HYPOTHESIS

TP53 rs1042522 and rs8064946 variants in myocardial infarction

Hakobjanyan A[1,2,3], Stahelova A[4], Mrazek F[4], Petrkova J[3,5], Navratilova Z[3], Petrek M[3,6]

NAS RA, Institute of Molecular Biology, Yerevan, Armenia. **martin.petrek@fnol.cz**

ABSTRACT

OBJECTIVE: This study investigated the hypothesis that the single nucleotide polymorphisms (SNPs) of *TP53* gene are related to a risk of myocardial infarction.

METHODS: The coding SNP at codon 72 (rs1042522) and non-coding rs8064946 SNP were genotyped by polymerase chain reaction with sequence specific primers in 205 Czech patients with myocardial infarction and 148 Czech control subjects.

RESULTS: The distribution of both SNPs was in agreement with the Hardy–Weinberg equilibrium and was similar to other European populations. Our power analysis showed 96 % of probability to detect an odd ratio equal to 2. Neither rs1042522 nor rs8064946 were associated with the risk of myocardial infarction. The haplotypes combined of rs1042522 and rs8064946 were not associated with myocardial infarction in the present study.

CONCLUSION: The *TP53* SNPs are not strongly associated with genetic predisposition to myocardial infarction *(Tab. 3, Fig. 3, Ref. 23).* Text in PDF *www.elis.sk.*

KEY WORDS: myocardial infarction, tumor suppressor protein p53, genes, polymorphism, single nucleotide, polymerase chain reaction.

Introduction

Myocardial infarction is a coronary artery disease (CAD) that is characterized by an irreversible necrosis of the cardiomyocytes suffering from prolonged hypoxia (1). The less hypoxic cardiomyocytes can undergo an apoptotic process during myocardial infarction. Experimental simulation of myocardial ischemia showed that the apoptotic process is mediated by a well-known protein p53 (2). An involvement of the protein p53 in CAD is also supported by human observation showing the elevated cell-free protein p53 in patients after myocardial infarction (3).

The protein p53 is encoded by a *TP53* gene that has a polymorphic character, more than 32 single nucleotide polymorphisms (SNPs) are known up to now (4). Among the common SNPs, one polymorphism with rs number 1042522 occurs at codon 72 of exon 4, with two alleles encoding either arginine (CGC) or proline (CCC). The non-mutated allele (Arg72) has been reported to induce apoptosis more effectively in comparison to the Pro72 allele (5). Another *TP53* SNP (rs8064946) is located in the strong linkage disequilibrium block within the intron 1 of the *TP53* gene (4). All the non-coding SNPs may theoretically affect an alternative splicing of RNA. However, biological function of the particular isoform corresponding to rs8064946 SNP has not been investigated so far.

The investigation of non-coding *TP53* SNPs is also omitted in clinical case-control studies on CAD. On the other hand, several studies have indicated that the coding SNP at codon 72 can play an important role in CAD (6-8). In the context of myocardial infarction, Al-Tu'ma et al observed that the Pro72Arg polymorphism was associated with the risk of myocardial infarction in the Iraqi patients (9). In particular, the carriers of the non-mutated Arg72 allele were hypothesised to be more susceptible to apoptosis that consequently promote the development of myocardial infarction. On the other hand, other studies observed neither CAD nor myocardial infarction to be associated with the coding SNP at codon 72 (10). We therefore investigated the hypothesis that the coding SNP at codon 72 or the non-coding rs8064946 SNP affect the risk of myocardial infarction in our Czech population

Materials and methods

Study population

353 Czech Caucasians in total were enrolled into the study. 148 of them were healthy controls (mean age ± SD: 29.8 ± 6.5

[1]NAS RA, Institute of Molecular Biology, Yerevan, Armenia, [2]NAS RA, G.S. Davtyan Institute of Hydroponics Problems, Yerevan, Armenia, [3]Department of Pathological Physiology, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic, [4]Department of Immunology, Faculty of Medicine and Dentistry, Palacky University, Olomouc and University Hospital Olomouc, Czech Republic, [5]Department of Internal Medicine I – Cardiology, Faculty of Medicine and Dentistry, Palacký University and University Hospital Olomouc, Olomouc, Czech Republic and [6]Institute of Molecular and Translational Medicine, Palacky University, Olomouc, Czech Republic

Address for correspondence: M. Petrek, Department of Pathological Physiology, Faculty of Medicine and Dentistry, Palacky University Olomouc, Hnevotinska 3, CZ-751 50 Olomouc, Czech Republic.
Phone: +420.58.8442285, Fax: +420.58.5415116

Tab. 1. Demographic characteristics of study groups.

		Controls	Patients		
			younger	older	whole
Age	Mean age±SD*	29.8±6.5	48.3±5.3	61.3±6	53.7±8.5
	Min/max	18/56	25/54	55/79	25/79
rs1042522	Number of subjects	148	121	82	205
	Mean age±SD*	29.8±6.5	48.3±5.3	61.4±6	53.6±8.5
	Gender (male/female)	86/62	106/16	68/15	174/31
rs8064946	Number of subjects	146	120	84	204
	Mean age±SD*	29.9±6.4	48.4±5.3	61.3±6	53.7±8.5
	Gender (male/female)	85/61	105/15	69/15	174/30

*SD – standard deviation

Tab. 2. Genotype, allele and phenotype (proportion of carriers) frequencies of the coding *TP53* SNP (single nucleotide polymorphism) at codon 72 (rs1042522) in our controls and patients with myocardial infarction.

(n)	Genotype frequency			Allele frequency		Phenotype frequency	
	CC%(*)	CG%(*)	GG%(*)	C%(*)	G%(*)	C%(*)	G%(*)
Controls (148)	9(14)	35(51)	56(83)	13(76)	87(219)	44(65)	91(134)
Myocardial infarction (205)	8(16)	4 (85)	51(104)	12(47)	88(361)	49(101)	92(189)

p > 0.05 for all comparisons, * absolute numbers

year) and 205 were patients with myocardial infarction (mean age ± SD: 53.7 ± 8.5). The patients were subdivided into the younger (121 patients, age allocation 25–54) and older (84 patients, age allocation 55–79) subgroups (Tab. 1). 42 % of control group were females, while younger and older patients' subgroups consisted predominantly of males (88 % and 82 % respectively) (Tab. 2).

Myocardial infarction was diagnosed by the criteria of international consensus (1). The study, realised within an institutional project (IGA PU LF), was approved by local research ethics committee. All participants provided written informed consent for use of samples in this study and anonymous publication of the summary data.

Method

Genomic DNA was extracted from the blood samples of all study participants using the standard salting-out procedure (11). The genotyping was carried out by SSP PCR (polymerase chain reaction with sequence-specific primers). The method was adopted from a "Phototyping" methodology (12). The primer sequences for rs1042522 were: allele C, reverse 5'-CCA GAG GCT GCT CCC CC, allele G, reverse 5'-CCA GAG GCT GCT CCC CG and forward constant 5'-TGC AAG TCA CAG ACT TGG CT. The primer sequences for rs8064946 (in intron part) were: allele G, forward 5'-GTA AGT ACG GCA CAA AGT GG, allele C, forward 5'-GTA AGT ACG GCA CAA AGT GC and reverse constant 5'-CTG AAC GTC GTG AAG CGG A. All primers for PCR SSP were designed using the genomic sequences available in the GenBank (http://www.ncbi.nlm.nih.gov, GenBank ID: 7157). The PCR SSP results were revealed by gel electrophoresis with ethidium bromide staining.

Statistical analysis

The distribution of genotypes was tested for conformity with Hardy-Weinberg equilibrium using the Chi-square test. Allelic and genotype frequencies were compared between the groups by
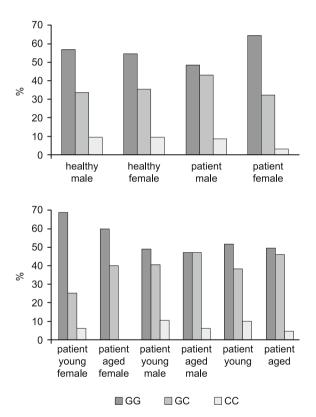

Fig. 1. Distribution (percentages) of the genotypes of the coding *TP53* SNP (single nucleotide polymorphism) at codon 72 (rs1042522) in the subgroups based on gender and age. a) gender subgroups of controls and patients; healthy male vs. healthy female; healthy male vs. patient male; healthy female vs. patient female and patient male vs. patient female: p > 0.05; b) younger and older subgroups of patients based on gender as well: patient young vs. patient aged; patient young female vs. patient young male and patient young male vs. patient aged male: p > 0.05). p value was not calculated for patient young female vs. patient aged female and patient aged female vs. patient aged male, because CC genotype was not identified in aged female patients.

Tab. 3. Genotype and allele frequencies of the non-coding *TP53* SNP (single nucleotide polymorphism, rs8064946) in our controls and patients with myocardial infarction.

(n)	Genotype frequency			Allele frequency	
	GG% (*)	GC%(*)	CC%(*)	G%(*)	C%(*)
Controls (146)	76(111)	22(32)	2(3)	87(254)	13(38)
Myocardial infarction (204)	78(159)	21(43)	1(2)	89(361)	11(47)

p > 0.05 for all comparisons, * absolute numbers

Pearson's Chi square test or Fisher's exact test (in case of low size of compared subgroups). p < 0.05 was considered statistically significant.

Results

In order to reveal plausible association between two selected SNPs of the *TP53* gene (rs1042522 and rs8064946) and myocardial infarction, 205 patients with myocardial infarction and 148 ethnically matched (Czech) control subjects were investigated. The rate of overall genotyping failure was acceptable, below 1 %, specifically 0.8 % (3/352). Accordingly, the samples with amplification failure were excluded from the analyses.

TP53 rs1042522 in the Czech population and its association with myocardial infarction

The distribution of rs1042522 genotypes was in agreement with the Hardy–Weinberg (HW)-equilibrium. The genotype, allele, and phenotype frequencies of rs1042522 did not differ between our patients with myocardial infarction and controls (p > 0.05). The rs1042522 SNP was not associated with myocardial infarction in our sub-analysis that was limited for Czech men. Further, it was not associated with myocardial infarction in Czech women. The distribution of the rs1042522 SNP did not differ between aged and young patients (Fig. 1).

TP53 rs8064946 in Czech population and its association with myocardial infarction.

Among 146 controls and 204 patients, each genotype and allele had very similar frequency in controls and patients, and, therefore, no significant difference between the groups was observed (p > 0.05) (Tab. 3, Fig. 2). It also applied to our sub-analysis based on the gender and age. The distribution of TP53 rs8064946 genotypes was in agreement with Hardy-Weinberg equilibrium (p > 0.05).

Frequency of combined TP53 rs1042522 and rs8064946 genotypes in healthy Czech population and patients with myocardial infarction

In female subgroup the GG/GC, GC/CC and CC/CC (rs1042522/rs8064946) combined genotypes were not range and GG/CC combination is specific for healthy females. GG/GG (rs1042522/rs8064946) combination was not detected in aged female patients. In the male subgroup GG/CC and CC/CC (rs1042522/rs8064946) combinations were specific for healthy males and GC/CC combination was found only in aged male patients (Fig. 3). Accordingly, females have less variation of combinations (they have 6 haplotypes) than males. Importantly, no
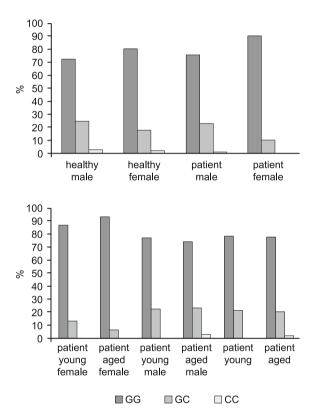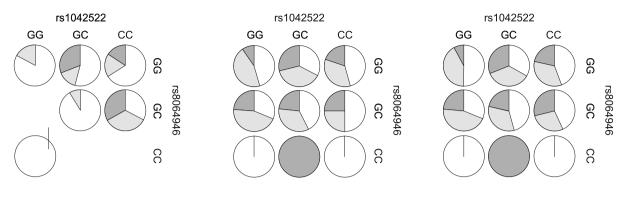

Fig. 2. Distribution (percentages) of the genotypes of the non-coding *TP53* SNP (single nucleotide polymorphism, rs8064946) in the subgroups based on gender and age. a) gender subgroups of controls and patients; healthy male vs. healthy female and healthy male vs. patient male: p > 0.05). P value was not calculated for healthy female:patient female and patient male:patient female, because there is no CC genotype in patient female subgroup. b) younger and older subgroups of patients based on gender as well: p value was not calculated because there CC genotype was identified only in aged male patients.

significant differences in the frequencies of combined genotypes of two investigated polymorphisms between the controls and patients were observed (Fig. 3) (p > 0.05).

Discussion

The present study showed that two *TP53* SNPs are not strongly associated with myocardial infarction in the Czech population. Our data on distribution of rs1042522 and rs8064946 polymorphisms (SNPs) among the healthy Czech controls confirm the earlier observations reported in Czech (13), German (14), Polish (15) and Irish (4) populations. To our best knowledge, this is the

Fig. 3. Genotype combinations of rs1042522 and rs8064946: a) female subgroup; b) male subgroup; c) whole studied group (control+patient):

first European case-control study on rs8064946 SNP in myocardial infarction.

Despite the experimental indications on the protein p53 being a risk factor for coronary artery disease (CAD) (2, 3) we observed neither rs1042522 nor rs8064946 to be associated with myocardial infarction in our Czech population. Our data are in line with the earlier study that genotyped the coding rs1042522 SNP in 1174 Swedish patients with myocardial infarction (10). However, the large Swedish study did not investigate another TP53 SNP, the non-coding rs8064946 SNP. We therefore extended the list of the investigated TP53 SNPs and calculated the combined haplotypes of rs1042522 and rs8064946. No association was observed between the haplotypes and myocardial infarction in the present study.

On the other hand, we cannot exclude the possibility that a weak genetic association exists and an insufficient power did not permit its detection in the present study. In the context of effect size, our power analysis showed 96 % of probability to detect an odds ratio (OR) equal to 2. However, our great probability would be decreased to 61 % if the OR would be only 1.5. Generally, a small effect size is likely, as other genetic studies on myocardial infarction have indicated the effect per one risk allele to be as small as OR = 1.3 (16). Various risk alleles with the small effect sizes are assumed to cooperate together and may modulate genetic predisposition to myocardial infarction according to a current hypothesis. In the context of apoptosis, the risk of myocardial infarction has already been reported to be associated with several alleles of the genes that stand up or down of the *TP53* gene (e.g. OLR1 gene, Fas) (17, 18). The association of the *TP53* SNPs with myocardial infarction may be therefore limited on the particular condition(s) that other genetic variants are present as well.

Regarding methodological approach, the STREGA recommendations including control on our genotyping failure and HW-equilibrium were considered in the study(19). Whereas genetic factors have been rather a subject of clinical investigation now, several non-genetic characteristics (e.g. gender and age) are well known to affect the risk of myocardial infarction (20, 21). To in-

vestigate the possibility that the association of two *TP53* SNPs with myocardial infarction is dependent on one gender, we performed two independent sub-analyses limited to either men or women. We observed neither men nor women to have the association between the *TP53* SNPs and the risk of myocardial infarction.

Further, age, as another risk factor, should be taken into consideration in myocardial infarction. It is a main limitation of the present study that our healthy controls were younger than the patients with myocardial infarction. One may therefore speculate that our healthy controls with their average age about 30 years can develop myocardial infarction in higher age. On the other hand, prevalence of myocardial infarction is lower than 1 % in European population of age group 55–64 years (22). Taken into consideration the total number of our controls, only 1–2 young controls may be speculated to develop myocardial infarction later. It is not likely that the low number of misfit individuals could have a great effect biasing our observation.

The risk of myocardial infarction was investigated in the present genetic study. On the other hand, we did not investigate the genetic predisposition to the outcome of myocardial infarction. The large Swedish study by Karin Leander et al. investigated the patients who survived at least 28 days from the day of diagnosis (10). More current study reported genetic predisposition to severe myocardial infarction with OR of mortality more than 3 (23).The matter of future study is the investigation whether *TP53* SNPs may substantially influence apoptotic activities after myocardial infarction episode and subsequent time of survival.

In summary, the *TP53* rs8064946 and rs1042522 SNPs have no strong association with myocardial infarction in the present study. Large studies with sufficient statistical power are desired to investigate the possible small effect of the *TP53* SNPs on genetic predisposition to the development of myocardial infarction. The *TP53* SNPs should also be a subject of other investigation to clarify whether they are able to affect the outcome of myocardial infarction such as the time of survival after myocardial infarction episode.

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