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Analysis of rs7895833 polymorphism of SIRT1 gene and its influence on the risk occurrence and progression of neurodegenerative disease, such as primary open-angle glaucoma in a Polish population

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Abstract

Introduction and Objective. A neurodegenerative disease, which is primary open-angle glaucoma (POAG) through damage of the optic nerve, leads to irreversible loss of vision. Sirtuins are responsible for regulating the metabolism involved in brain aging and neurodegenerative disorders. Previous studies revealed that upregulation of SIRT1 has an important protective effect against various ocular diseases, such as cataract, retinal degeneration, optic neuritis and uveitis. Moreover, some experimental studies in animal models demonstrated a neuroprotective effect of SIRT1 against retinal and optic nerve damage. Therefore, the purpose of this study was to explore, for the first time, rs7895833 polymorphism of SIRT1 gene and its influence on the risk occurrence and progression of POAG in the Polish population.

Materials and methods. The study included 187 glaucoma patients and 171 controls.DNA was isolated from peripheral blood. Gene polymorphism was analyzed by restriction of the fragment length polymorphism-polymerase chain reaction (RFLP-PCR).

Results. A statistically significant correlation was observed between the AG variant of the rs7895833 polymorphism of the SIRT1, and the occurrence of POAG. Moreover, a statistically significant correlation was observed between the rs7895833 polymorphism of the SIRT1, depending on the nerve fibre layer analyzer (GDx) ($p = 0.034$).

Conclusions. Analysis of the rs7895833 polymorphism SIRT1 gene in the Polish population with POAG shows a higher prevalence of heterozygote A/G polymorphism than the control group, and the correlation between the nerve fibre layer analyzer (GDx) and SIRT1 gene polymorphism, which suggest that variant A / G polymorphism rs7895833 of the SIRT1 gene may have a protective effect on the occurrence of JPOK in the Polish population.

Key words

polymorphism, neurodegeneration, sirtuins, SIRT1, primary open angle glaucoma (POAG)

INTRODUCTION

Primary open-angle glaucoma (POAG) is a neurodegenerative eye disease of complex etiology, leading to inevitable blindness [1]. The population of patients with POAG was estimated in 2013 at almost 44 million and is expected to increase to 53 million by 2020 [2]. The clinical course of this disease is connected with degeneration of the optic nerve head, which leads to a defective field of vision. Progression of POAG is connected with many factors, such as intraocular pressure, genetic and environmental risk factors [3]. Typical POAG damage of the optic nerve head is caused by apoptosis of retinal neurons; therefore, researchers connect the pathomechanism of typical neurodegenerative disease of the central nervous system with POAG [4].

Sirtuins are a highly conserved family of nicotinamide adenine dinucleotide (NAD+)-dependent histone deacetylases responsible for regulation of the lifespan of diverse organisms. The human genome contains seven different sirtuins (SIRT1–7) [5–8]. The process of ageing involves retinal cell damage that leads to visual dysfunction. Sirtuin 1 (Sirt1) can prevent oxidative stress, DNA damage and apoptosis. The ability of Sirt1 to stabilize DNA and

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chromatin in yeast and mammalian cells may determine cell apoptosis and survival. SIRT1 mediated through the neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) or heat shock protein 70 (HSP70), promotes neuron survival and protects neurons from cell death under multiple environmental stimuli and metabolic stress during the ageing process [9–11].

Several experimental studies in animal models have demonstrated a neuroprotective effect of SIRT1 against retinal and optic nerve damage. Intravitreal injections of SIRT1 activators prevent RGC loss in a dose-dependent manner through stimulating SIRT1 enzymatic activity in mice with optic neuritis [9,12,13].

OBJECTIVE

The results of research and previous knowledge prompted the research for the first time of the correlation between risk, clinical course POAG, and polymorphism rs7895833 of the promoter region of *SIRT1* gene in the Polish population. The reason for the selection of rs7895833 polymorphism was its prominent presence in the European population [including Turkey], and numerous studies describing expression of the SIRT gene and association of the mentioned polymorphism with cardiovascular disease, multiple sclerosis and energy management of the body [14–16].

MATERIALS AND METHOD

Blood samples were taken from 187 glaucoma patients and 171 health individuals (control group). Clinical data of JPOK patients are presented in Table 1. All patients and controls were age-matched (no difference was calculated at p>0.05). All patients and controls were examined in the Department of Ophthalmology at the Medical University of Warsaw, Poland. The study design was approved by the Committee for Bioethics of the Medical University of Łódź, Poland, and met the tenets of the Declaration of Helsinki. Informed written consent was explained and signed by all participants before initiation of the study.

The blood was collected in EDTA tubes. DNA was isolated form peripheral blood using commercial extraction kit (A&A Biotechnology, Gdańsk, Poland) as per the manufacturer's instructions. Analyses of the polymorphic variants of the *SIRT1* genes were conducted using PCR-RFLP method (Polymerase Chain Reaction – Restriction Length Polymorphism). The total volume of the reaction mixture was 25 μl which contained 10 ng of genomic DNA, 1 U of Taq polymerase, PCR buffer 1x solution (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 11 mM MgCl2), 1.5 mM MgCl2 50 mM dNTPs and 250 nM of each of the two designed primers (using Probe Finder). The temperature conditions of the reactions were as follows: initial denaturation at 95°C for 5 min. and 35 cycles of denaturation at 95°C for 30 sec and primers annealing for 30 sec at 66°C, elongation at 72°C for 1 minute. Final elongation was performed for 7 mins at 72°C. The obtained PCR product was digested with the restriction enzyme Acil (New England Biolabs, Inc, Beverly, MA, USA), which was then separated by electrophoresis in 3% agarose gel, stained in ethidium bromide and visualized in UV light.

Using the $\chi 2$ test, the distribution of genotypes and frequencies of alleles with Hardy-Weinberg distribution were checked. Statistical analysis also included the risk assessment of the event (odds ratio – OR) and the confidence interval (CI 95%) using the linear regression model. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Statistical analysis showed that the distribution of genotypes for the *SIRT1* gene in the control group was consistent with the Hardy-Weinberg law (p>0.05; χ^2=0.019). The clinical parameters of open-angle glaucoma patients are shown in Table 1. The frequency of genotypes (A/A, A/G, and G/G) and allele (A, G) was assessed on the research and controls group.

Table 1. Clinical parameters characteristic of open-angle glaucoma (POAG) patients and control groups

	Parameters	Patient groups n=187
Mean±SD	Cup disk ratio (c/d) right eye /left eye	0.60±0.40/0.60±0.40
	Rim area (RA) right eye / left eye	1.15±1.04/1.13±0.85
	Retinal Nerve Fibre Layer (RNFL) right eye/left eye	0.23±0.22/1.04±1.04

c/d – cup disc ratio; RA – rim area; RNFL – retinal nerve fibre layer

The SIRT1 gene A/A, A/G, G/G genotype frequencies were 40%, 55%, 5% on POAG patient group and 53%, 39%, 8% on control group, respectively. Odds ratio (OR) for A/G genotype frequency was 0.55 (95% CI 0.36–0.85; p = 0.0089), for G/G genotype frequency – 1.21 (95% Cl 0.49 – 2.98; p = 0.86). The frequencies of allele A and G in POAG patients were 68%, 32% and 73%, 27% in the control group, respectively. However, the distribution of A and G allele was not statistically significant (95% Cl OR=0.79; p=0.17) (Tab. 2).

Table 2. Genotype and allele frequency, odds ratio and 95% interval (95% CI) of *SIRT1* polymorphism for POAG

Genotype allele	Patients N=187		Controls N=171		OR (95% CI)*	p
	/no	/frequency	/no	/frequency		
A/A	76	0.40	91	0.53	1 ref	-
A/G	102	0.55	67	0.39	0.55 (0.36 – 0.85)	**0.0089**
G/G	9	0.05	13	0.08	1.21 (0.49 – 2.98)	0.86
allel A	254	0.68	249	0.73	1ref	-
allel G	120	0.32	93	0.27	0.79 (0.57 – 1.09)	0.17

Analysis of rs7895833 polymorphism of the *SIRT1* gene depending on the clinical parameters in POAG patients is shown in Table 3. There was a statistically significant correlation between the rs7895833 polymorphism of the SIRT1 depending on the nerve fibre layer analyzer (GDx; p = 0.034). However, other clinical parameters describing the progression of POAG, such as cup-to-disc ratio (c/d), rim area (RA) and retinal nerve fibre layer (RNFL), were not correlated with the above-mentioned gene polymorphism (p>0.05).

In the presented study, a possible association was found between the rs7895833 polymorphism of *SIRT1* gene

Table 3. Analysis of *SIRT1* (rs7895833) gene polymorphism depending on the clinical parameters in patients with primary open angle glaucoma (POAG) for each eye counted

Clinical parameter	Genotype	POAG patients	Quartile 25%	Median	Quartile 75%	p
c/d	A/A	226	0.60	0.75	0.85	0.313
	A/G	279	0.60	0.74	0.80	
	G/G	48	0.70	0.80	0.90	
RA	A/A	189	0.91	1.25	1.48	0.922
	A/G	212	0.97	1.24	1.45	
	G/G	34	0.96	1.28	1.38	
RNFL	A/A	188	0.13	0.19	0.26	0.257
	A/G	212	0.13	0.19	0.26	
	G/G	34	0.12	0.16	0.22	
GDx	A/A	147	17.0	27.0	45.5	**0.034**
	A/G	171	16.0	23.0	31.8	
	G/G	33	19.0	27.0	52.3	

c/d – cup disc ratio; RA – rim area; RNFL – retinal nerve fibre layer; GDx – nerve fibre layer analyzer

genotype A/G prevalence (p=0.0089), and a correlation between the nerve fibre layer analyzer (GDx) and SIRT1 gene polymorphism (rs7895833) (p=0.034).

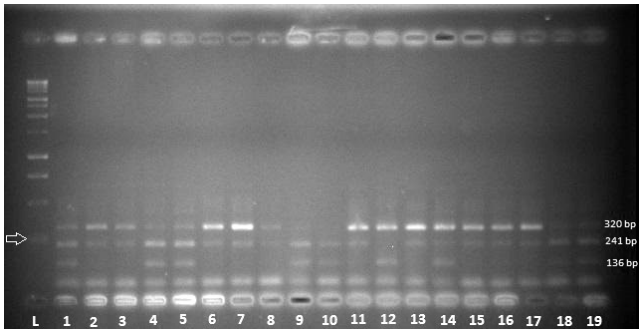

Figure 1. Representative PCR gel picture of each SIRT1 gene polymorphisms. DNA ladder in the first lane; arrows point at 250 bp band; slot 1 = AG; slot 2 = AA; slot 10 = GG

DISCUSSION

Glaucoma is the second cause of vision loss worldwide after cataracts. POAG is characterized by retinal ganglional cell death (RGC), axonal loss, and a change in the appearance of the optic disc. The consequence of these changes is deterioration of vision that ultimately can lead to irreversible vision loss. However, molecular mechanisms of glaucoma pathogenesis have not yet been fully understood.

Sirtuins are responsible for regulating the metabolism involved in ageing of the brain and neurodegenerative disorders [17], and animal studies have been conducted to examine the role of sirtuins in ocular ageing. Up-regulation of SIRT1 has been shown to have an important protective effect against various ocular diseases, such as cataract, retinal degeneration, optic neuritis and uveitis [9–11]. Furthermore, one of the targets of SIRT1 are histones through which SIRT1 regulates the transcription of several genes indirectly by histone-dependent epigenetic silencing [18].

The current study reveals that the polymorphism rs7895833 promoter region of *SIRT1* has significant correlation with the progression of POAG in the Polish population. Frequency of heterozygotes (AG) of POAG patients have a statistically higher SIRT1 protein expression level than the control group, and *SIRT1* polymorphism correlates with the nerve fibre layer analyzer (GDx). The results of the current study are in harmony with Kilic et al. who observed the influence of polymorphism promotor SNP rs7895833 on a statistically significant higher expression level of the SIRT1 protein in the patients with cardiovascular disease with wild-type (AA) and heterozygote (AG) [19]. Moreover, Kalemci et al. observed more AG genotype in the group with chronic obstructive pulmonary disease [20]. Similarly, Chen et al. show significantly different genotype frequency of *SIRT1* rs7895833 in the ApoE E4 between the mild cognitive impairment research and control group [21], where ApoE E4 was the major genetic risk factor for neurodegenerative disease, e.g. Alzheimer Disease [22]. These results are consistant with the regulatory role of SIRT1 and indirect influence the transcription of several genes.

The roles of rs7895833 polymorphism of the SIRT1 gene are still under investigation. The influence of *SIRT1* gene polymorphism on the level expression in older people is highly correlated. Carrying the AG genotype for rs7895833 is connected with an elevated level of SIRT1 protein, which may explain the compensatory mechanism for oxidative stress in the elderly [15]. In addition, the above-mentioned SNP genotype *SIRT1* gene was statistically significant in rheumatoid arthritis (RA) [23]. Recent studies have focused on the impact of rs7895833 polymorphism on cardiovascular diseases, such as coronary artery disease [24,25], calcification [26], risk of obese in children [27, 28], body mass index [14, 29, 30] diabetes type 2 [31] and polycystic ovary syndrome [32]. However, many studies have provided contradictory information about the impact of the SIRT1 gene SNP allele and genotype on neurological diseases, such as multiple sclerosis [16]. Therefore, its influence should be investigated on a larger population.

To fully understand the impact of polymorphism rs7895833 *SIRT1* gene to patomechanism of development POAG, we need to focus on two issues: the clinical parameters which are a markers of the developing illness and the mechanism of regulation gene expression by SNP polymorphism.

The presented study shows a correlation between the nerve fibre layer analyzer (GDx) and the polymorphism rs7895833 genotype (p=0.034), but others – cup-to-disc ratio (c/d), rim area (RA) and retinal nerve fibre layer (RNFL), have no significant correlation. The diagnostic accuracy of scanning laser polarimeter (GDx) varies from 83.8% – 95.9% [33], which is useful in an early glaucomatous damage screening [34]. Thus, it can be assumed that the polymorphism rs7895833 *SIRT1* gene is one of the factors relevant to the process in the glaucomatous damage development, although the mechanism of its action is as yet unknown.

As mentioned above, the polymorphism rs7895833 has an influence on the SIRT1 gene expression. It is located at 21 kb upstream of the SIRT1 gene in the promoter region, where it can regulate the expression. Thus, a promoter-dependent altered expression mechanism seems to be involved in regulation of the expression of SIRT1, and can therefore influence the clinical course of neurological disease or metabolism in the elderly [15].

CONCLUSIONS

Analysis of the rs7895833 polymorphism SIRT1 gene in the Polish population with primary open-angle glaucoma shows a higher prevalence of heterozygote A/G polymorphism than the control group, and the correlation between the nerve fibre layer analyzer (GDx) and SIRT1 gene polymorphism. It is assumed that in the early stage of glaucoma, rs7895833 polymorphism upregulate the SIRT1 expression, which leads to the activation of neuroprotective mechanisms which compensate for neurodegenerative factors, such as oxidative stress. However, more studies are needed to better understand the altered mechanism of SIRT1 expression with the participation rs7895833 polymorphism.

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