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Novel KCNQ1 mutations in patients after myocardial infarction

Marlena Olszak-Waśkiewicz[1], Mirosław Dziuk[1], Leszek Kubik[1], Radosław Kaczanowski[2] and Krzysztof Kucharczyk[2]

[1]Department of Cardiology, Military Institute of Health Service, Warsaw, Poland
[2]Kucharczyk Techniki Elektroforetyczne Co., Warsaw, Poland

Abstract

Background: *Patients after myocardial infarction (MI) are at greater risk of sudden cardiac death (SCD) than people in the overall population. The aim of this study was to detect mutations, including intronic ones, in the KCNQ1 gene coding for proteins of cardiac potassium channels and evaluate their possible effects on the clinical course in patients after MI.*

Methods: *The study group was composed of 100 Polish patients after MI, which included 27 women (mean age 69 years) and 73 men (mean age 67 years). All patients underwent clinical examinations and genetic tests. The genetic test results have been correlated with the clinical data. The following parameters have been chosen as endpoints for this survey: sudden cardiac arrest (SCA) or SCD, complex ventricular arrhythmia, QT interval and QT dispersion values assessed during 24-hour Holter ECG monitoring in relation to ventricular arrhythmias as well as the minimum and maximum heart rate (HR) observed during the examination.*

Results: *Six new mutations in the KCNQ1 gene: C2505734T, A2753831C in exons and C2505846A, G2753881A, T2755854C, T2755875G in introns. Detected intronic mutations in patients after MI were related to a worse clinical course and frequent occurrence of SCA.*

Conclusions: *The novel intronic mutations may have a significant influence on the clinical course of the disease.* (Cardiol J 2008; 15: 252–260)

Key words: myocardial infarction, mutation, arrhythmias

Introduction

Mutations in potassium channel genes are the most frequent genetic abnormalities in patients with congenital long QT syndrome (LQTS). To date, more than 100 families with mutations in this gene have been reported, most of them with their own specific mutations. In general the majority of these mutations are missed. However, other types of mutations, such as deletions, frame-shifts and splice-donor errors have also been reported [1–5]. The protein produced from the KCNQ1 gene interacts with proteins in the KCNE family (such as KCNE1) to form functional potassium channels. Four alpha subunits, each produced from the KCNQ1 gene, form the structure of each channel. A combination of normal and mutant KCNQ1 alpha-subunits has been found to form abnormal channels; hence mutations associated with the KCNQ1 gene may result in the prolongation of the cellular repolarization phase that may clinically predispose patients with mutations to arrhythmic events. In patients after myocardial infarction the arrhythmic events are more common than in the normal population [6]. It is important to define the subgroup of patients who may be prone to life-threatening arrhythmias.

Address for correspondence: Marlena Olszak-Waśkiewicz, MD, PhD, Ass. Prof., Department of Cardiology, Military Institute of Health Service, Szaserów 128, 00–909 Warszawa, Poland, tel./fax: +48 22 68 16 356, e-mail: meridiap@interia.pl

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It is possible that the presence of specific alterations in the genotype, occurring in the form of mutations or polymorphisms of single nucleotides, may constitute such indicators for higher-risk groups, independently of other clinical factors. The genetic abnormalities found in those patients may help us to understand the mechanism leading to arrhythmic events and serve as an indicator of high risk for sudden cardiac death.

The aim of this study was therefore to detect mutations in the KCNQ1 gene in patients after myocardial infarction in order to see whether they have any effect on electrophysiological predictors of arrhythmia, such as QT interval length and QT dispersion, and subsequently to assess the relationship between the detected genetic alterations and the clinical course of the disease. In addition, we decided to try and assess the clinical significance of the detected mutations in introns of the KCNQ1 gene.

Methods

The protocol of this research study has been approved by the Commission for Bioethics of the Military Medical Chamber in Warsaw. All patients expressed in writing an informed consent to take part in this study.

The studied population included 27 women (age range: 54–79 year old) and 73 men (age range: 43–83 year old) after non-recent (> 3 months) myocardial infarction (MI). The diagnosis of MI was made if elevated troponin or CK-MB levels and either ischemic symptoms or electrocardiographic changes were noted. The exclusion criteria were as follows: recent MI, anginal symptoms at rest, electrolyte imbalance, QT prolonging medications, non-interpretable ECG. All patients were examined (past and present history, physical examination, standard blood tests including sodium, calcium, potassium and magnesium concentrations, 12-lead ECG, 12-lead Holter ECG monitoring and echocardiography) at baseline and after 12 months of follow-up. The blood was drawn for the genetic analysis at baseline visit.

24-hour Holter ECG monitoring

Twelve lead Holter monitoring systems (Mortara Instruments H-Scribe 2) at the sweep speed of 25 mm/s was employed. The following parameters were assessed:

— heart rhythm disturbances with the ventricular arrhythmia classification according to the Lown classification;
— ST segment changes suggesting ischemia;
— QT interval length and dispersion.

The QT interval (from the start of the QRS complex to the end of the T wave) was measured in all leads. If the U wave was present the end of the QT interval was defined as the intersection point of the tangent to the visible part of the descending part of the T-wave. The QT interval duration was corrected for heart rate (QTc) according to the Bazzet's formula if the heart rate (HR) was between 50–120/min or Hodges formula if it was outside this range. The QT dispersion (QTd) was calculated as the difference between the maximal QT (QTmax) and minimal (QTmin) interval duration.

The measurements of QTmax, maximal QTc and QTd were performed during daytime and between 10 PM and 6 AM:

— at minimal and maximal heart rate;
— in the sinus beat before the last normal sinus beat preceding the ventricular extrasystole, ventricular couplet or ventricular tachycardia — called "pre-sinus beat" and designated by "QT1";
— in the last sinus beat before the above mentioned ventricular arrhythmia — called "pre-ventricular beat" and designated by "QT2".

Then the ΔQT for each patient was calculated as the difference between QT2 and QT1 time points for QTmax, QTc max, QTd respectively. The mean difference of ΔQT for all the patients in the group was subsequently estimated.

Echocardiography

The echocardiographic evaluation (M-mode, 2D and Doppler) included the global and regional contractility, left ventricular ejection fraction (EF) calculation according to the Simpson formula, valvular and pericardial assessment. The General Electric device (System Five) was used for all studies.

DNA analysis

The genetic analysis was conducted in the Kucharczyk TE Company (Warsaw, Poland). Genomic DNA from 100 patients were extracted from peripheral blood leucocytes (100 μl of frozen blood was used) with Blood Mini Kit (A&A Biotech, Gdańsk, Poland). Isolation was performed according to the manufacturer's procedure. Regions which were known to contain genetic alterations were chosen for amplification. Twelve regions of KCNQ1 gene were amplified. Amplification was performed according to the procedure listed in [5, 7, 8] The primers are presented in Table 1.

Table 1. Primers used for PCR reaction for amplification of fragments of KCNQ1 gene.

Exon	Primer sequence	Reference
1	F:TCGCCTTCGCTGCAGCTC R:TCCCCCACCAGCTCTCAG	[2]
1a	F:CTACCTGGGGGCGGGGCTGA R:GTGTGGGCAAGGGGCTGGGA	[2]
3	F:CCCTTCCCCAGACGAGAGCA R:CTCCACCCATCCCAGCACAT	[2]
4	F:AGGGGCAGGGGCAGGGACAC R:CGGGGCCTCAGCGCATCTCA	[2]
5	F:TCGCTGGGACTCGCTGCCTT R:TGTCCTGCCCACTCCTCAGCCT	[2]
6	F:GGGTTTGGGTTAGGCAGTTGG R:AGCCACCCCAGGACCCCAG	[2]
11	F:CAGGGGCAGTGAGGGGATGA R:GTGGCTTGGGGGCGGAGG	[2]
12	F:CACTGCCTGCACTTTGAGCC R:GTGAGGAGAAGGGGGTGGTT	[2]
14	F:CCCCCAGCCCTACCACCC R:GCAGGAGCTTCACGTTCACA	[2]
15–1	F:TTCCCACCACTGACTCTCT R:ACTCTTGGCCTCCCCTCT	[2]
15–2	F:GGGATGGGGCTGGGGGA R:CTGTGCTACTTCCTGGCCAT	[2]
15–3	F:TCACTGGCATGGTGGTTGGG R:GGGGAAATGGTGAGACTGTC	[2]

The PCR products were separated on agarose electrophoresis to check for their specificity. Next 2800 PCR products were analyzed by multitemperature single strand conformation polymorphism (MSSCP) [9] technique for single mutation and polymorphisms presence.

The MSSCP conditions were optimized for each analyzed region. MSSCP was performed on 6 to 10%T polyacrylamide gels, 3.3%C in 0.75 × × TBE buffer. For some regions glycerol was added to polyacrylamide gel 5% w/v. MSSCP analysis was performed in DNA Pointer System in 0.5 × × TBE buffer. Temperature profile of electrophoresis was 35–15–5°C. Electrophoresis was performed with 40 W of electrical power, 10 min of preelectrophoresis (40 W at 35°C) was performed before applying samples onto the gel. At the start samples were separated for 10 min at 100 V for concentration. After that step MSSCP separation was performed. Optimized MSSCP electrophoresis conditions are presented in Table 2.

The PCR products that have altered MSSCP mobility were selected and sequenced. Twenty ng DNA/100 bp of PCR product was used as a matrix for sequencing reaction. Both strands were sequenced in PCR products the sequencing of which revealed a genetic alternation.

Genetic alterations were identified using the BLAST (Basic Local Alignment Search Tool) program and its BLASTN version as well as UCSC (University of California Santa Cruz) Genome Bioinformatics and NCBI (National Center for Biotechnology Information) databases of single nucleotide polymorphisms (SNPs).

Study's clinical endpoints

The following parameters have been chosen as endpoints for this study:

— occurrence of sudden cardiac arrest or sudden cardiac death (SCD);
— presence of complex ventricular arrhythmia in the form of non-sustained or sustained ventricular tachycardia (respectively nsVT and sVT) or ventricular fibrillation (VF);

Table 2. Optimised multitemperature single strand conformation polymorphism (MSSCP) conditions for KCNQ1 gene.

Exon	PCR product size	Acrylamide concentration (%)	Glycerole concentration (%)	MSSCP temperature profile [°C]
1a	510	6	5	35–15–5
1	310	9	5	35–15–5
3	330	8	0	35–15–5
4	300	9	5	35–15–5
5	291	9	5	35–15–5
6	247	9	5	35–15–5
11	270	9	5	35–15–5
12	302	9	5	35–15–5
14	249	9	5	35–15–5
15–1	328	8	0	35–30–25
15–2		9	5	35–15–5
15–3		9	5	35–15–5

— QT interval and QT dispersion values assessed during 24-hour Holter ECG monitoring in relation to ventricular arrhythmias as well as minimum and maximum HR observed during the examination.

Statistical analysis

The distribution of the data subjected to analysis was not normal so the non-parametric tests were used. Spearman's rank test correlation was used for interval data. In order to assess the statistical relationship between interval and nominal data the Mann-Whitney test was employed. The χ^2 statistical analysis was used for the nominal data subsets.

Results

In the population of 100 patients after myocardial infarction several genetic alterations in the KCNQ1 gene were found. We searched for their existence both in SNP databases (such as NCBI or UCSC) and in scientific literature, and found that they were neither reported as common polymorphisms nor as known mutations. Therefore, since the variants we detected in our study must be quite rare in the wild-type population, we conclude that these genetic alterations are novel mutations.

Among all discovered mutations in the KCNQ1 gene the most widespread was the T2755875G mutation. According to the UCSC Genome Bioinformatics the alteration is located within the gene's intron 14. It was found in 19 patients. All patients were heterozygotes (T/G). The remaining 81 patients were free from this mutation. The clinical data characterizing both groups are presented in Table 3.

The comparison of the groups with and without the T2755875G mutation showed a significantly higher rate of sudden cardiac arrest, as depicted in Figure 1, in patients with this mutation despite a significantly higher left ventricular ejection fraction (55 ± 5% *vs*. 50 ± 11.6%, $p < 0.03$). There were no differences in the SCD rates between groups.

The arrhythmic episodes were not significantly different apart from the higher percentage of sustained VT in history found in patients with the mutation (10.5% *vs*. 2.5% in the control group).

On the other hand, in the analysis of Holter ECG QT interval parameters we corrected the QT interval duration for HR using Bazett's or Hodges' formule. In the baseline examination the percentage of Hodges-corrected QT measurements was 9.6% in the group with the mutation and 7.9% in the group without, whereas in the follow-up examination these percentages were respectively 8.2% and 9.4%.

Thus, the analysis of the QT interval observed in Holter ECG recordings obtained during the baseline examination revealed that Bazzet-corrected QT1c max was shorter in patients with this mutation than in those without. The QT2c max was not significantly different (Fig. 2, 3). In contrast, Holter ECG recordings obtained during the follow-up examination revealed a trend towards longer values at peak heart rate in patients with the T2755875G mutation (436 ± 40 ms *vs*. 412 ± 46 ms, $p = 0.06$ for QTmax and 70 ± 30 ms *vs*. 54 ± 21 ms, $p = = 0.06$ for QTd). There were no statistical differences in the QT interval, QT dispersion in relation to complex ventricular arrhythmia between groups. The remaining data observed in Holter ECG recordings are presented in Table 4.

An example of MSCCP results is shown in Figure 4. They concern the KCNQ1 exon 14 and its bordering intronic regions (mutation T2755875G in samples 58, 59).

Other mutations

During DNA sequencing a novel mutation C2505734T was found in the first exon of the KCNQ1 gene. This is a silent mutation in the 129th position (V129V). It was found in a patient with a history of sudden cardiac arrest (SCA). This patient had also mutations in the KCNQ1 gene introns — G2753881A in intron 12 and T2755875G in intron 14. He was heterozygotic with regard to all his mutations (C/T, G/A, T/G) respectively.

In the exon 12 another new mutation — A2753831C — was found in 2 patients. Both patients suffered from ventricular arrhythmia after myocardial infarction. They were heterozygotic with regard to this mutation (A/C). This is a silent mutation in the positions 552 (leu552leu), 452 (leu452leu) and 425 (leu425leu), depending on the protein isoforms.

Furthermore the C2505846A mutation located in intron 1 has been detected in a heterozygotic patient who had a SCD during the follow-up. Another mutation (T2755854C) was found in the KCNQ1 gene intron 14 in a patient with ventricular arrhythmia Lown IVa. This patient was heterozygotic with regard to the above mentioned mutation (T/C).

Discussion

The patients after myocardial infarction represent a non-homogenous group with an increased risk for cardiovascular adverse events including

Table 3. Clinical data characterizing groups with and without the T2755875G mutation.

Feature			Group with mutation	Group without mutation
Gender (N)	Women		2	24
	Men		17	57
Mean age (years)	Women		68	69
	Men		70	66
Myocardial infarction (%)	Inferior		73.7	51.9
	Anterior		42.1	51.9
	Lateral		31.6	19.8
Hypertension (%)			79	65.4
Diabetes (%)			36.8	14.8
PTCA (%)			57.9	45.7
Coronary artery bypass graft (%)			21.1	16.1
Sudden cardiac arrest (%)			21.1	5
Sudden cardiac death (%)			11	4
IVS [cm]	Baseline examination	< 1.2	68.4	70
		> 1.2	31.6	30
	Follow-up examination	< 1.2	66.7	77.1
		> 1.2	33.3	23
LVEDD [cm]	Baseline examination	< 5.6	84.2	77.5
		5.6–6.0	15.8	12.5
		> 6.0	0	10
	Follow-up examination	< 5.6	93.3	80.3
		5.6–6.0	6.7	13.1
		> 6.0	0	5
LVEF (%)	Baseline examination		52 ± 10	49 ± 11
	Follow-up examination		55 ± 5	50 ± 11,6
Complex ventricular arrhythmia in history (%)	nsVT		11	15
	sVT		10.5	2.5
	VF		11	5
Patients requiring treatment due to complex ventricular arrhythmia (%)			23.3	20.9

IVS — intraventricular septum (< 1.2 cm — normal, > 1.2 cm — left ventricular hypertrophy), PTCA — percutaneous transluminal coronary angioplasty; LVEDD — left ventricular end-diastolic diameter (< 5.6 cm — normal, > 5.6 cm — left ventricular dilation), LVEF — left ventricular ejection fraction, nsVT — nonsustained ventricular tachycardia, sVT — sustained ventricular tachycardia, VF — ventricular fibrillation

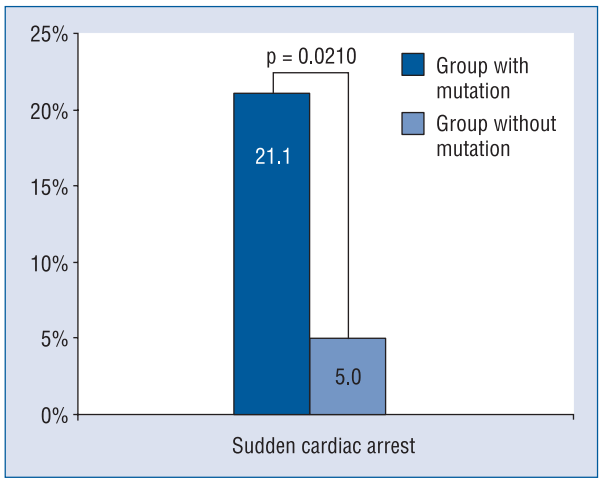

Figure 1. Percentage of patients with sudden cardiac arrest episodes in groups with and without T2755875G mutation.

arrhythmic episodes and sudden cardiac death. The QT interval on the surface ECG is a representation of repolarization time in the ventricle. QT intervals in humans vary as a function of age, sex, heart rate, heart disease, and drugs and are generally less than 480 ms.

"Acquired LQTS" describes not one end of a physiologic spectrum, but rather pathologic QT interval prolongation, generally to greater than 550––600 ms, upon exposure to an environmental stressor and reversion back to normal following withdrawal of the stressor. When QT intervals are markedly prolonged, the polymorphic ventricular tachycardia — torsade de pointes becomes a real risk. Torsade de pointes can be self-limited or can degenerate to fatal arrhythmias such as ventricular fibrillation. It is the potential for torsade de pointes and sudden cardiac death that has generated such

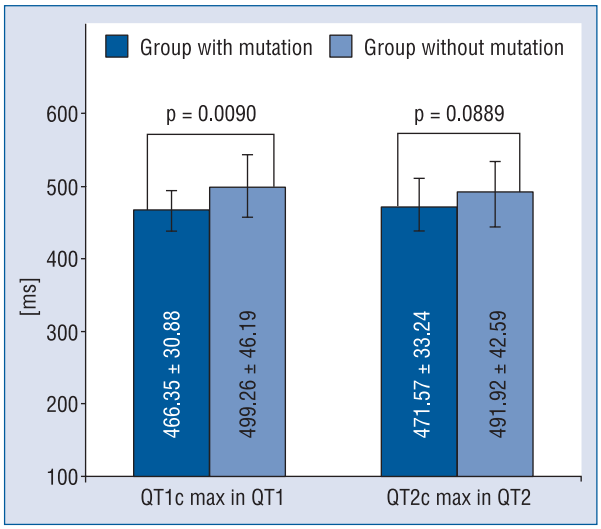

Figure 2. Average values of QTc max in QT1 and QT2 before a single ventricular ectopic heartbeat at daytime.

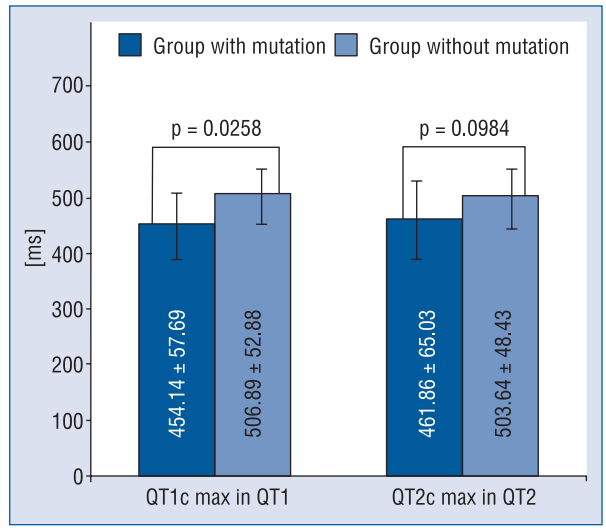

Figure 3. Average values of QTc max in QT1 and QT2 before a couplet of ventricular ectopic heartbeats at daytime.

Table 4. Holter ECG data in groups with and without the T2755875G mutation.

	Variable		Group with mutation	Group without mutation
HRmin [min]	Baseline examination		46 ± 8	47 ± 8
	Follow-up examination		47 ± 8	47 ± 8
HRmax [min]	Baseline examination		107 ± 15	112 ± 24
	Follow-up examination		104 ± 17	114 ± 19
HRmean [min]	Baseline examination		67 ± 11	69 ± 11
	Follow-up examination		66 ± 9	68 ± 10
ST level (%)	Baseline examination		11	11
	Follow-up examination		13	16
Ventricular arrhythmia (Lown class) (%)	Baseline examination	0	10.5	6.3
		I	26.3	20
		II	10.5	13.8
		IVa	26.3	26.3
		IVb	26.3	33.8
	Follow-up examination	0	6.3	4.3
		I	37.5	20.3
		II	25	10.1
		III	6.3	1.4
		IVa	12.5	34.8
		IVb	12.5	29

HRmin — minimum heart rate, HRmax — maximum heart rate, HRmean — mean heart rate, ST level — myocardial ischemia

attention to acquired LQTS [10, 11]. There are only limited data on the role of genetic abnormalities in the pathophysiology of the acquired LQTS. Kubota et al. [12] discovered a missense mutation in the KCNQ1 gene with hypokalemia linked long QT and ventricular tachycardia. Napolitano et al. [13] documented a heterozygotic mutation in the KCNQ1 gene with SCA after cisapride ingestion.

In patients after MI the prolonged QTc interval greater than 440 ms worsens the prognosis of the disease [14]. It is necessary to find the clinical factors which will be able to define the subgroup of high risk patients, who may benefit from arrhythmia preventive measures such as implantable cardioverter defibrillators. The QT measurement on ECG is not always a reliable surrogate marker for prolonged

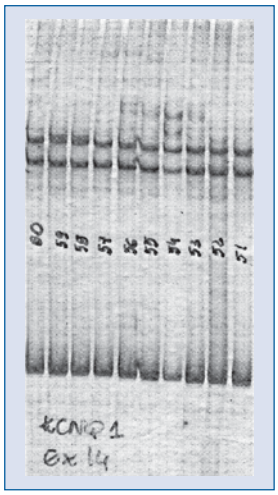

Figure 4. An example of multitemperature single strand information polymorphism results.

repolarisation. It is not an ideal metric for accurate indication of clinical outcome as not all patients who present the arrhythmia phenotype exhibit prolonged QT interval and some unaffected individuals have prolonged QTc value. Moreover, QT interval varies with gender, age, concurrent drug administration, electrolyte abnormalities and other diseases [15–17]. Molecular genetics thus may play a complementary role in defining arrhythmic risk in this subgroup of patients.

In this study we report novel mutations in the KCNQ1 potassium channel gene. It is the first report attempting to assess the genotype changes in patients after prior myocardial infarction. We have identified DNA mutations associated with post infarction ventricular arrhythmia by testing the hypothesis that variants in the congenital LQTS disease genes might contribute to risk in post MI patients. Patients with the target phenotype were screened for variants in this gene and the frequency of these variants was then determined and correlated with clinical data.

In our study, in order to obtain as reliable results as possible, we used the DNA Pointer System which enabled us to carry out the MSSCP analysis. As a matter of fact, this is a genotyping method characterized by both a significantly higher sensitivity in detecting point mutations in comparison to the commonly used SSCP and high reproducibility of test results [9, 18–20].

In this study the new mutations were found both in exons and introns. Most of the available data concern exonic mutations [5]. However there are more and more data on intronic mutations and their role in phenotypic variants [21, 22].

The intronic mutation T2755875G found in 19 out of 100 patients after MI was associated with SCA and sustained ventricular tachycardia. This is the first study that describes this intronic mutation. It seems that this novel mutation may promote adverse ventricular arrhythmic events in patients after myocardial infarction. Moreover in patients with T2755875G, QTmax and QTd at peak heart rate are significantly longer compared to controls.

The explanation of the observed correlation between mutations in KCNQ1 introns and the clinical data appears to be very interesting. It is a commonly accepted fact that the effects of mutations depend on their location in the gene. However, all too often, it is taken for granted that only genetic alterations in the coding sequences, i.e. in exons, have an impact on the clinical course of the disease. Indeed, almost up to the present day, any alterations in the non-coding sequences, i.e. in introns, have been discarded beforehand as irrelevant because of the assumption that if intronic they do not take part in the protein biosynthesis process. On the other hand, recent studies and findings have shown that intronic mutations may play a major role in the splicing process, alter its course, lead to coding sequence abnormalities and consequently influence the structure and function of the encoded proteins. Numerous data reported in scientific papers show that both intronic and exonic alterations may result in an aberrant splicing process, leading to the formation of abnormal proteins, what in turn affects the severity of the disease symptoms. These data concern not only conventional splice sites and regulatory splicing elements such as exonic splicing enhancers (ESE), exonic splicing silencers (ESS), intronic splicing enhancers (ISE), intronic splicing silencers (ISE), but even deep intronic mutations [23–26]. Consequently, genetic alterations occurring in introns, in particular those related to clinical symptoms, as in the case of post-MI patients having the T2755875G intronic mutation, can no longer be ignored. The role of this mutation in the clinical course of the disease is corroborated by the values of statistical p-levels obtained while comparing this group of subjects to that without any such genetic alterations in the KCNQ1 gene.

It is also worthwhile to turn our attention to the influence of additional factors (such as drugs, ischemia, electrolyte imbalance, increased adrenergic activity) on the risk of life-threatening ventricular arrhythmia in subjects with mutations. This issue has been largely discussed in scientific papers [12, 13, 27–29]. It is not known, in fact, whether the carriers of detected genetic alterations in the group

of patients after MI incur a greater risk of malignant ventricular arrhythmia on exposure to additional environmental factors propitious to electrical instability of the myocardium, in comparison with healthy subjects. And although the coronary artery disease, including the MI, is partly genetically determined (influence of several genes), the impact of a single mutation may prove to be insufficient to engender a life-threatening ventricular arrhythmia. On the other hand, if the said mutation is concomitant with additional environmental factors, there is an increase of the risk of life-threatening ventricular arrhythmia and consequently of sudden cardiac death. It is therefore important to assess not only traditional but also genetic SCD risk factors in patients after myocardial infarction.

Genetic tests performed to this end might reveal the presence of genetic alterations in these patients and allow the undertaking of preventive steps such as: lifestyle modification, early implementation of adequate therapeutic measures in the treatment of life-threatening ventricular arrhythmia (cardioverter-defibrillator implantation), or creation of new drugs improving the ion channel function. Gene therapy could be the ultimate method of treatment. It is worth extending genetic testing to the relatives of probands and, in case they should have the same genetic alterations, implementing primary prevention measures that they might avoid life-threatening arrhythmia.

Conclusions

Summing up we discovered six new mutations in the cardiac potassium gene KCNQ1: C2505734T in exon 1, A2753831C in exon 12, C2505846A in intron 1, G2753881A in intron 12, T2755854C and T2755875G in intron 14. Detected intronic alterations in patients after myocardial infarction were related to a worse clinical course of the disease and frequent occurrence of sudden cardiac arrests. The novel intronic mutations in KCNQ1 found in our study may have a significant influence on the clinical course of the disease.

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