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Mutation analysis of *RyR2* gene in patients after arrhythmic storm

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ABSTRACT

Introduction: Mutations of *RyR2* gene encoding calcium channel of sarcoplazmatic reticulum are the cause of congenital catecholaminergic polymorphic ventricular tachycardia. The aim of this study was to test the hypothesis that *RyR2* variants can increase occurrence of malignant arrhythmias in patients with structural heart diseases.
Methods: The investigated group consisted of 36 patients with structural heart diseases with ICD implanted who suffered arrhythmic storm. In the control group there were 141 patients with coronary artery disease who were hospitalized at our department owing to an acute coronary event and they were alive at least 3 years after the index event. Thus, they could be considered as a group with a low risk of sudden cardiac death. In all of them mutation analysis of *RyR2* gene was performed.
Results: We detected 16 different sequence changes of *RyR2* gene in both groups. None of found nucleotide polymorphisms led to amino acid changes, were located close to splice sites or had any similarity to known splicing enhancer motifs. The occurrence of these variants was not different in both groups.
Conclusions: The prevalence of *RyR2* gene variants was not different in cases versus controls suggesting a limited role of this gene in the arrhythmogenesis in structural heart disease patients.

Klíčová slova:
Arytmická bouře
Gen *RyR2*
Mutační analýza
Náhlá srdeční smrt

SOUHRN

Úvod: Mutace genu *RyR2* kódujícího vápníkový kanál sarkoplazmatického retikula jsou příčinou kongenitální arytmie katecholaminergní polymorfní komorové tachykardie. Cílem práce je ověřit hypotézu, zda varianty genu *RyR2* nemohou zvyšovat výskyt maligních arytmií u pacientů se strukturálním onemocněním srdce.
Metody: Soubor vyšetřených tvořilo 36 pacientů se strukturálním onemocněním srdce zajištěných implantabilním kardioverterem-defibrilátorem (ICD), kteří prodělali arytmickou bouři. Kontrolní skupinu tvořilo 141 jedinců s ischemickou chorobou srdeční hospitalizovaných na našem pracovišti z důvodu akutního koronárního syndromu a dobou přežití delší než tři roky od příhody, tedy pacienti s relativně nízkým rizikem náhlé srdeční smrti. U všech zařazených byla provedena mutační analýza genu *RyR2*.
Výsledky: V obou skupinách jsme detekovali celkem 16 sekvenčních změn DNA genu *RyR2*. Odhalené nukleotidové polymorfismy nezahrnují žádnou změnu, která by vedla k zařazení odlišné aminokyseliny.
Závěr: Výskyt sekvenčních změn byl stejný v obou skupinách vyšetřených pacientů.

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Introduction

The leading cause of cardiovascular mortality is sudden cardiac death (SCD). Estimated incidence of SCD is more than 3 million people in the world per year. It means about 200–400 thousand in the U.S. population per year and in Europe it accounts for about 2,500 deaths per day. Nevertheless, the SCD incidence in the general population is low (0.1–0.2% per year, i.e. 1–2 patients/1,000 population). Approximately 50% of all coronary heart disease deaths are sudden [1]. In most cases, the underlying cause of death is the rapid onset of lethal ventricular arrhythmias in the setting of acquired heart disease. In our study we considered the common knowledge, that the risk of sudden cardiac death is higher in patients with structural heart disease (it means chronic ischemic heart disease or dilated cardiomyopathy) and with systolic dysfunction of left ventricle. Data from MADIT-II study showed that during 3 years only one third of patients with primary preventive implantation of ICD needed the therapy from the device due to malignant arrhythmias. The remaining two thirds of patients were only ICD carriers [2]. Observations from population-based studies demonstrate a strong genetic component of SCD [3–6]. It is clear that there must be genetically determined variations of physiological processes that increase the risk of SCD. It is well known that myocardial ion channel gene mutations lead to increased SCD risk in patients with relatively rare diseases, such as long QT syndrome (LQTS), Brugada syndrome and catecholaminergic polymorphic ventricular tachycardia (CPVT).

Ventricular premature complexes (VPCs) could initiate malignant arrhythmias. High occurrence of VPCs is typical for rare hereditary disease called CPVT. For this disorder stress syncope or sudden cardiac death are typical, manifested in young patients most often before 40 years of age. The mortality is high, almost 50%. Mutations of *RyR2* gene are found in about 60% of patients with clinical diagnosis of CPVT [7]. An important fact is that the resting ECG is completely normal. But during stress VPCs are becoming more frequent and can progress in typical bidirectional ventricular tachycardia. In our study we tried to test the hypothesis, that mutation of RyR2 receptor could increase the risk of malignant arrhythmias in patients with structural heart disease.

Table 1 – Clinical characteristics of investigated individuals.

	Arrhythmic storm (n = 36)	CAD controls (n = 141)	*p*
Male/Female	33/3	129/12	0,4676
Age (years)	69,4 ± 11,4	66,7 ± 10,7	0,4610
Left ventricle ejection fraction	32 ± 9 %	42 ± 9 %	< 0,0001
CAD	69%	100%	< 0,0001
History of myocardial infarction	21 (58%)	129 (90%)	< 0,0001
DCM	11 (31%)	0	< 0,0001

CAD – coronary artery disease; DCM – dilated cardiomyopathy.

Table 2 – *RyR2* gene variants in investigated individuals.

Nucleotide change	Exon/intron	Allelic frequency %		*p*	References
		Arrhythmic storm n = 36	Control group n = 141		
c.[268-100G>T]	intron 3-4	4,0	6,2	0,5414	–
c.[458-8A>C]	intron 7-8	16,2	12,1	0,2991	12
c.[671-11T>A]	intron 9-10	43,2	53,8	0.1003	12
c.[1000-31T>A]	intron 12-13	1,3	2,7	0,4860	–
c.[1353C>T]	exon 15	33,8	40,5	0,2708	12
c.[6682+72T>G]	intron 43-44	36,5	47,0	0,0924	12
c.[6682+93C>A]	intron 43-44	27,0	16,2	0,0487	–
c.[6900T>C]	exon 45	94,5	87,8	0,1117	13
c.[11320-23C>T]	intron 82-83	35,1	29,7	0,4182	12
c.[12957T>C]	intron 89-90	58,1	68,9	0,0928	–
c.[13470+16A>G]	intron 92-93	33,8	28,3	0,4091	12
c.[13470+47G>A]	intron 92-93	36,5	43,2	0,2722	12
c.[13777-21G>A]	intron 94-95	55,4	42,5	0,0479	12
c.[13777-6A>G]	intron 94-95	50,0	36,2	0,0318	12
c.[13907+12A>C]	intron 95-96	59,5	46,0	0,0390	12
c.[14085-(11-22delT)]	intron 97-98	47,3	40,8	0,3231	–

Methods

Investigated subjects

The investigated group consisted of 36 Caucasian patients from the region of south Moravia in the Czech Republic. These individuals were identified from the ICD registry of our department, partially retrospectively (years 1998–2006) and from 2007 new consecutive patients were included prospectively. The inclusion criteria were: 1. history of arrhythmic storm defined as 3 or more sustained ventricular tachyarrhythmias within 24 hours detected and treated from ICD; 2. structural heart disease such as dilated cardiomyopathy (DCM) or CAD verified by echocardiography and coronary angiography.

The control group consisted of 141 patients with CAD who were hospitalized at our department owing to an acute coronary event. For all patients the diagnosis was confirmed by coronary angiography. All patients had depressed left ventricle ejection fraction (LVEF) and they were alive at least 3 years after the index event. Thus, they could be considered as a CAD group with a low risk of SCD. Clinical characteristics of both groups are summarized in Table 1.

Informed consent was obtained from all patients and peripheral blood samples were taken for genomic DNA preparation. The study protocol was approved by Ethical Committee of the University Hospital Brno and conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

Mutation analysis

Mutation analysis of the *RyR2* gene was performed in all members of the arrhythmic storm group. Genomic DNA samples were isolated from peripheral blood lymphocytes and PCR amplified fragments covering hot spot areas of *RyR2* exons 2-4, 6-15, 17-20, 39-49, 83, 84, 87-97-105 were analyzed by direct sequencing on the instrument ABI PRISM 3130 (Life Technologies, USA). For mapping of exons 3, 97 and 105 deletions multiplex ligation-dependent probe amplification analysis (MLPA) was used. Detailed methodology has been described elsewhere [8–10]. The regions containing sequence variants found in the arrhythmic storm group were sequenced in the control group of 141 patients with CAD without arrhythmias.

Statistics

Values are given as mean ± SD. Demographic data were analyzed by the F test for variance and Student's *t*-test. The distributions of allelic frequencies and their differences were calculated using χ^2 tests. The program package Statistica v. 8.0 (Statsoft Inc., Tulsa, OK, USA) was used for all statistical analyses.

Results

We detected 16 different sequence changes in both groups. Twelve of these DNA changes were already described in the study of Bagattin et al. [11] or in the database Ensembl [12]. The other have not been described yet. None of the found nucleotide polymorphisms led to amino acid changes and were located close to splice sites or had any similarity to known splicing enhancer motifs. The occurrence of these variants was not different in both groups. The results are summarized in Table 2.

Discussion

In the present study, we performed a mutation analysis of a cardiac ion channel gene important in arrhythmogenesis. We test the hypothesis that mutation of *RyR2* gene could increase risk of malignant arrhythmias in patients with structural heart diseases. Our hypothesis was based on findings of previous population studies that demonstrated a strong genetic component of SCD.

Recently, several studies have investigated the issue of the influence of genetic variants on the risk of arrhythmias in patients with structural heart diseases. In these studies the prevalence of rare coding variants in so called long QT genes was significantly higher in patients suffering from ventricular fibrillation as compared to control group [13–15].

The *RyR2* gene was chosen because of its well known role in the arrhythmogenesis in CPVT. This disease is characterized by high occurrence of VPCs. We hypothesized that such *RyR2* mutations can exist which remain usually latent, but under stress conditions, such as structural heart disease, they can increase the risk of ventricular arrhythmias also in normal population.

The *RyR2* gene is one of the largest genes in the human genome and its analysis is time consuming. The majority of mutations appear to cluster in three regions of the predicted *RyR2* protein topology, and about 65% of published mutations of the *RyR2* gene are located in these regions [9,16]. Therefore we used a tiered targeting strategy suggested by groups in the Mayo Clinic and the Netherlands [9]. Although we expected only rare finding of sequence changes of *RyR2* gene, the opposite was true. The occurrence of sequence variants was common in both groups. This suggests that these regions are probably rather polymorphic in population. In our study 16 sequence changes of *RyR2* gene were detected in both groups. None of the found nucleotide polymorphisms led to amino acid changes, were located close to splice sites or had any similarity to known splicing enhancer motifs. Nevertheless, there is emerging evidence that even synonymous DNA variants can play important role in protein structure changes. On the other side there were no statistical differences in their allelic frequencies, thus the probability of any functional effect of these variants is low.

The most important limitation of our study is the small number of investigated individuals. Although the control group was age and sex matched and all the control individuals have depressed left ventricular systolic function and coronary artery disease confirmed by angiography, there were differences in ejection fraction and the etiology of heart failure. This could have possible impact on the results. Nevertheless, the prevalence of DNA variants was high also in the control group. Using the tiered targeting strategy of *RyR2* gene mutation analysis can have an impact on the robustness of the estimate of the prevalence of *RyR2* mutations among the cohort. Due to the

small number of women included in the present study, it was not possible to perform any sex-related associations.

Conclusions

The prevalence of *RyR2* gene variants was not different in cases versus controls suggesting a limited role of this gene in the arrhythmogenesis in structural heart disease patients.

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