Analysis of SCN5A Gene Variants in East Slovak Patients with Cardiomyopathy

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Objective: Mutations in ion channels genes are potential cause of cardiomyopathy. The SCN5A gene (sodium channel, voltage gated, type V alpha subunit gene; 3p21) belongs to the family of cardiac sodium channel genes. Mutations in SCN5A gene lead to decreased Na⁺ current and ion unbalance. The SCN5A gene mutations are found in approximately 2% of patients with dilated cardiomyopathy (DCM), and they may be potential phenotype modifiers in hypertrophic cardiomyopathy (HCM). The role of SCN5A gene mutations in cardiomyopathy is not fully elucidated. **Methods:** Three selected exons (12, 20, and 21) of the SCN5A gene in the cohort of 58 East Slovak

patients with dilated and HCM were analyzed by the Sanger sequencing method in order to detect etiopathogenic mutations associated with dilated and HCM.

Results: The mutation screening of three selected exons of SCN5A gene in the cohort of 27 DCM, 12 HCM patients, and 16 controls identified 10 missense genetic variants. Three of them (T1247I, A1260D, and G1262S), all in exon 21 of the SCN5A gene, were potentially damaging and disease-causing variants. **Conclusion:** Data from this study demonstrate that SCN5A gene variants have important role in the etiopathogenesis of dilated and HCM. *J. Clin. Lab. Anal.* 31:e22037, 2017. © 2016 Wiley Periodicals, Inc.

Key words: cardiomyopathy; East Slovak patients; potentially damaging variants; SCN5A gene

INTRODUCTION

Cardiomyopathies (CMPs) are diseases of the heart muscle, characterized by abnormality in chamber size and wall thickness, functional contractile systolic or diastolic dysfunction in the absence of coronary artery disease, hypertension, valvular disease, or congenital heart disease (1). CMPs are one of the leading causes of sudden death and heart failure (2, 3). The question of pathogenesis is actual, and more than 100 single genes have been reported to be linked to the molecular-genetic background of cardiomyopathies (4).

The SCN5A gene (sodium channel, voltage gated, type V alpha subunit gene) is located on 3p21 and belongs to a family of genes encoding sodium channels. The gene contains 28 exons, and it encodes

the α -subunit of the cardiac sodium channel (NaV1.5) which plays a critical role in cardiac electrophysiology, in impulse formation and propagation through the cardiac conduction system and muscle (5, 6). NaV1.5 is predominantly expressed in the heart where it is responsible for the rapid initiating phase of the action potential (7).

Hundreds of genetic variants and mutations in the SCN5A gene have been detected, with responsibility

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for multiple pathologic cardiac phenotypes including broad spectrum of cardiac rhythm disorders, long QT syndrome type 3 (LQT3), Brugada syndrome (progressive) conduction disease, sick sinus syndrome, atrial standstill, atrial fibrillation, dilated cardiomyopathy, or sudden infant death syndrome (8). The common genetic variants in the SCN5A gene are suspected to contribute to the pathogenesis of dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM), accelerated age of onset and clinical progression. Genetic variants can affect cardiomyopathy penetrance and modulate phenotypic expression (9).

Presence of multiple mutations can be responsible for disease severity (10). The SCN5A gene mutations are characterized by heterogeneity and by overlapping clinical and in-vitro phenotypes. The SCN5A gene mutations are inherited in an autosomal dominant pattern, often with incomplete penetrance and male predominance (11). It is not clear how mutations in the same gene may cause different clinical syndromes and what is the link between these symptoms (12). Functional mechanisms of SCN5A gene mutations lead to a decreased Na⁺ current. A number of mutations cause failure of the channel, by affecting the pore conductance, or disrupting transfer to the cell membrane. Other mutations cause an alteration in channel kinetics by a shift in voltage and time dependence of gating, slow recovery, or accelerated inactivation (5, 10, 12).

The SCN5A gene mutations are found in approximately 2% of patients with DCM (13). Mutations are predominantly localized to the region of the channel's voltage sensor, but mechanisms by which SCN5A gene mutations lead to DCM are not elucidated (13, 14). Relation of SCN5A gene mutations to HCM is not completely described. It has been suggested that mutations in the SCN5A gene may be potential phenotype modifiers in the pathophysiology of HCM (15).

The aim of this study was to analyze selected exons of the SCN5A gene in patients with DCM and HCM with the purpose to detect etiopathogenic variants associated with cardiomyopathy. The selection of exons was based on our previous results obtained from whole exome sequencing (not published).

PATIENTS AND METHODS

The molecular screening was performed on a selected cohort of 58 unrelated patients from eastern Slovakia, recruited with an original clinical diagnosis of DCM ($n = 39$) or HCM ($n = 19$). A total of 42 male and 16 female patients with mean age of 60.81 ± 13.34 (range of 22–82 years) were included. The diagnosis of DCM and HCM was made by specialists in the cardiology according to criteria provided

by the World Health Organization and ESC Guidelines. A total of 26 healthy, age, sex, and ethnicity similar controls without any history of cardiovascular disease were enrolled in our study.

Written informed consent, clinical and anthropological characteristics according to our protocol designed for this purpose were obtained from all probands.

Blood samples were obtained from patients with diagnosis DCM or HCM and from controls. Genomic DNA was extracted from peripheral blood lymphocytes by a commercially available RepliaPrep™ Blood gDNA isolation kit (Promega, Madison, WI) following the manufacturer's instructions.

Exons 12, 20, and 21 of the SCN5A gene were selected for direct Sanger sequencing. This selection was based on our previous investigation of cardiomyopathy patients by next-generation sequencing technology by which we identified potentially etiopathogenic variants in these three exons of the SCN5A gene. Exons were amplified by polymerase chain reaction and sequenced from both sides in all individuals.

Sequencing was performed on the automated sequencer 3500xL Genetic Analyzer (Life Technologies, Foster City, CA) using a terminator kit BigDye version v3.1 (Life Technologies). Sequences of primers and specific reaction conditions are available on request.

The results were analyzed by the SeqScape v.2.7 and Sequencing Analysis v5.4 software (Life Technologies) and revised manually with Ensembl sequence database (reference SCN5A ENSG00000183873, www.ensembl.org). Damaging effect of missense variants of the SCN5A gene was predicted by SIFT (Sorting Intolerant From Tolerant) and PolyPhen-2 score, using a variant effect predictor (www.ensembl.org/HOMO_sapiens/Tools/VEP) and Mutation taster (www.mutationtaster.org).

RESULTS

The mutation screening of three exons of the SCN5A gene in the cohort of 27 DCM and 12 HCM patients identified 15 genetic variants. From total number of variants, 10 variants were missense and five variants were synonymous (Table 1). Three missense variants in the exon 21 of the SCN5A gene were identified as potentially damaging or disease causing according to SIFT, PolyPhen-2 scores, and mutation taster predictor.

No variants in exons 12, 20, and 21 of the SCN5A gene were found in 32.76% of cardiomyopathy patients. However, one variant was detected in 31.03% of patients and coexistence of two or more variants was confirmed in 36.21% of patients. The most

TABLE 1. SCN5A gene Variants Detected in Slovak Patients with Cardiomyopathy

Exon	rs	Nucleotide changes	Amino acid changes	Phenotype or disease	Patients (n = 58)	Controls (n = 26)	SIFT	PP2	MT
12	rs199473118	c.1535C>T	T512I	Brugada syndrome, cardiac conduction defect, progressive familial heart block type IA	2 (3.45%)	0	T (0.19)	Ben (0.002)	DC
12	rs145733679	c.1537C>T	R513C	Not specified	2 (3.45%)	0	T (0.16)	Ben (0.311)	P
12	rs397517951	c.1538G>A	R513H	Not specified	1 (1.72%)	0	T (0.53)	Ben (0)	P
12	rs370684004	c.1575C>T	S525S	Not specified	2 (3.45%)	0	—	—	DC
12	rs45627438	c.1577G>A	R526H	Brugada syndrome	4 (6.90%)	0	T (0.7)	Ben (0.002)	DC
12	rs749857735	c.1641A>G	E547E	Not specified	1 (1.72%)	0	—	—	DC
12	rs1805124	c.1673A>G	H558R	Progressive familial heart block type IA	18 (31.03%)	10 (38.46%)	T (1)	Ben (0.001)	P
12	rs45522138	c.1681C>T	L561L	Brugada syndrome	3 (5.17%)	0	—	—	DC
12	rs780825701	c.1709C>A	T570N	Not specified	1 (1.72%)	1 (3.85%)	T (0.76)	Ben (0.051)	P
20	rs752243560	c.3546C>T	D1182D	Not specified	2 (3.45%)	0	—	—	DC
21	rs753677814	c.3735G>A	M1245I	Not specified	7 (12.07%)	2 (7.69%)	T (0.52)	Ben (0.281)	P
21	rs764018276	c.3740C>T	T1247I ^a	Not specified	19 (32.76%)	0	D (0.01)	Pos (0.844)	DC
21	rs199540275	c.3779C>A	A1260D ^a	Not specified	3 (5.17%)	1 (3.85%)	D (0)	Pro (0.999)	DC
21	rs750678689	c.3783C>T	Y1261Y	Not specified	2 (3.45%)	2 (7.69%)	—	—	DC
21	rs137854616	c.3784G>A	G1262S ^a	Brugada syndrome	3 (5.17%)	0	D (0.01)	Pro (0.972)	DC

Ben, benign; D, damaging; DC, disease causing; MT, mutation taster predictor; P, polymorphism; Pos, possible damaging; PP2, PolyPhen-2; Pro, probably damaging; T, tolerated.

^aBold indicates the results of predictor software that are potentially damaging or disease causing according to at least two predictor software.

frequent variants of the selected exons were T1247I (32.76%), H558R (31.03%) (Fig. 1), and M1245I (12.07%). The most common variant combinations detected in our case study were H558R/T1247I (20.5%) and H558R/M1245I (10.2%).

R513H, S525S, E547E, L561L, T570N, A1260D, and Y1261Y variants were identified only in DCM patients. R513C and D1182D were detected only in HCM patients. T512I, R526H, H558R, M1245I, T1247I, and G1262S nonsynonymous genetic variants were confirmed in both DCM and HCM patients. Some identified variants of exons 12, 20, and 21 in our Slovak cardiomyopathy patients were previously detected in association with specific phenotypes—Brugada syndrome, cardiac conduction defect, atrial fibrillation (Table 1). Four variants (T570N, M1245I, A1260D, and Y1261Y) of the SCN5A gene were found in cardiomyopathy patients and also in controls. Variants directly associated with cardiomyopathy phenotype were not detected in our cohort of patients.

DISCUSSION

Although the pathophysiology of DCM and HCM is well known, the underlying genetic mechanism for this disorder has remained unclear (16, 17).

This is the first report of genetic analysis of the SCN5A gene in Slovak cardiomyopathy patients, and we focused on the pathogenic mutations and their importance in cardiomyopathy etiology. Our data support the earlier findings that the SCN5A gene can be associated with cardiomyopathy (18–22).

In this study, we used Sanger sequencing to identify etiopathogenic variants of the SCN5A gene in DCM and HCM patients. It is suggested that SCN5A gene mutations play an important role in multiple phenotypes including cardiomyopathies (23). The SCN5A gene mutations have been found in approximately 2% of DCM patients (13). Information about the presence of SCN5A gene mutations in patients diagnosed with HCM is limited, but we verified and confirmed the presence of SCN5A gene variants in the group of East Slovak patients with HCM.

We sequenced 58 unrelated patients and 26 controls DNA, and we identified 39 SCN5A variant carriers after systematic mutation screening. All 15 variants have already been reported. Ten detected variants were nonsynonymous. Four of the nonsynonymous variants (T512I, R526H, H558R, and G1262S) were associated with specific phenotypes, and four variants (H558R, T570N, M1245I, and A1260) of the SCN5A gene were found in cardiomyopathy patients and also in controls. All SCN5A gene variants were categorized by scientific

Fig. 1. Results of Sanger sequencing showing heterozygous genotype of H558R polymorphism in a patient with HCM (A), DCM (B) in comparison with wild-type genotype in a control subject (C).

approach based on SIFT, PP2, and Mutation taster predictor. Tolerated, benign polymorphisms were 50% of the nonsynonymous variants based on scores of all three predictor software, whereas 20% of the variants were classified as disease causing according to Mutation taster predictor. Only three variants were potentially damaging or disease causing (T1247I, A1260D, and G1262S) according to at least two predictor software. All potentially pathogenic variants were identified in the exon 21 of the SCN5A gene. The variant T1247I of the SCN5A gene was detected in 11 DCM (10 males, 1 female) and 7 HCM (6 males, 1 female) patients and was absent in controls. The variant A1260D was detected in three male DCM patients and was also present in one control. The variant G1262S of the SCN5A gene was observed in two male DCM and one female HCM patients and was absent in the control group.

In our study, the percentage of at least one detected variant was higher in DCM patients than in HCM patients. The SCN5A-cardiomyopathy-affected patients with potentially damaging effect of variants were predominantly male. The clinical phenotype of the analyzed variants was not directly related with cardiomyopathy based on data of Ensembl database, except for G1262S which was associated with the Brugada syndrome (24). Other authors detected two cardiomyopathy associated missense variants D1275N

and V1279I in the exon 21 of the SCN5A gene (13, 25, 26) which were absent in our cohort of cardiomyopathy patients.

Other identified variants in the cohort of East Slovak patients were benign genetic variations or were identified at low frequency in controls, but they could still be relevant for cardiomyopathy pathogenesis. These findings require next validations.

CONCLUSION

This is the first study that describes variants of the SCN5A gene in Slovak cardiomyopathy patients. Despite the relatively small number of patients with cardiomyopathy enrolled in our study, we confirmed 15 variants in the exons 12, 20, and 21 of the SCN5A gene. From the total number of variants, 10 were missense. Five-identified variants (T512I, R526H, H558R, L561L, and G1262S) of exons 12, 20, and 21 in our Slovak cardiomyopathy patients were previously detected in association with specific phenotypes, and they were absent in controls, except for H558R, which is common polymorphism. Four variants of the SCN5A gene were found in cardiomyopathy patients and also in controls. Variants directly associated with cardiomyopathy phenotype were not detected in Slovak cardiomyopathy patients, but predicting software indicated that three variants (T1247I, A1260D, and G1262S) can be potentially damaging and disease causing. Our findings require validation in a larger cohort of patients.

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ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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