction

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inheritable cardiomyopathy pathologically characterized by fibrofatty infiltrations in the myocardium leading to thinning of the wall and aneurysms. Clinical presentation is mainly due to ventricular arrhythmias and may include palpitations, syncope, and sudden cardiac death (Basso et al., 2009). Diagnosis is based on Task Force criteria (Marcus et al., 2010), which encompass functional and anatomical characteristics of the right ventricle, electrical abnormalities, family history, and genetic information. The original Task Force criteria were published in 1994, and in 2010 revised criteria were published (Marcus et al., 2010).

To this date ARVC has been associated with mutations in five desmosomal (*DSC2*, *DSG2*, *DSP*, *JUP*, and *PKP2*) and five non-desmosomal genes (*TGFβ3*, *RYR2*, *TMEM43*, *DES*, and *TTN*) (Basso et al., 2009; Taylor et al., 2011; van Tintelen et al., 2009). Although ten genes have been associated with ARVC, these only account for approximately 40% of

the patients with identifiable mutations (Christensen et al., 2010; Bhuiyan et al., 2009) underlining the genetic heterogeneity of the disease.

In the present study we aimed at investigating the involvement of six candidate genes which have not previously been investigated in the pathogenesis of ARVC: *SCN1B-SCN4B* (Sodium channel, voltage gated, type I-IV, beta), encoding β-subunits for the cardiac sodium channel, *FHL1* (Four and a half LIM domains 1), encoding a member of the four-and-a-half LIM only protein family, and *LMNA* (Lamin A/C), encoding a nuclear envelope protein. All are highly expressed in the heart and are known to interact with ARVC-associated genes. They have also been associated with heritable arrhythmia syndromes and/or cardiomyopathy (Brackenbury and Isom, 2008; Cowling et al., 2011; Lu et al., 2011).

2. Methods

2.1. Study subjects

The study comprised 65 unrelated patients recruited from Rigshospitalet, Copenhagen University Hospital, Denmark. All patients were of Northern European descent. The clinical characteristics of the study population have previously been published (Christensen et al., 2010). All patients have previously been screened for mutations

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in the known ARVC-related genes (*DSC2*, *DSG2*, *DSP*, *JUP*, *PKP2*, *TGFb3*, and *TMEM43*) and mutations were identified in 36% of patients fulfilling the 1994 Task Force criteria (Christensen et al., 2010, 2011). Since compound inheritance has been described (Bhuiyan et al., 2009) we chose not to exclude patients with known mutations from further screening. The study was approved by the local ethics committee, followed the Helsinki II declaration, and all subjects gave written informed consent before inclusion.

2.2. Genetic screening

Genomic DNA was isolated from blood samples using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). The coding sequences and splice sites of *SCN1B* (NM_001037.3, NM_199037.2), *SCN2B* (NM_004588.4), *SCN3B* (NM_018400.3), *SCN4B* (NM_174934.2), *FHL1* (NM_001449, NM_001159700, NM_001159704, NM_001167819, NM_001159702, NM_001159703), and *LMNA* (NM_005572, NM_017070.2), corresponding to a total of 38 exons, were amplified with intronic primers and bidirectionally sequenced using Big Dye chain-termination chemistry (DNA analyzer 3730, Applied Biosystems, CA, USA). Some amplicons were prescreened with a LightScanner high-resolution melting curve analysis system (Idaho Technology, UT, USA). All fragments with an abnormal melting profile were sequenced. Primers and PCR conditions are available on request.

3. Results

3.1. Study cohort

A total of 65 (38 men) patients were included in the study. 55 of the patients fulfilled 1994 Task Force criteria for ARVC: \geq two major criteria ($n=15$), one major and \geq two minor criteria ($n=36$), and four minor criteria ($n=4$). Ten patients had some ARVC characteristics but did not fulfill the criteria: one major and one minor criterion ($n=5$), three minor criteria ($n=4$), and two minor criteria ($n=1$).

3.2. Genetic screening

The variant analysis identified a total of 28 sequence variants: Seven in *SCN1B*, three in *SCN2B*, two in *SCN3B*, two in *SCN4B*, four in *FHL1*, and ten in *LMNA*. The identified variants are listed in Table 1. Three of the variants were novel. All novel variants were positioned in flanking introns and did not affect a canonical splice site. One non-synonymous variant was identified (*FHL1* p.D275N). No disease-causing mutations were identified.

4. Discussion

This study is the first comprehensive attempt to associate ARVC with genetic variation in the accessory subunits of the cardiac $Na_v1.5$ channel, *FHL1*, and *LMNA*. Screening of these genes, representing 38 exons, revealed no disease-causing mutations and these genes are therefore unlikely to be common causes of ARVC.

The channels carrying the cardiac sodium current ($Na_v1.5$) is composed of an α -subunit, encoded by *SCN5A*, and two of the four β -subunits (Brackenburg and Isom, 2008). The β subunits ($\beta1$ – $\beta4$), encoded by *SCN1B*–*4B*, are multifunctional and members of the immunoglobulin superfamily. Some of their functions may be independent of the α -subunit (Brackenburg and Isom, 2008) and several studies have demonstrated that $\beta1$ and $\beta2$ both play important roles in homophilic and heterophilic cell adhesion and function as cell adhesion molecules (Brackenburg and Isom, 2008; Chioni et al., 2009). A recent study demonstrated a physical and functional interaction between $Na_v1.5$ and the ARVC-associated gene *PKP2* at the intercalated disc and an altered sodium current after knockdown of *PKP2* (Sato et al., 2009). In addition, Deo et al. (2011) showed that

Table 1
Identified sequence variants.

Gene	Variant	Exon/intron	Amino acid	Minor allele	MAF ^a	dbSNP
<i>SCN1B</i>	c.40+15G>T	Intron		T	0.238	rs72556351
	c.448+181T>C	Intron		C	0.423	rs55742440
	c.448+296C>A	Intron		A	0.169	rs67701503
	c.448+301G>C	Intron		C	0.169	rs67486287
	c.860T>C	Exon	p.I167I	C	0.046	rs16969930
	c.591-25T>G	Intron		G	0.008	rs28365107
<i>SCN2B</i>	c.591-14C>A	Intron		A	0.008	rs28365109
	c.70+32G>A	Intron		A	0.023	–
	c.449-12C>A	Intron		A	0.454	rs8192613
<i>SCN3B</i>	c.648+38C>T	Intron		T	0.092	rs8192614
	c.438C>T	Exon	p.T146T	T	0.054	rs1275085
<i>SCN4B</i>	c.445+37T>G	Intron		G	0.008	–
	c.174C>T	Exon	p.C58C	T	0.046	rs45539032
<i>FHL1</i>	c.639C>T	Exon	p.N213N	T	0.023	rs72544155
	c.688+37G>A	Intron		A	0.008	–
	c.689-8C>T	Intron		T	0.546	rs2076705
<i>LMNA</i>	c.689-13C>T	Intron		C	0.031	rs11798700
	c.823G>A	Exon	p.D275N	A	0.008	–
	c.612G>A	Exon	p.L204L	A	0.015	rs12117552
	c.369+56G>T	Intron		T	0.031	rs11264442
	c.639+73C>T	Intron		T	0.031	rs11264443
	c.640-52C>T	Intron		T	0.008	rs41314033
	c.861T>C	Exon	p.A287A	C	0.054	rs538089
	c.937-83G>T	Intron		T	0.054	rs80356810
	c.1157+16G>A	Intron		A	0.054	rs534807
	c.1338T>C	Exon	p.D446D	C	0.054	rs505058
c.1489-41C>T	Intron		T	0.054	rs553016	
c.1698C>T	Exon	p.H566H	T	0.231	rs4641	

^a Minor allele frequency in the 130 patient alleles tested.

these alterations of the sodium current complex lead to an increased susceptibility to arrhythmias due to reentrant activity, even without anatomical obstacles. Furthermore, it has been demonstrated that 16% of the ARVC patients exhibit inducible coved-type ST elevations in right precordial leads (Peters, 2008), an ECG finding usually characteristic of Brugada Syndrome, and that mutations affecting $Na_v1.5$ are prevalent in Brugada syndrome patients with structural heart abnormalities resembling ARVC (Frustaci et al., 2005; Frigo et al., 2007). Both *SCN1B* and *SCN3B* have recently been associated with Brugada Syndrome (Olesen et al., 2012). These findings thus suggest that the sodium current complex could play a role in the pathogenesis of ARVC. A variety of other diseases have also been associated with mutations in *SCN1B*–*4B*, including cardiac arrhythmia, cancer, and neurological diseases (Brackenburg and Isom, 2008; Chioni et al., 2009; Olesen et al., 2011).

The *FHL1* gene, located on the X chromosome, is characterized by a half LIM domain in the N-terminus and four following complete LIM domains. The protein is localized both in the cytosol and the nucleus and has several functions including transcription regulation, cell signaling, and sarcomere assembly (Cowling et al., 2011). *FHL1* interacts with *TTN* recently associated with ARVC4 (Taylor et al., 2011; Cowling et al., 2011). A wide variety of human muscle diseases including non-compaction, hypertrophic and dilated cardiomyopathies, have been associated with *FHL1* mutations (Cowling et al., 2011). In our study the non-synonymous variant *FHL1* p.D275N was present in one patient. The variant affects an unconserved residue located in the C-terminus of the *FHL1* peptide. The variant has previously been described in control populations (Schoser et al., 2009) and was present in 31 out of 1669 alleles of American/European descent in the NHLBI GO Exome Sequencing Project (Exome Variant Server). We therefore consider it to be a polymorphism.

The *LMNA* gene, encoding the intermediate filament proteins lamin A and C, plays a role in maintaining the structural integrity of the inner nuclear membrane, in gene expression, and in organization of chromatin (Lu et al., 2011). The lamins interact with several proteins, including *TMEM43*, associated with ARVC5 (Basso et al., 2009;

Bengtsson and Otto, 2008). The ARVC-genes *TMEM43* and *JUP* are believed to cause dysregulation of the adipogenic pathway regulated by PPAR- γ , resulting in adipogenesis, fibrogenesis and myocyte apoptosis (Mermer et al., 2008), and may explain the fibrofatty replacement in the myocardium characteristic of ARVC. Also *LMNA* have been suggested to affect PPAR- γ . (Capanni et al., 2005). Mutations in *LMNA* have been associated with numerous extracardiac disorders, conduction defects, ventricular arrhythmia, and dilated cardiomyopathy (Lu et al., 2011). Dilated cardiomyopathy may mimic ARVC and a predominantly left-sided form of ARVC is becoming increasingly recognized (Sen-Chowdhry et al., 2008) making *LMNA* a plausible candidate gene for ARVC.

This study has the following limitations: We only analyzed the coding regions of *SCN1B-4B*, *FHL1*, and *LMNA*, and the possibility of variants occurring in other regions of the genes cannot be excluded. Our study cohort has a limited size and all patients were of Northern European descent, thus we cannot exclude the possibility of different results in cohorts of different ethnicity. The diagnosis of our cohort is based on the 1994 Task Force criteria. We were not able to apply the revised criteria published in 2010 to our cohort, due to lack of necessary clinical data, e.g. all myocardial biopsies are obtained from the septum.

In conclusion, we found no disease-causing mutations in ARVC patients within the *SCN1B-4B*, *FHL1*, and *LMNA* genes. The present study is to our knowledge the first attempt to associate genetic variation in these genes with ARVC. Our results indicate that these six genes are not common causes of ARVC.

Disclosures

None.

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