

Sirtuin gene polymorphisms are associated with chronic obstructive pulmonary disease in patients in Muğla province



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Abstract

Introduction: Chronic obstructive pulmonary disease (COPD) is an irreversible progressive chronic inflammatory disease that causes shortness of breath in consequence of a decrease in pulmonary functions. The pulmonary inflammatory pathogenesis is multifactorial. We have too little up-to-date information about the relation between COPD and genetics. In our study, the relation with the *SIRT1* gene's mononucleotide polymorphisms (SNP) rs7895833, rs7069102 and rs2273773 was analyzed through various laboratory data.

Material and methods: One hundred COPD patients from the archive records of the Chest Diseases Department of Muğla Sitki Kocman University Medical Faculty were included in the study. A control group was constituted from 100 healthy individuals who live in the same geographical region. The *SIRT1* genotypes for these patients were determined using polymerase chain reaction (PCR) and confronting two-pair primers (CTPP) methods. The *SIRT1* gene polymorphisms rs7895833, rs7069102 and rs2273773 were analyzed. GG, AG, AA genotypes and G and A alleles of rs7895833, TT, TC, CC genotypes and T and C alleles of rs2273773, and CC, CG, GG genotypes and C and G alleles of rs7069102 were examined. The data in both groups were compared.

Conclusions: A significant difference between GG, AG and AA genotypes of rs7895833 was found. Especially, the AG genotype was observed more in the group with COPD, with a significant difference. A significant difference between TT, TC and CC genotypes of rs2273773 was found. There was a significant difference between two groups with regards to C and G alleles of rs7069102. A significant difference was not found between the groups with regards to G and A alleles of rs7895833. A difference was not found for both groups between T and C alleles of rs2273773. It shows that these polymorphisms of the *SIRT1* gene may be associated with COPD.

Key words: COPD, *SIRT1* gene.

Streszczenie

Wstęp: Przewlekła obturacyjna choroba płuc (POChP) jest nieodwracalną postępującą przewlekłą chorobą zapalną. Powoduje duszność wynikającą ze zmniejszonej czynności płuc. Patogeneza płucnej reakcji zapalnej jest wieloczynnikowa. Nie ma pełnych informacji na temat genetycznych uwarunkowań POChP. W opisywanym badaniu związek pomiędzy jednonukleotydowymi polimorfizmami genu *SIRT1*: rs7895833, rs7069102 oraz rs2273773, został zbadany poprzez analizę różnych danych laboratoryjnych.

Materiał i metody: Do badania włączono dane 100 pacjentów z POChP z archiwum Oddziału Chorób Klatki Piersiowej Wydziału Medycyny Uniwersytetu Muğla Sitki Kocman. Grupę kontrolną stanowiło 100 zdrowych osób mieszkających w tym samym regionie geograficznym. Genotypy *SIRT1* pacjentów ustalono przy użyciu reakcji łańcuchowej polimerazy (PCR) i (CTPP) PCR i CTPP. Analizowano polimorfizmy rs7895833, rs7069102 oraz rs2273773 genu *SIRT1*. Zbadano genotypy GG, AG i AA oraz allele G i A wariantu rs7895833, genotypy TT, TC i CC oraz allele T i C wariantu rs2273773, jak również genotypy CC, CG i GG oraz allele CG wariantu rs7069102. Dane z obu grup zostały porównane.

Wnioski: Znaleziono istotną różnicę pomiędzy genotypami GG, AG i AA wariantu rs7895833. W szczególności genotyp AG obserwowany był zdecydowanie częściej w grupie z POChP. Wykazano istotną różnicę między genotypami TT, TC i CC wariantu rs2273773. Pomiędzy grupami wystąpiła również istotna różnica w odniesieniu do alleli C i G wariantu rs7069102. Nie wykazano istotnej różnicy pomiędzy grupami w odniesieniu do alleli G i A wariantu rs7895833; grupy nie różniły się też względem alleli T i C wariantu rs2273773. Przedstawione wyniki wskazują na to, że opisywane polimorfizmy genu *SIRT1* mogą być związane z POChP.

Słowa kluczowe: POChP, gen *SIRT1*.

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Introduction

Chronic obstructive pulmonary disease (COPD) is an irreversible progressive chronic inflammatory disease that causes shortness of breath in consequence of a decrease in pulmonary functions. Chronic obstructive pulmonary disease is the fourth most frequent cause of death in the world and it is still a serious global public health concern. While the COPD prevalence is about 10% in Turkey, its prevalence in the EU countries is 3.1%. The prevalence in the developing countries is higher than in the developed countries because of bad living conditions and excessive smoking. In the end, chronic airflow obstruction and other physiological abnormalities incident to COPD occur. The pulmonary inflammatory pathogenesis is multifactorial. Chronic obstructive pulmonary disease progresses slowly. Long-term exposure to the affecting factors causes this disease and therefore this illness is observed in elderly patients. The A1-antitrypsin (a1-AT) gene, some oxidants, chemical compounds, cigarette smoke, toxic gases, free radicals and environmental factors play a role in COPD development. Cigarette smoking is the most important among these risk factors for COPD. However, we have too little up-to-date information about the relation between COPD and genetics [1-5].

Sirtuin 1 (SIRT1) is a protein/histone deacetylase dependent NAD. It plays a crucial role in various human diseases such as cardiovascular diseases, cancer, inflammation, aging, neurodegenerative disease, obesity and type 2 diabetes. Seven isoforms of the *SIRT1* gene (SIRT1-SIRT7) have been determined in mammals. Expression of the *SIRT1* gene is regulated by transcription factors such as CREB, FOXO3, HIC1, NF-KB, p53, PARP-2 and PPAR. SIRT1 deacetylates FOXO3, which has a regulatory role in the pulmonary inflammatory response. The *SIRT1* gene, which has a role in the nucleus and cytoplasm, encodes deacetylase enzyme, and this enzyme is important for energy metabolism, fatty tissue and bone tissue of mammals [6, 7]. Some recent research shows that SIRT1 transcription affects gluconeogenesis, insulin secretion, cellular life, prevention of gene damage, response to DNA damage, stress management, aging and metabolism [7, 8].

The aim of this study is to analyze the relation between rs7895833, rs7069102 and rs2273773, mononucleotide polymorphisms of the *SIRT1* gene, between 100 COPD patients and a control group consisting of 100 healthy persons, by using various laboratory data and polymerase chain reaction (PCR) with the confronting 2-pair primers (CTPP) assay method.

Material and methods

Participants

The protocol was approved by the regional ethical committee and made according to the principles of the Helsinki Declaration. One hundred COPD patients, who were diagnosed through GOLD guideline standards, were analyzed. The onset of illness, the character, place, ache time

and history of illness, the accompanying symptoms and the medication reports were examined in the analysis. One hundred COPD patients from the archive records of the Chest Diseases Department of Muğla Sitki Kocman University Medical Faculty, who did not have any other chronic disease, were included in the study. A control group was formed from 100 healthy individuals living in the same geographical region.

Genotyping

After receiving written informed consent, 20 cc of blood was taken from both groups. Peripheral blood leucocytes were separated by using the DNA Isolation Kit (Thermo K0772) according to the instructions of Genomic DNA producer and it was kept at -20°C . SIRT1 genotypes were determined using PCR and CTPP methods [9] (Table I). Genotyping was implemented for the rs7895833 polymorphism of the *SIRT1* gene: 320, 241 bp for AA genotype; 320, 241, 136 bp for AG genotype; and 320, 136 bp for GG genotype. For the rs7069102 polymorphism of the *SIRT1* gene, genotyping was performed as follows: 391, 277 bp for CC genotype; 391, 277, 167 bp for CG genotype; and 391, 167 bp for GG genotype. Also, for the rs2273773 polymorphism of the *SIRT1* gene, genotyping was performed as follows: 314, 228 bp for CC genotype; 314, 228, 135 bp for CT genotype; and 314, 135 bp for TT genotype (Figs. 1A-C).

Statistical analysis

The program Statistical Package for the Social Sciences (SPSS) 21 was used in the analysis of the data. For the analysis of the conformity of quantitative data to the normal distribution, the Shapiro-Wilk test was used. In the comparison of 2 independent groups, the independent-samples *t*-test was used. In the comparison of categorical data, Pearson's χ^2 (exact) test was used. The quantitative values are shown in the tables as mean \pm SD. (standard deviation) values. The categorical values are shown as *n* (number) and percentage (%). The data were analyzed at the 95% confidence level, and the *p* value was accepted as significant if less than 0.05.

Analysis results

All patients were taken from Muğla Sitki Kocman University Medical Faculty, Department of Chest, Turkey. Among 100 patients with COPD, 60 were men and 40 were women. The average age was 65.92. In the control group 55 were men and 45 were women; the average age was 49.54. While there was no significant difference between groups with regards to gender, a significant difference was found with regard to age. As the study carried out was a genotype study, the significant difference in age was not considered remarkable. The age and gender distribution of the persons included in the study and their *p* values are given in Table II. The *SIRT1* gene genotypes and allele distributions of the patients with COPD and the control group in the Muğla region are presented in Tables III and IV. The distribution of

Tab. I. Primers and annealing temperature used for rs7895833, rs7069102, and rs2273773 polymorphisms of SIRT1 gene

Gene	Polymorphism	Primers sequence 5'-3'	Ann. temp. (°C)	Fragment size (bp)
<i>SIRT1</i>	rs7895833	CCCAGGGTTCAACAAATCTATGTTG	64	AA; 320, 241 bp
		GGTGGTAAAGGCCTACAGGAAA		
		GCTTCTAATCTCCATTACGTTGAC		
		CCTCCCAGTCAACGACTTTATC		
	rs7069102	GTAGCAGGAACACTACAGGCCTG	64	CC; 391, 277 bp
		GAGAAGAAAGAAAGGCATAATCTCTGC		
		CTATCTGCAGAAATAATGGCTTTTCTC		
		GATCGAGACCATCCTGGCTAAG		
	rs2273773	GTGTGTCGCATCCATCTAGATAC	63	CC; 314, 228 bp
		CTCTCTGCACAAATTCATAGCCT		
		GTAGTTTTCTTCTTATCTGACAG		
		CTGAAGTTTACTAACCATGCACTG		

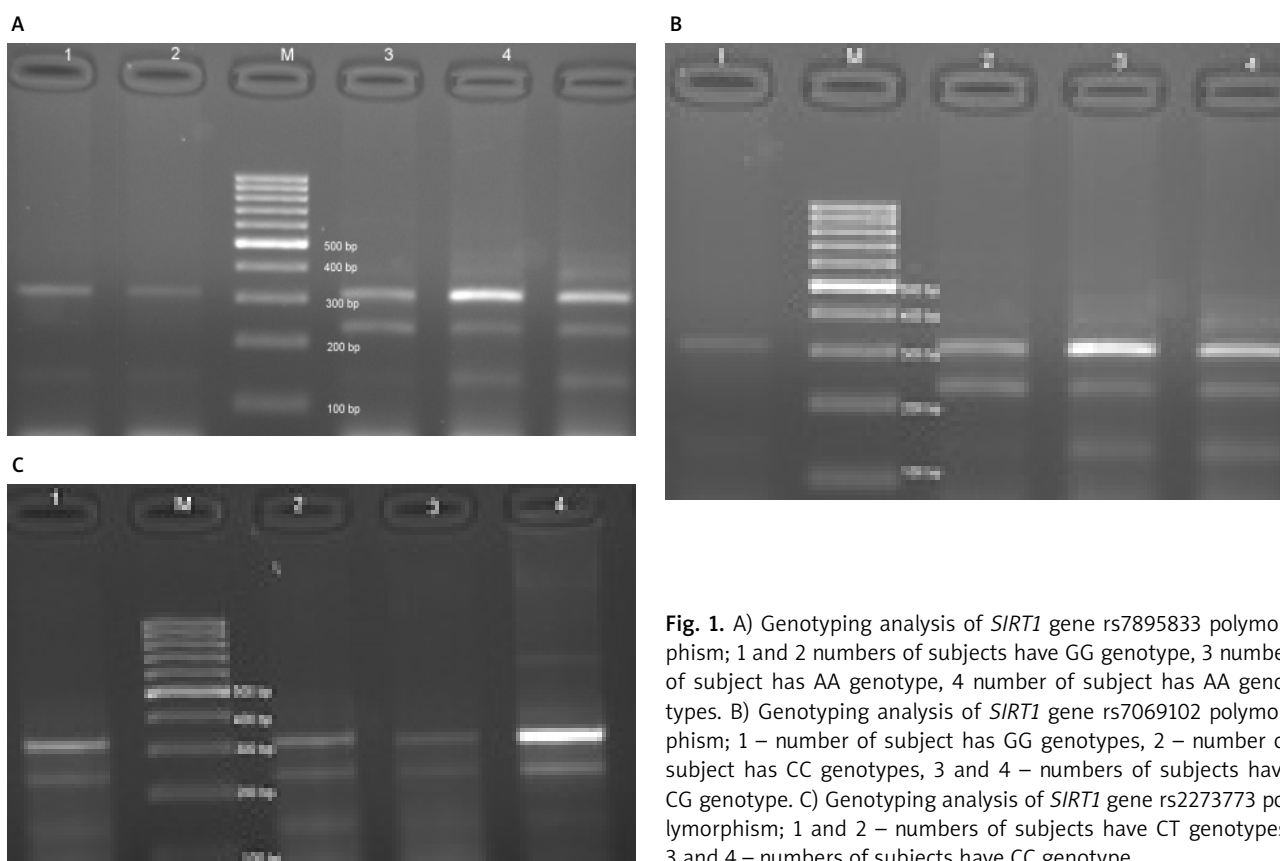


Fig. 1. A) Genotyping analysis of *SIRT1* gene rs7895833 polymorphism; 1 and 2 numbers of subjects have GG genotype, 3 number of subject has AA genotype, 4 number of subject has AA genotypes. B) Genotyping analysis of *SIRT1* gene rs7069102 polymorphism; 1 – number of subject has GG genotypes, 2 – number of subject has CC genotypes, 3 and 4 – numbers of subjects have CG genotype. C) Genotyping analysis of *SIRT1* gene rs2273773 polymorphism; 1 and 2 – numbers of subjects have CT genotypes, 3 and 4 – numbers of subjects have CC genotype

Tab. II. Demographic characteristics of patients and controls

Factors	COPD (n = 100)	Control (n = 100)	p value
Age (mean ± SD)	65.92 ± 8.76	49.54 ± 10.80	< 0.001
Sex (male/female)	60/40	55/45	0.567

Independent t test – Pearson χ^2 (exact), SD – standard deviation

the *SIRT1* gene for the persons in the COPD and control groups was in Hardy-Weinberg equilibrium.

While GG, AG and AA genotype frequencies of rs7895833 were 20%, 60% and 17% respectively for the group with COPD, these were 36%, 47% and 17% for the control group ($p = 0.033$). While the frequencies of G and A alleles of the group with COPD were 51.5% and 48.5%, these were 59.5%

Tab. III. Distribution of genotypes of patients and controls

Gene name	SNP	Genotypes	Control, n (%)	COPD, n (%)	p value
<i>SIRT1</i>	rs7895833	GG	36 (36%)	20 (20%)	0.033
		AG	47 (47%)	63 (63%)	
		AA	17 (17%)	17 (17%)	
	rs7069102	CC	43 (43%)	41 (41%)	0.981
		CG	41 (41%)	43 (43%)	
		GG	16 (16%)	16 (16%)	
	rs2273773	TT	33 (33%)	25 (25%)	0.01
		TC	50 (50%)	39 (39%)	
		CC	17 (17%)	36 (36%)	

*Pearson χ^2 (exact)**Tab. IV.** Allele frequencies for COPD patients and control subjects

SNP	Allele	Control, n (%)	Patient, n (%)	p value
rs7895833	G	119 (59.5%)	103 (51.5%)	0.131
	A	81 (40.5%)	97 (48.5%)	
rs7069102	C	127 (63.5%)	125 (62.5%)	0.918
	G	73 (36.5%)	75 (37.5%)	
rs2273773	T	116 (58%)	89 (44.5%)	0.009
	C	84 (42%)	111 (55.5%)	

* Pearson χ^2 (exact)

and 40.5% for the control group ($p = 0.131$). While CC, CG and GG genotype frequencies of rs7069102 were 41%, 43% and 16% respectively for the group with COPD, these were 43%, 41% and 16% for the control group ($p = 0.981$). While the frequencies of C and G alleles of the group with COPD were 62.5% and 37.5%, these were 63.5% and 36.5% for the control group ($p = 0.918$). While TT, TC and CC genotype frequencies of rs2273773 were 25%, 39% and 36% respectively for the group with COPD, these were 33%, 50% and 17% for the control group ($p = 0.981$). While the frequencies of T and C alleles of the group with COPD were 44.5% and 55.5%, these were 58% and 42% for the control group ($p = 0.009$) (Tables III and IV).

A significant difference was found between GG, AG and AA genotypes of rs7895833. Especially the AG genotype was found in the group with COPD with a significant difference. While there was no change in the percentages of AA genotype in the same group, the analysis showed that the percentages were significantly lower in the GG genotype. A significant difference was found between TT, TC and CC genotypes of rs2273773. As the TC genotype's rate of incidence was decreasing, it was determined that the rate of incidence for the CC gene increased more than twice. A significant difference was not found in both groups for CC, CG and GG genotypes of rs7069102. It shows that these polymorphisms of the *SIRT1* gene could

be associated with the COPD tendency of the population from Muğla.

There was a significant difference between the two groups with regards to C and G alleles of rs7069102. While the percentage of the T allele, which was dominant in the control group, was decreasing in the group with COPD, the C allele, which was seen less often in the control group, was determined as more dominant in the group with COPD. While no difference was found in both groups with regards to G and A alleles of rs7895833, a high rate of G allele was determined in both groups. A difference was not found in both groups with regards to T and C alleles of rs2273773. A dominant rate of C allele was also found in this group.

Discussion

Chronic obstructive pulmonary disease is one of the important public health concerns. The COPD pathophysiology is associated with an inflammatory cell profile that contains macrophages, neutrophils and T lymphocytes. The early diagnosis of COPD is important, because it is a remediable illness with early diagnosis. Oxidative stress, proteinases, proinflammatory mediators, cytokines and chronic irritants play an important role in chronic inflammation development for COPD [1-3]. Oxidative stress increases transcription factors. The *SIRT1* gene plays an important role in the

formation of NF- κ B dependent proinflammatory cytokines. However, this role of SIRT1 is not known for COPD.

The *SIRT1* gene is located on the 10q21.3 chromosome [6]. It encodes the enzyme group that has tasks such as gene transcription, DNA replication and DNA repair. The relation between SIRT1 polymorphisms and some diseases such as cardiovascular diseases, obesity and type 2 diabetes has been researched. A single gene polymorphism has been found in the *SIRT1* gene for these illnesses and reported [8-11]. Botden *et al.* reported that there was an interaction between type 2 diabetes, which is seen in adults, and rs7895833 [12]. With the studies carried out, it was documented that the *SIRT1* gene was involved in COPD pathogenesis [7, 8]. In the study performed by Yao *et al.*, they determined that there was a decrease in SIRT1 levels in the lungs of patients with COPD [13]. Additionally, Rajendrasozhan *et al.* reported that nuclear SIRT1 protein was at low levels in lung epithelial cells of patients with COPD [14]. These results show that SIRT1 protein is a safeguard against inflammation.

In company with these studies, we planned to investigate the changes in genotype and alleles of the *SIRT1* gene in patients with COPD. In our study, we analyzed rs7895833, rs7069102 and rs2273773 polymorphisms in the *SIRT1* gene. As far as we know, this is the first study where *SIRT1* gene polymorphism has been investigated in patients with COPD.

In our study, a significant difference was found between GG, AG and AA genotypes of rs7895833 and particularly the AG genotype was significantly more frequent in the group with COPD. Moreover, a significant difference was also found between TT, TC and CC genotypes of rs2273773. As the incidence rate of TC genotype decreases, it has been determined that the incidence rate had increased more than twice in CC gene. It shows that these polymorphisms of the *SIRT1* gene could be associated with the COPD tendency of the population from Muğla.

There was a significant difference between the two groups with regards to C and G alleles of rs7069102. As the T allele, which is dominant in the control group, was decreasing in the group with COPD, the C allele, which is seen less frequently in the control group, was determined as more dominant in the group with COPD.

These studies, performed at low cost through easily accessible equipment, support the usability of these genotypes and alleles for a more precise and objective diagnosis of COPD.

At the end of this study, we found an association between rs7895833 and rs2273773 polymorphisms of the *SIRT1* gene and COPD illness. No association between the *SIRT1* gene and COPD could be shown in any previous study. In more prospective studies, the *SIRT1* gene expression and mutations should be analyzed with a bigger COPD patient group. Moreover, additional studies are needed to clarify the SIRT1 gene's role in the pathogenesis of COPD.

Conclusions

The study showed that rs7895833 and its polymorphisms in the *SIRT1* gene and T and C alleles of the rs2273773 polymorphism could be important for the diagnosis of COPD in Muğla province and the surrounding region.

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Disclosure

The authors report no conflict of interest.

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