Original Research Article

Mutation analysis of ion channel genes promoters in ventricular fibrillation survivors with coronary artery disease

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Introduction: Strong evidence suggests that sudden cardiac death (SCD) is genetically determined. In our previous study we found that the prevalence of selected, rare coding variants in 5 long QT genes was significantly higher in ventricular fibrillation (VF) survivors with coronary artery disease (CAD) than in controls. In the present study we performed mutational analysis of the promoters of 5 LQTS-related myocardial ion channel genes in the same group of patients and in control populations.

Methods: The promoters of KCNQ1, KCNH2, SCN5A, KCNE1 and KCNE2 genes were analyzed in 45 CAD individuals – survivors of documented VF. The allelic frequencies were compared either to data from the 1000 Genomes Project or from a local DNA bank of patients with coronary artery disease and no malignant arrhythmia (141 individuals).

Results: In 34 (75.5%) of 45 VF survivors 9 different promoter variants were found: 2 in KCNQ1 gene promoter, 1 in KCNE1 promoter, and 6 in SCN5A promoter. Statistically significant differences were found in the allelic frequencies of both KCNQ1 gene promoter variants: 1-182C>T (P=0.008), 1-119G>A (P=0.007). Nevertheless, these variants did not segregate with long QT phenotype in a previous study. While the allelic frequency of the SCN5A gene promoter variant 225-1072T>C significantly differed in VF survivors compared to the 1000 Genomes Project (P=0.001), this allelic frequency was not different when compared to the group of local CAD controls.

Conclusions: Our findings demonstrated that variants of ion channel gene promoters are common, both in VF survivors and control groups. These results suggest that promoter variants are geographically-specific and are not a common cause of SCD.

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1. Introduction

There is strong evidence that sudden cardiac death (SCD) is genetically determined [1–4]. In our previous study we found that the prevalence of selected, rare coding variants in 5 long QT genes was significantly higher in cases compared to controls, confirming a mechanistic role for these genes in a subgroup of patients with coronary artery disease (CAD) who had suffered ventricular fibrillation (VF) [5].

Ten years after human genome sequencing it has become clear that coding sequences represent only about 1.5% of human genome sequence [6]. The remaining portion is either so called “DNA junk” or, more likely, contains important regulatory sequences.

In the present study we performed mutational analysis of the non-coding parts of genes – the promoters – of 5 LQTS-related myocardial ion channel genes in patients with CAD who had suffered documented VF at the time of SCD event and in control population.

2. Methods

2.1. Investigated subjects

The group of patients was identical to our previous study [5], consisting of 45 Caucasian patients (5 females, 40 males) from the region of south Moravia in the Czech Republic (population approximately 1.5 million). The primary inclusion criterion was VF documented at the time of circulatory arrest not related to acute phase of myocardial infarction (i.e. more than 48 h after index event). Such individuals were identified from ICD registry of our department, partially retrospectively (years 1998–2006), from 2007 new consecutive patients were included prospectively. Two patients suffered VF while staying in a hospital for more than 72 h after acute myocardial infarction. All other individuals suffered circulatory arrest in the out-of-hospital setting – VF was recorded by emergency car staff. Patients were successfully resuscitated and then transferred to our department, either directly or via regional hospitals. After circulatory stabilization and improvement in mental status, all patients underwent comprehensive cardiological examination. None of the patients had an acute myocardial infarction at the time of arrhythmia according to electrocardiogram (ECG) and serum troponin level analysis. In all patients coronary artery disease was confirmed by coronary angiography findings of significant stenoses (>70%) or chronic occlusions. The left ventricle ejection fraction (LVEF) was calculated from echocardiography analysis. In three patients, QT interval assessment was not possible due to left bundle branch block or atrial fibrillation. None of the other 45 patients showed substantial QT interval prolongation or Brugada-like ECG.

As a control, we used data from the 1000 Genomes Project, which sequences the genomes of a large number of people, to provide a comprehensive resource on human genetic variation. To date, the database contains data on genetic variation in 1092 human genomes from various populations [7].

In cases where the data from the 1000 Genomes Project were not available, another control group was used, consisting 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 LVEF and were alive at least 4 years after the index event and, accordingly, they could be considered as a CAD group with a low risk of SCD. Clinical characteristics of both groups of patients are summarized in Table 1.

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

2.2. Genomic DNA samples and polymerase chain reaction amplification

The genomic DNA of all cases was analyzed for mutations in the promoter sequences of KCNQ1, KCNH2, SCN5A, KCNE1 and KCNE2 genes. Genomic DNA was extracted from peripheral blood samples according to a standard protocol using DNA BloodSpin Kit and the classical method of ethanol progression. The promoter sequences of KCNQ1, KCNH2 and KCNE1 genes (GenBank accession nos. NG008935.1, NG008916.1, NG009091.1), were amplified by multiplex polymerase chain reaction (PCR). The following primer pairs were designed and used: 7 primer pairs for KCNQ1 promoter, 6 primer pairs for KCNH2 promoter and 1 primer pair for KCNE1 promoter. The PCR amplifications were performed using HotStarTaq Master Mix (Qiagen, Inc., Valencia, CA, USA). Each PCR was performed in 200-μl thin-walled PCR tubes in a total reaction volume of 25 μl using Verity Thermal Cycler (Applied Biosystems, Foster City, CA, USA).

A total of 12 oligonucleotide primer pairs was designed and used to amplify the promoter area of the SCN5A gene (GenBank accession no. AY313163). The core promoter

<table>
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<th>Table 1 – Clinical characteristics of investigated individuals.</th>
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<td>VF survivors (n = 45)</td>
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VF – ventricular fibrillation, CAD – coronary artery disease.
sequence of KCNE2 gene was predicted to correspond to base pairs 7481–7746 of sequence DQ784804 using the Web Promoter Scan Service program, version 1.7. This region was amplified using one oligonucleotide primer pair. PCR amplifications were performed using Taq DNA polymerase (Fermen tas, Inc., Glen Burnie, MD, USA) and HotStarTaq Master Mix (Qiagen, Inc., Valencia, CA, USA). Each PCR was performed in 200-μl thin-walled PCR tubes in a total reaction volume of 25 μl. Each PCR amplification was performed using a Senso Quest Labcycler (Progen Scientific, Ltd., Mexborough, UK) and a Perkin Elmer 2400 machine (Applied Biosystem, Foster City, CA, USA).

2.3. DNA sequencing

DNA sequence analysis was carried out in an ABI 3130 (Applied Biosystem, Foster City, CA, USA) to screen for KCNQ1, KCNH2, SCN5A, KCNE2 and KCNE1 promoter sequence variations. Sequencing analyses were performed using forward and reverse primers.

For purification of the amplified samples, the MinElute PCR Purification Kit (Qiagen, Inc., Valencia, CA, USA) was used. Cycle sequencing was performed using the Big Dye Terminator Kit (Applied Biosystems, Foster City, CA, USA) and the DyeEx2.0 Spin Kit (Qiagen, Inc., Valencia, CA, USA) was used for purification of samples after cycle sequencing.

The resulting sequences of the screened KCNQ1, KCNH2, SCN5A, KCNE2, and KCNE1, gene promoters were aligned with the wild-type sequences deposited in NCBI (GenBank accession nos. NG008935.1, NG008916.1, NG009091.1, AY313163, DQ784804). The numbering was based on Yang et al. [8] and the allelic frequencies were compared to data from the 1000 Genomes Project, if available [7]. Otherwise the regions containing sequence variants found in VF survivors were sequenced in the control group of 141 patients with CAD without arrhythmias (described above).

2.4. Statistics

The distributions of allelic frequencies and their differences were calculated using χ² tests. Odds ratio (OR) and 95% confidence interval (CI) were calculated to estimate the risks associated with the detected polymorphisms. To calculate the significance of OR, Fisher’s exact test was used. The program package Statistica v. 8.0 (Statsoft Inc., Tulsa, OK, USA) was used for all statistical analyses.

3. Results

In 34 (75.5%) of 45 VF survivors 9 different DNA sequence variants were found: 2 in KCNQ1 gene promoter, 1 in KCNE1 promoter, and 6 in SCN5A promoter. No variants were detected in the promoters of KCNH2 and KCNE2 genes. Variants in more than one gene promoter were present in 6 patients, and multiple variants in single gene promoter were found in 16 patients.

3.1. KCNQ1 gene promoter variants

In KCNQ1 gene promoter variants 1-182C>T and c. 1-119G>A were found in 15 and 3 patients, respectively. The c. 1-119G>A polymorphism segregated with the c. 1-182C>T in all 3 cases. These variants were present in the 1000 Genomes Project data.

3.2. KCNE1 gene promoter variant

A single variant, c. 1-107insG, was found in the KCNE1 gene promoter in 3 VF survivors. Data on this particular variant were not available from then 1000 Genomes Project, and its allelic frequency was tested in the CAD control group.

3.3. SCN5A gene promoter variants

Six promoter variants were found in the SCN5A gene in 22 VF survivors. More than one SCN5A promoter variant was present in 13 VF survivors. The following variants have been described in the 1000 Genomes Project previously: c. -225-2038G>T, c. -225-1823C>T, c. -225-834T>C, c. -225-1744G>C, c. -225-1072T>C. For the c. -225-775T>A variant, no data were available from the 1000 Genomes Project and, therefore, its allelic frequency was tested in the CAD control group.

3.4. Statistic results

The allelic frequencies of all promoter variants in the cases and control groups are summarized in Table 2. Statistically significant differences were found in the allelic frequencies of both KCNQ1 gene promoter variants: c. 1-182C>T (OR=2.57, CI=1.18–5.57 for the C allele in VF survivors compared to the CAD group, P=0.008) and c. 1-119G>A (OR=7.76, 95% CI=1.06–56.77 for the G allele in VF survivors compared to the control group, P=0.007). The allelic frequency of SCN5A gene promoter variant c. 225-1072T>C was significantly different in VF survivors compared to the S1000 Genomes Project (OR=7.28, 95% CI=1.00–53.28 for the T allele in VF survivors compared to the control group, P=0.001), but was not different between the cases and the group of local CAD controls.

4. Discussion

In the present study, we performed a mutation analysis of promoters of five cardiac ion channel genes in VF survivors with CAD to test the hypothesis that polymorphisms in these regions are more common in VF survivors compared with a control population. This study was an extension of our previous study in which we found that the carrier frequency of rare coding variants of these genes was significantly higher in the VF survivors (8/45, 17.8%) than in CAD controls (3/141, 2.2%, P=0.001). In the promoter regions of the same genes we found 9 different DNA sequence variants. The allelic frequencies of three of these variants were significantly different compared to data from 1000 Genomes Project, which was used as a primary control due its statistical power to detect
most genetic variants with frequencies of at least 1%. A key goal of the 1000 Genomes Project is to identify more than 95% of single nucleotide polymorphisms at 1% frequency in a broad set of populations and to date it contains data on genetic variation from 1092 human genomes in 14 populations drawn from Europe, East Asia, sub-Saharan Africa and the Americas [7].

Data on the prevalence of ion channel promoter polymorphisms in arrhythmic patients are limited [9]. The variants we detected in KCNQ1 gene promoter were also present in the 1000 Genome Project dataset and the occurrences of both polymorphisms in this promoter were significantly different in VF survivors group compared to the 1000 Genomes Project dataset. Data concerning the possible functional effect of these polymorphisms are lacking; in our previous work these variants were found also in patients with clinical diagnosis of long QT syndrome (LQTS) but in no case did the particular variant segregate with LQTS phenotype [10]. Thus, any possibility of their significant functional effect is very limited.

The variant we detected in the KCNE1 gene promoter has not been described previously [11], and its allelic frequency did not differ between VF survivors and CAD patients without malignant arrhythmias.

The SCNSA gene promoter is highly polymorphic, reflected in the fact that in this study we identified 6 different polymorphisms in this promoter. With the exception of c. -225-1072T>C all these variants were the subject of a study by Yang et al. [8], which found that study only promoter activity in c. -225-775 T>A variant was significantly reduced in cardiomyocytes. Such variant may modulate sodium-channel-related physiology in the face of environmental stressors such as transient myocardial ischemia. Nevertheless, allelic frequency of this variant did not differ between VF survivors and CAD group in our study, indicating that the potential for a significant functional effect is limited.

No functional data are available for the variant c. -225-1072T>C. While the allelic frequencies of this variant were significantly different in VF survivors compared to the 1000 Genomes Project dataset, its allelic frequency in VF survivors was not different when compared to the group of local CAD controls. These results indicate that this variant may be a regionally specific, and likely to be a non-functional polymorphism.

### 5. Limitations

There are several limitations to our study, the most important of which is the small number of investigated individuals. Owing to the incidence of SCD events and the capacity of individual centers to perform routine genetic methods, larger numbers could only be provided by a multicenter study. Due to the small number of women included in the present study it was not possible to perform any sex-related associations. Allelic frequencies of variants found in VF survivors were compared with data from the 1000 Genomes Project, which represents a non-specific population. In the case of KCNQ variants, the missing functional studies were substituted by data from genotype–phenotype analyses in LQTS subjects.

### 6. Conclusion

While variants of ion channel genes promoters are common both in SCD individuals and control groups, our results suggest that promoter variants of ion channel genes are not a common cause of SCD.
Acknowledgments

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