The role of SIRT1 level and SIRT1 (rs3818292, rs3758391, rs7895833) gene polymorphisms in patients with optic neuritis and multiple sclerosis

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Abstract

The aim: To investigate the role of SIRT1 level and *SIRT1* (rs3818292, rs3758391, rs7895833) gene polymorphisms in patients with optic neuritis (ON) and multiple sclerosis (MS).

Methods: We studied 79 patients with ON and 225 healthy subjects. We divided ON patients into 2 subgroups: patients with MS (n=30) and patients without MS (n=43). 6 ON patients did not have sufficient data for MS diagnosis and were excluded from subgroup analysis. The subjects' DNA was extracted from peripheral blood leukocytes and genotyped by real-time polymerase chain reaction. Results were analyzed using the program "IBM SPSS Statistics 26.0".

Results: We discovered that *SIRT1* rs3758391 was associated with a 2-fold increased odds of developing ON under the codominant (p=0.007), dominant (p=0.011), and over-dominant (p=0.008) models. Also, it was associated with a 3-fold increased odds ofON with MS development under the dominant (p=0.010), 2-fold increased odds under the over-dominant (p=0.032) models and a 1.2-fold increased odds of ON with MS development (p=0.015) under the additive model. We also discovered that the *SIRT1* rs7895833 was significantly associated with a 2.5-fold increased odds of ON development under the codominant (p=0.001), dominant (p=0.006), and over-dominant (p<0.001) models, and a 4-fold increased odds of ON with MS development under the codominant (p<0.001), dominant (p=0.001), over-dominant (p<0.001) models and with a 2-fold increased odds of ON with MS development (p=0.013) under the additive genetic model. There was no association between SIRT1 levels and ON with/without MS development.

Conclusions: We discovered that the *SIRT1* rs3758391 and rs7895833 polymorphisms are associated with ON and ON with MS development.

Introduction

Optic neuritis (ON) - inflammation of the optic nerve characterized by painful, usually monocular, visual disturbances, visual field defects, and loss of color contrast sensitivity in young people (mostly women), usually associated with multiple sclerosis (MS) (1). MS is characterized by central nervous system lesions that cause neurologic dysfunction and other complaints such as fatigue, pain, depression, and anxiety. The disease usually relapses in the early stages, but most people develop the secondary progressive disease over time. Treatment has not been shown to affect long-term outcomes (2). An association between ON and MS has been known for many years. In 15–20% of patients with MS, optic neuritis is the first inflammatory event, and half of MS patients have had at least one ON attack within the past 15 years (3). Like ON, MS is a multifactorial disease with many genes and environmental factors involved in its pathogenesis.

SIRT1 is known to be expressed in the cornea, lens, iris, ciliary body, inner nuclear layer, outer nuclear layer, and retinal ganglion cell layer of mice (4). It may impact the development of ON and other neurodegenerative diseases in many experimental models (5–15). Devin S. McDougald et al. demonstrated a significant role of the SIRT1 gene in the pathogenesis of ON and MS in experimental

models (5). Sirtuin 1 (SIRT1) is an evolutionarily conserved NAD +-dependent deacetylase that regulates various components of cellular metabolism related to aging, DNA repair, mitochondrial biogenesis, and apoptosis (6). There is growing evidence that modulation of SIRT1 activity by pharmacological induction or transgenic overexpression may be of therapeutic value in various forms of neurodegenerative diseases (7). SIRT1 mediates neuroprotection from mutant huntingtin by activating the TORC1 and CREB transcriptional pathways (8). SIRT1-activating compounds reduce oxidative stress-mediated neuronal loss in virus-induced CNS demyelinating diseases (9–15). In experimental optic neuritis, small-molecule activators of SIRT1, including resveratrol and related polyphenolic compounds, effectively preserve visual acuity and RGC survival in EAE and virus-related demyelinating diseases (7–9). The results suggest that SIRT1-activating drugs may play a specific role in preventing traumatic optic nerve damage and suggest a broader role for this strategy in treating various optic nerve diseases that may contain an oxidative stress component (15).

Therefore, our study aimed to investigate the role of SIRT1 levels and *SIRT1* (rs3818292, rs3758391, rs7895833*)* gene polymorphisms in patients with optic neuritis and multiple sclerosis in the Caucasian population.

Materials And Methods

Kaunas Regional Biomedical Research Ethics Committee approved the study (No. BE-2-13). The study was conducted in the Department of Ophthalmology of the Hospital of the Lithuanian University of Health Sciences and the Neuroscience Institute of the Lithuanian University of Health Sciences. The study participants consisted of 79 subjects diagnosed with optic neuritis and 225 subjects from the control group.

The inclusion criteria for subjects with optic neuritis were described in our previous study (16).

Dna Extraction, Genotyping, And Enzyme Immunoassay

We extracted DNA samples from peripheral venous blood using the DNA salting-out method. Genotyping of all three SNPs was performed using TaqMan® genotyping assays (Applied Biosystems Foster City, CA, USA): *SIRT1* (rs3818292, rs3758391, and rs7895833) according to the manufacturer's instructions using real-time polymerase chain reaction (PCR). Serum SIRT1 levels were determined in control subjects and patients using the commercial enzyme-linked immunosorbent assay (ELISA) kit for human SIRT1 (Human SIRT1 ELISA Kit, Abcam, Cambridge, United Kingdom) according to the manufacturer's instructions, and optical density was measured immediately at a wavelength of 450 nm using a microplate reader (Multiskan FC microplate photometer, Thermo Scientific, Waltham, MA). The SIRT1 level was calculated using the standard curve; the sensitivity range of the standard curve: 0.63-40 ng/ml, sensitivity 132 pg/ml.

Quality Control Of Genotyping

Repeated analysis of 5% randomly selected samples was performed for all SNPs to confirm the same rate of genotypes from initial and repeated genotyping.

Statistical analysis

Statistical analysis was performed with SPSS/W 27.0 software (Statistical Package for the Social Sciences for Windows, Inc, Chicago, Illinois, USA). Data on subjects' ages were expressed as mean with standard deviation (SD) and median with interquartile range (IQR). The Student t-test was performed to compare the mean age of the study groups, and the Mann Whitney U test was used to compare serum SIRT1 levels between the study groups. Hardy-Weinberg equilibrium analysis compared the observed and expected frequencies of *SIRT1* rs3818292, rs3758391, and rs7895833. The distributions of the genotypes and alleles in the study groups and subgroups were compared using the χ^2 test. Binomial logistic regression analysis was performed to estimate the effects of genotypes on the development of ON, and ON subgroups: with MS and without MS. Odds ratios and 95% confidence intervals are shown. The best genetic model selection was based on the Akaike Information Criterion (AIC); therefore, the best genetic models were those with the lowest AIC values. Differences were considered statistically significant when $p < 0.05$.

Results

Our study population included data from 304 individuals. To investigate the frequency of selected gene polymorphisms, subjects were divided into two groups. The group of ON patients included 79 subjects: 26 (32.9%) males and 53 (67.1%) females. The control group included 225 subjects: 91 (40.4%) males and 134 (59.6%) females. There was no statistically significant difference between males and females with ON and the control groups ($p = 0.236$). The mean age was 37 years in the patients with ON and 32 years in the control group. No statistically significant differences were found between the groups by age ($p = 0.066$). The distribution of subjects by gender and age is shown in Table 1.

Table 1
Demographic characteristics of study subjects.

Characteristic	Group		p-value
	Patients with ON	Reference group	
Males, n (%)	26 (32.9)	91 (40.4)	0.236
Females, n (%.)	53 (67.1)	134 (59.6)	
The median age (IQR)	37 (23)	32 (17)	0.066
ON – optic neuritis; IQR – interquartile range; p-value – significance level (statistically significant, when $p < 0.05$);			

Associations between SIRT1 **concentration, optic neuritis, and multiple sclerosis.**

Blood serum SIRT1 concentrations were determined in patients with ON (n = 23) and the control group (n = 24). Statistically significant differences were not observed between these groups (IQR: 2.130 ng/ml (1.68) vs. 2.130 ng/ml (0.61), respectively, p = 0.856).

Also, serum SIRT1 levels were compared between ON patients with MS and ON patients without MS subgroups. We also found no statistically significant differences between these two groups (IQR: 3.821 ng/ml (4.35) vs. 2.124 ng/ml (0.61) respectively, p = 0.593).

No statistically significant differences in serum SIRT1 levels were found between ON patients with MS and the control group (IQR: 3.821 ng/ml (4.35) vs. 2.130 ng/ml (0.61), p = 0.695) or between ON patients without MS and the control group (IQR: 2.124 ng/ml (0.61), 2.130 ng/ml (0.61), p = 0.989).

SIRT1 **rs3818292, rs3758391, rs7895833 genotypes associations with ON**

We determined the frequency of genotypes and alleles of *SIRT1* rs3818292, rs3758391, and rs7895833 SNPs compared between ON patients and control groups. The distribution of genotypes and alleles in the control group was in accordance with the Hardy-Weinberg equilibrium ($p > 0.001$).

There was no statistically significant difference in the frequency of genotypes and alleles of *SIRT1* rs3818292 in ON patients and control groups (p = 0.067 and p = 0.383, respectively) (Table 2).

There was a statistically significant difference in C/C and C/T genotypes distribution between patients with ON and control groups. The C/C genotype of *SIRT1* rs3758391 was less frequent and the C/T genotype was more frequent in the patients of ON than in the control group (39.2% vs. 56.0%, p = 0.010; 53.2% vs. 36.0%, p = 0.007, respectively) (Table 2).

In addition, a statistically significant difference was found between the distribution of *SIRT1* rs7895833 A/A and A/G genotypes. In patients with ON, the A/A genotype was less frequent, and the A/G genotype was more frequent than in the control group (57.0% vs. 73.8%, p = 0.007; 41.8% vs. 20.9%, p = 0.001, respectively) (Table 2).

Table 2
SIRT1 rs3818292, rs3758391, rs7895833 genotypes and alleles frequencies.

Polymorphism	Genotype/allele	Frequency (%)			
		Control group, n (%) N = 225	HWE p-value	Patients with ON, n (%) N = 79	p-value
rs3818292	Genotype				
	A/A	192 (85.3)	0.003	62 (78.5)	0.067
	A/G	28 (12.4)		17 (21.5)	
	G/G	5 (2.2)		0 (0)	
	Total	225 (100)		79 (100)	
	Allele				
	A	412 (91.6)		141 (89.2)	0.383
	G	38 (8.4)		17 (10.8)	
rs3758391	Genotype				
	C/C	126 (56.0)[1]	0.334	31 (39.2)[1]	**0.024**
	C/T	81 (36.0)[2]		42 (53.2)[2]	
	T/T	18 (8.0)		6 (7.6)	
	Total	225 (100)		79 (100)	
	Allele				
	C	333 (74.0)		104 (65.8)	0.062
	T	117 (26.0)		54 (34.2)	
rs7895833	Genotype				
	A/A	166 (73.8)[3]	0.001	45 (57.0)[3]	**0.001**
	A/G	47 (20.9)[4]		33 (41.8)[4]	
	G/G	12 (5.3)		1 (1.3)	
	Total	225 (100)		79 (100)	
	Allele				

ON – optic neuritis; p value - significance level (statistically significant, then $p < 0.05$); HVE p-value – significance level according to Hardy-Weinberg equilibrium principle (differences are considered significant, when $p < 0.001$); 1 – $p = 0.010$; 2 – $p = 0.007$; 3 – $p = 0.007$; 4 – $p = 0.001$;

	A	379 (84.2)	123 (77.8)	0.069
	G	71 (15.8)	35 (22.2)	

ON – optic neuritis; p value - significance level (statistically significant, then $p < 0.05$); HVE p-value – significance level according to Hardy-Weinberg equilibrium principle (differences are considered significant, when $p < 0.001$); 1 – p = 0.010; 2 – p = 0.007; 3 – p = 0.007; 4 – p = 0.001;

Binary logistic regression analysis of *SIRT1* rs3818292, rs3758391, and rs7895833 was performed. It was found that *SIRT1* rs3758391 C/T genotype was associated with a 2.1-fold increased probability of developing ON compared to C/C genotype (OR = 2.108; 95%. CI 1.226–3.622; p = 0.007). The C/T + T/T genotypes were associated with a 2-fold increased odds of developing ON compared to the C/C genotype (OR = 1.971; 95%. CI 1.168–3.324; p = 0.011) and the C/T genotype was associated with a 2.7-fold increased odds of developing ON compared to the C/C + T/T genotype (OR = 2.717; 95%. CI 1.566–4.712; p = 0.008) (Table 3).

The *SIRT1* rs7895833 A/G genotype was associated with a 2.6-fold increased likelihood of developing ON than the A/A genotype (OR = 2.590; 95% CI 1.489–4.506; p = 0.001). The A/G + G/G genotypes were associated with a 2.1-fold increased odds of developing ON compared to the A/A genotype (OR = 2.126; 95%, CI 1.245–3.631; p = 0.006), and the A/G genotype was associated with a 2.7-fold increased odds of developing ON compared to the A/A + G/G genotype (OR = 2.717; 95%, CI 1.566–4.712; p < 0.001) (Table 3).

Table 3
SIRT1 rs3818292, rs3758391, rs7895833 binary logistic regression between ON and control groups.

Polymorphism	Model	Genotype	OR (95% CI)	p-value	AIC
rs3818292	Codominant	A/A	1	0.064	345.990
		A/G	1.880 (0.865–3.664)	1	
		G/G	–		
	Dominant	A/A	1	0.160	348.426
		A/G + G/G	1.595 (0.832–3.060)		
	Recessive	AA + A/G	1	1	347.296
		G/G	–		
	Overdominant	A/A + G/G	1	0.053	346.261
		A/G	1.929 (0.990–3.758)		
	Additive	G	1.275 (0.718–2.264)	0.407	349.667
rs3758391	Codominant	C/C	1	**0.007**	344.935
		C/T	2.108 (1.226–3.622)	0.553	
		T/T	1.355 (0.496–3.698)		
	Dominant	C/C	1	**0.011**	343.729
		C/T + T/T	1.971 (1.168–3.324)		
	Recessive	C/C + C/T	1	0.909	350.322
		T/T	0.945 (0.361–2.473)		
	Overdominant	C/C + T/T	1	**0.008**	343.274
		C/T	2.018 (1.201–3.391)		
	Additive	T	1.482 (0.999–2.199)	0.051	346.545
rs7895833	Codominant	A/A	1	**0.001**	338.196
		A/G	2.590 (1.489–4.506)	0.574	
		G/G	0.574 (0.025–7.691)		
	Dominant	A/A	1	**0.006**	342.826
		A/G + G/G	2.126 (1.245–3.631)		

p-value – significance level (differences are considered significant when $p < 0.05$); OR – odds ratio; CI – confidence interval; AIC – Akaike information criterion.

Polymorphism	Model	Genotype	OR (95% CI)	p-value	AIC
	Recessive	AA + A/G	1	0.158	347.368
		G/G	0.228 (0.029–1.779)		
	Overdominant	A/A + G/G	1	**< 0.001**	337.908
		A/G	2.717 (1.566–4.712)		
	Additive	G	1.470 (0.950–2.276)	0.084	347.415

p-value – significance level (differences are considered significant when $p < 0.05$); OR – odds ratio; CI – confidence interval; AIC – Akaike information criterion.

SIRT1 **rs3818292, rs3758391, rs7895833 genotypes associations with ON according to gender and morbidity of MS.**

We compared the distribution of genotypes and alleles of *SIRT1* rs3818292, rs3758391, and rs7895833 in ON and control groups by sex.

The analysis showed that *SIRT1* rs7895833 A/A genotype was less frequent, while A/G genotype was more frequent in women with ON than in the control group (56.6% vs. 74.6%, $p = 0.022$; 41.5% vs. 20.9%, $p = 0.006$, respectively. We found no statistically significant differences in the male groups (Table 4).

Table 4
SIRT1 rs3818292, rs3758391, rs7895833 genotypes and alleles frequencies in patients with ON by gender.

Polymorphism	Genotype	Frequency, n (%)					
		Males		p-value	Females		p-value
		Control group, n (%) N = 91	Patients with ON, n (%) N = 26		Control group, n (%) N = 134	Patients with ON, n (%) N = 53	
rs3818292	A/A	80 (87.9)	21 (80.8)	0.788	112 (83.6)	41 (77.4)	0.492
	A/G	7 (7.7)	5 (19.2)		21 (15.7)	12 (22.6)	
	G/G	4 (4.4)	0 (0)		1 (0.7)	0 (0)	
	Allele						
	A	167 (91.8)	47 (90.4)	0.755	245 (91.4)	94 (88.7)	0.412
	G	15 (8.2)	5 (9.6)		23 (8.6)	12 (11.3)	
rs3758391	Genotype						
	C/C	48 (52.7)	10 (38.5)	0.385	78 (58.2)	21 (39.6)	0.056
	C/T	32 (35.2)	13 (50)		49 (36.6)	29 (54.7)	
	T/T	11 (12.1)	3 (11.5)		7 (5.2)	3 (5.7)	
	Allele						
	C	128 (70.3)	33 (63.5)	0.346	205 (76.5)	71 (67.0)	0.594
	T	54 (29.7)	19 (36.5)		63 (23.5)	35 (33.0)	
rs7895833	Genotype						
	A/A	66 (72.5)	15 (57.7)	0.067	100 (74.6)[1]	30 (56.6)[1]	**0.014**

ON – optic neuritis; p-value - significance level (differences are considered significant, when $p < 0.05$); 1 – (A/A vs. A/G + G/G) p = 0.022; 2 – (A/G vs. A/A + G/G) p = 0.006;

	A/G	19 (20.9)	11 (42.3)		28 (20.9)[2]	22 (41.5)[2]	
	G/G	6 (6.6)	0 (0)		6 (4.5)	1 (1.9)	
	Allele						
	A	151 (83.0)	41 (78.8)	0.495	228 (85.1)	82 (77.4)	0.074
	G	31 (17.0)	11 (21.2)		40 (14.9)	24 (22.6)	

ON – optic neuritis; p-value - significance level (differences are considered significant, when $p < 0.05$); 1 – (A/A vs. A/G + G/G) p = 0.022; 2 – (A/G vs. A/A + G/G) p = 0.006;

Binary logistic regression analysis of *SIRT1* rs3818292, rs3758391, and rs7895833 in patients with ON and control groups by sex was performed. In the male group, we observed that the A/G genotype of *SIRT1* rs7895833 was associated with a 2.6-fold increased likelihood of developing ON compared to the A/A genotype (OR = 2.547; 95%. CI 1.005–6.459; p = 0.049) and the A/G genotype was associated with a 2.8-fold increased odds of developing ON in males compared to the A/A and G/G genotypes (OR = 2.779; 95%. CI 1.099–7.028; p = 0.031) (Table 5).

In the female group, we found that the C/T genotype of *SIRT1* rs3758391 was associated with a 2.2-fold increased likelihood of developing ON compared with the C/C genotype (OR = 2.198; 95%. CI 1.130–4.277; p = 0.020). The C/T + T/T genotypes were associated with a 2.1-fold increased odds of developing ON compared to the C/C genotype (OR = 2.122; 95%. CI 1.109–4.060; p = 0.023) and the C/T genotype was also associated with a 2.1-fold increased odds of developing ON compared to the C/C and T/T genotypes in females (OR = 2.096; 95%. CI 1.100–3.995; p = 0.025) (Table 6).

The A/G genotype of *SIRT1* rs7895833 was associated with a 2.6-fold increased probability of developing ON compared to the A/A genotype (OR = 2.619; 95%. CI 1.312–5.230; p = 0.006). Genotypes A/G + G/G were associated with a 2.2-fold increased odds of developing ON compared to genotype A/A (OR = 2.255; 95%. CI 1.156–4.399; p = 0.017), and genotype A/G was associated with a 2.7-fold increased odds of developing ON in females compared to genotype A/A + G/G (OR = 2.68; 95%. CI (1.352–5.340); p = 0.005) (Table 6).

Table 5
Binary logistic regression for *SIRT1* rs3818292, rs3758391, between males of ON and control groups.

Polymorphism	Model	Genotype	OR (95%. CI)	p-value	AIC
rs3818292	Codominant	A/A	1	0.115	123.561
		A/G	2.721 (0.784–9.443)	1	
		G/G	–		
	Dominant	A/A	1	0.354	125.135
		A/G + G/G	1.732 (0.542–5.531)		
	Recessive	AA + A/G	1	1	123.901
		G/G	–		
	Overdominant	A/A + G/G	1	0.098	123.358
		A/G	2.857 (0.824–9.906)		
	Additive	G	1.134 (0.455–2.825)	0.788	125.881
rs3758391	Codominant	C/C	1	0.163	125.976
		C/T	1.950 (0.763–4.982)	0.715	
		T/T	1.309 (0.308–5.564)		
	Dominant	C/C	1	0.202	124.228
		C/T + T/T	1.786 (0.733–4.353)		
	Recessive	C/C + C/T	1	0.939	125.945
		T/T	0.949 (0.244–3.689)		
	Overdominant	C/C + T/T	1	0.173	124.106
		C/T	1.844 (0.764–4.450)		
	Additive	T	1.326 (0.714–2.460)	0.371	125.162
rs7895833	Codominant	A/A	1	**0.049**	121.054
		A/G	2.547 (1.005–6.459)	1	
		G/G	–		
	Dominant	A/A	1	0.152	123.941
		A/G + G/G	1.936 (0.784–4.781)		

p-value - significance level (differences are considered significant when $p < 0,05$); OR – odds ratio; CI – confidence interval; AIC – Akaike information criterion.

Polymorphism	Model	Genotype	OR (95%. CI)	p-value	AIC
	Recessive	AA + A/G	1	1	122.844
		G/G	–		
	Overdominant	A/A + G/G	1	**0.031**	121.416
		A/G	2.779 (1.099–7.028)		
	Additive	G	1.267 (0.614–2.611)	0.522	125.551

p-value - significance level (differences are considered significant when p < 0,05); OR – odds ratio; CI – confidence interval; AIC – Akaike information criterion.

Table 6
Binary logistic regression for *SIRT1* rs3818292, rs3758391, between females of ON and control groups.

Polymorphism	Model	Genotype	OR (95%. CI)	p-value	AIC
rs3818292	Codominant	A/A	1	0.272	225.119
		A/G	1.561 (0.705–3.455)	1	
		G/G	–		
	Dominant	A/A	1	0.322	224.006
		A/G + G/G	1.490 (0.677–3.280)		
	Recessive	AA + A/G	1	1	224.294
		G/G	–		
	Overdominant	A/A + G/G	1	0.262	223.741
		A/G	1.575 (0.712–3.485)		
	Additive	G	1.379 (0.648–2.937)	0.405	224.285
rs3758391	Codominant	C/C	1	**0.020**	221.480
		C/T	2.198 (1.130–4.277)	0.526	
		T/T	1.592 (0.379–6.690)		
	Dominant	C/C	1	**0.023**	219.682
		C/T + T/T	2.122 (1.109–4.060)		
	Recessive	C/C + C/T	1	0.905	224.948
		T/T	1.089 (0.271–4.377)		
	Overdominant	C/C + T/T	1	**0.025**	219.862
		C/T	2.096 (1.100–3.995)		
	Additive	T	1.684 (0.995–2.849)	0.052	221.181
rs7895833	Codominant	A/A	1	**0.006**	218.788
		A/G	2.619 (1.312–5.230)	0.593	
		G/G	0.556 (0.064–4.798)		
	Dominant	A/A	1	**0.017**	219.336
		A/G + G/G	2.255 (1.156–4.399)		

p-value - significance level (differences are considered significant when $p < 0,05$); OR – odds ratio; CI – confidence interval; AIC – Akaike information criterion;

Polymorphism	Model	Genotype	OR (95%. CI)	p-value	AIC
	Recessive	AA + A/G	1	0.415	224.157
		G/G	0.410 (0.048–3.492)		
	Overdominant	A/A + G/G	1	**0.005**	217.111
		A/G	2.687 (1.352–5.340)		
	Additive	G	1.627 (0.933–2.837)	0.086	222.068

p-value - significance level (differences are considered significant when p < 0,05); OR – odds ratio; CI – confidence interval; AIC – Akaike information criterion;

We studied the genotypes and allele distribution of *SIRT1* rs3818292, rs3758391, and rs7895833 in ON patients and control groups with and without MS.

We found that the *SIRT1* rs3758391 C/C was less frequent, and the C/T genotype was more frequent in ON patients with MS than in the control group (30%. vs. 60%, p = 0.007; 56.7%. vs. 36%, p = 0.029, respectively). The T allele was more frequent in ON patients with MS compared to the control group (41.7% vs. 26.0%, p = 0.011) (Table 7).

In addition, statistically significant differences were found in the distribution of *SIRT1* rs895833 genotypes A/A, A/G, and G/G in ON patients with MS and the control group (p = 0.001). The A/A genotype was less frequent, and the A/G genotype was more frequent in ON patients with MS than in the control group (43.4% vs. 73.8%, p = 0001; 53.3% vs. 20.9%, p < 0.001, respectively). The G allele was also more frequent in ON patients with MS than in the control group (30.0% vs. 15.8%, p = 0.006) (Table 7).

Table 7

SIRT1 rs3818292, rs3758391, rs7895833 genotypes and alleles frequencies for ON patients with and without MS and control group.

Polymorphism	Genotype	Frequencies, n (proc.)				
		Control group, n (%) N = 225	ON patients with MS, n (%) N = 30	p-value	ON patients with MS, n (%) N = 43	p-value
rs3818292	Genotype					
	A/A	192 (85.3)	23 (76.7)	0.243	34 (79.1)	0.250
	A/G	28 (12.4)	7 (23.3)		9 (20.9)	
	G/G	5 (2.2)	0 (0)		0 (0)	
	Allele					
	A	412 (91.6)	53 (88.3)	0.408	77 (89.5)	0.544
	G	38 (8.4)	7 (11.7)		9 (10.5)	
rs3758391	Genotype					
	C/C	126 (56.0)[1]	9 (30.0)[1]	**0.023**	20 (46.5)	0.270
	C/T	81 (36.0)[2]	17 (56.7)[2]		21 (48.8)	
	T/T	18 (8.0)	4 (13.3)		2 (4.7)	
	Allele					
	A	333 (74.0)	35 (58.3)	**0.011**	61 (70.9)	0.554
	G	117 (26.0)	25 (41.7)		25 (29.1)	
rs7895833	Genotype					
	A/A	166 (73.8)[3]	13 (43.4)[3]	**0.001**	29 (67.4)	0.107
	A/G	47 (20.9)[4]	16 (53.3)[4]		14 (32.6)	
	G/G	12 (5.3)	1 (3.3)		0 (0)	
	Allele					
	A	379 (84,2)	42 (70,0)	**0.006**	72 (83.7)	0.907
	G	71 (15,8)	18 (30,0)		14 (16.3)	

ON – optical neuritis; MS – multiple sclerosis; p-value - significance level (differences are considered significant, when $p < 0,05$); 1 – $p = 0,007$; 2 – $p = 0,029$; 3 – $p = 0,001$; 4 – $p < 0,001$

We performed binary logistic regression analysis to evaluate the influence of *SIRT1* rs3818292, rs3758391, and rs7895833 on the development of ON in patients with and without MS.
We found that the C/T and T/T genotypes *SIRT1* rs3758391 together were associated with a 3-fold increased odds of ON patients with MS (OR = 2.970; 95%. CI 1.303–6.770; p = 0.010), and the C/T genotype was associated with a 2.2-fold increased odds of ON patients with MS while compared with C/C and T/T genotypes (OR = 2.235; 95% CI 1.075–5.030; p = 0.032). The T allele was associated with a 1.2-fold increased odds of ON with MS (OR = 1.199; 95% CI 1.143–3.450; p = 0.015) (Table 8).
The A/G genotype of *SIRT1* rs7895833 was associated with a 4.4-fold increased odds of developing ON with MS compared with the A/A genotype (OR = 4.347; 95%. CI 1.953–9.677; p < 0.001), A/G + G/G genotypes were associated with 3.7-fold increased odds of ON in patients with MS compared to A/A genotype (OR = 3.679; 95% CI 1.685–8.033; p = 0.001) and A/G genotype was associated with 4.3-fold increased odds of ON in patients with MS compared to A/A and G/G genotype (OR = 4.328; 95% CI 1.972–9.499; p < 0.001). At least one G allele was associated with a 2.1-fold increased odds of ON with MS (OR = 2.058; 95% CI 1.162–3.645; p = 0.013) (Table 8).

Table 8
Binary logistic regression for *SIRT1* rs3818292, rs3758391, between ON patients with MS and the control group.

Polymorphism	Model	Genotype	OR (95% CI)	p-value	AIC
rs3818292	Codominant	A/A	1	0.123	185292
		A/G	2.087 (0.820 – 5.312)	1	
		G/G	–		
	Dominant	A/A	1	0.225	185.362
		A/G + G/G	1.771 (0.703–4.457)		
	Recessive	AA + A/G	1	1	185.462
		G/G	–		
	Overdominant	A/A + G/G	1	0.110	184.409
		A/G	2.141 (0.841–5449)		
	Additive	G	1.364 (0617–3.013)	0.443	186.178
rs3758391	Codominant	C/C	1	0.082	181.417
		C/T	0.321 (0.090–1.153)	0.926	
		T/T	0.994 (0.284–3.145)		
	Dominant	C/C	1	**0.010**	179.425
		C/T + T/T	2.970 (1,303–6.770)		
	Recessive	C/C + C/T	1	0.334	185.879
		T/T	1.769 (0.556–5.630)		
	Overdominant	C/C + T/T	1	**0.032**	182.090
		C/T	2.235 (1.075–5.030)		
	Additive	T	1.199 (1.143–3450)	**0.015**	178.919
rs7895833	Codominant	A/A	1	**< 0.001**	175.665
		A/G	4.347 (1.953–9.677)	0.954	
		G/G	1.064 (0.128–8.836)		
	Dominant	A/A	1	**0.001**	176.008
		A/G + G/G	3.679 (1.685–8.033)		

p-value - significance level (differences are considered significant when p < 0,05); OR – odds ratio; CI – confidence interval; AIC – Akaike information criterion;

Polymorphism	Model	Genotype	OR (95% CI)	p-value	AIC
	Recessive	AA + A/G	1	0.643	186.483
		G/G	0.612 (0.077–4.882)		
	Overdominant	A/A + G/G	1	**< 0.001**	173.668
		A/G	4.328 (1.972–9.499)		
	Additive	G	2.058 (1.162–3.645)	**0.013**	181.025

p-value - significance level (differences are considered significant when p < 0,05); OR – odds ratio; CI – confidence interval; AIC – Akaike information criterion;

Table 9
Binary logistic regression for *SIRT1* rs3818292, rs3758391, between ON patients without MS and the control group.

Polymorphism	Model	Genotype	OR (95% CI)	p-value	AIC
rs3818292	Codominant	A/A	1	0.162	236.466
		A/G	1.815 (0.788–4.183)	1	
		G/G	–		
	Dominant	A/A	1	0.303	237.056
		A/G + G/G	1.540 (0.677–3.505)		
	Recessive	AA + A/G	1	1	236.294
		G/G	–		
	Overdominant	A/A + G/G	1	0.144	236.077
		A/G	1.862 (0.808–4.291)		
	Additive	G	1.230 (0.602–2.516)	0.570	237.752
rs3758391	Codominant	C/C	1	0.153	237.368
		C/T	1.633 (0.833–3.201)	0.649	
		T/T	0.700 (0.151–3.249)		
	Dominant	C/C	1	0.254	236.755
		C/T + T/T	1.464 (0.761–2.816)		
	Recessive	C/C + C/T	1	0.450	237.405
		T/T	0.561 (0.125–2.511)		
	Overdominant	C/C + T/T	1	0.115	235.592
		C/T	1.697 (0.880–3.273)		
	Additive	T	1.162 (0.701–1.927)	0.599	237.724
rs7895833	Codominant	A/A	1	0.144	233.706
		A/G	1.705 (0.834–3.487)	1	
		G/G	–		
	Dominant	A/A	1	0.394	237.351
		A/G + G/G	1.358 (0.672–2.745)		

p-value - significance level (differences are considered significant when p < 0,05); OR – odds ratio; CI – confidence interval; AIC – Akaike information criterion;

Polymorphism	Model	Genotype	OR (95% CI)	p-value	AIC
	Recessive	AA + A/G	1	1	233.757
		G/G	–		
	Overdominant	A/A + G/G	1	0.098	233.447
		A/G	1.828 (0.895–3.735)		
	Additive	G	1.033 (0.576–1.853)	0.913	238.049

p-value - significance level (differences are considered significant when p < 0,05); OR – odds ratio; CI – confidence interval; AIC – Akaike information criterion;

SIRT1 **serum levels and** SIRT1 **rs3818292, rs3758391, rs7895833 associations with ON.**

We compared the serum levels of ON patients and the control group according to the *SIRT1* SNPs genotypes. Because of the small subject group, we formed two groups: homozygous with the more common allele and heterozygous and homozygous with the less common allele together.

First, serum SIRT1 levels were compared in all subjects. There was no statistically significant difference in SIRT1 serum levels between subjects with the A/A genotype and with the A/G and G/G genotypes for the *SIRT1* rs3818292 (IQR: 2.130 ng/ml (0.54) vs. 2.124 ng/ml (1.68), p = 0.551). Similarly, serum SIRT1 levels did not differ between SIRT1 rs3758391 C/C genotype and C/T + T/T genotypes (IQR: 2.130 ng/ml (0.54) vs. 1.124 ng/ml (1.53), p = 1). No statistically significant difference was found between SIRT1 rs7895833 polymorphism A/A genotype and A/G + G/G genotypes (IQR: 2.130 ng/ml (0.51) vs. 2.130 ng/ml (1.57), p = 0.915).

No statistically significant difference in SIRT1 serum levels was found between subjects with A/A genotype and with A/G and G/G genotypes for *SIRT1* rs3818292 (IQR: 2.174 ng/ml (1.51) vs. 1.911 ng/ml (1.85), respectively, p = 0.379). Serum SIRT1 levels did not differ between *SIRT1* rs3758391 C/C genotype and C/T and T/T genotypes (IQR: 2.130 ng/ml (1.85) vs. 2.134 ng/ml (1.77), p = 0.525). In addition, there was no statistically significant difference in SIRT1 serum levels between the *SIRT1* rs7895833 A/A genotype and the A/G + G/G genotypes (IQR: 2.130 ng/ml (1.85) vs. 2.134 ng/ml (1.77), p = 0.525.

No statistically significant differences in serum SIRT1 levels were found between subjects with A/A genotype and with A/G + G/G genotypes for the *SIRT1* rs3818292 (IQR: 2.130 ng/ml (0.58) vs. 2.130 ng/ml (1.65), respectively, p = 0.910). Serum SIRT1 levels did not differ between SIRT1 rs3758391 C/C genotype and C/T and T/T genotypes (IQR: 2.066 ng/ml (0.55) vs. 2.188 ng/ml (1.26), respectively, p = 0.472). In addition, there was no statistically significant difference in SIRT1 serum levels between the *SIRT1* rs7895833 A/A genotype and the A/G + G/G genotypes (IQR: 2.130 ng/ml (0.52) vs. 2.130 ng/ml (1.57), p = 0.424).

Discussion

As we know, optic neuritis is an inflammatory optic neuropathy often associated with multiple sclerosis (17, 18). Typically, myelin ensures that electrical impulses travel rapidly from the eye to the brain, where they are converted into visual information. Optic neuritis disrupts this process and impairs vision (19). It is well known that genetic factors may influence ON and MS pathogenesis. Therefore, our study investigated the role of SIRT1 level and *SIRT1* (rs3818292, rs3758391, rs7895833) gene polymorphisms in patients with optic neuritis and multiple sclerosis.

Currently, there is growing interest that modulation of SIRT1 activity by pharmacological induction or transgenic overexpression may be of therapeutic value in various forms of neurodegenerative diseases. These experimental models are discussed in the following discussion (5, 7, 10, 14, 15, 20–22).

SIRT1 is one of the targets of resveratrol, which has been shown to increase longevity and protect various organs from aging. Available data suggest that SIRT1 is localized in the nucleus and cytoplasm of cells that form all typical ocular structures, including the cornea, lens, iris, ciliary body, and retina, and that it may provide protection against diseases related to oxidative stress-induced ocular damage (23) and also plays a role in DNA repair, mitochondrial biogenesis, and apoptosis (6).

There are many animal studies, but there are no studies investigating the role of SIRT1 level and *SIRT1* (rs3818292, rs3758391, rs7895833) gene polymorphisms in patients with optic neuritis and multiple sclerosis. However, only one study links the *SIRT1* rs12778366 gene polymorphism to optic neuritis and multiple sclerosis, but this study found no association between this SNP and ON and MS (24). Our research found that *SIRT1* rs3758391 and rs7895833 polymorphisms are associated with increased ON and ON with MS development. However, it is known from experimental studies that activation of SIRT1 deacetylase prevents retinal ganglion cell (RGC) loss in experimental optic neuritis, an inflammatory optic neuropathy (15). Intravitreal injection of SIRT1 agonists inhibits the loss of RGCs in a dose-dependent manner by inducing SIRT1 activity in mice with optic neuritis. This neuroprotective effect is blocked by sirtinol (25).

In contrast to SIRT1 overexpression, SIRT1 inactivation in an established mouse model of multiple sclerosis increased the production of new oligodendrocyte progenitor cells in the adult mouse brain, improved remyelination, and delayed paralysis (26). Sirtuins have received considerable attention since the discovery that Silent Information Regulator 2 (Sir2) extends yeast lifespan (24). Sir2, a nicotinamide adenine dinucleotide (NAD -) dependent histone deacetylase, serves as both a transcriptional effector and an energy sensor. Oxidative stress and apoptosis are associated with the pathogenesis of neurodegenerative eye diseases. Sirtuins provide protection against oxidative stress and retinal degeneration (26).

In mammals, the SIRT family consists of seven proteins. These differ in tissue specificity, subcellular localization, enzymatic activity, and targets. A possible role of specific therapeutic targets is currently being explored (27).

Khan and colleagues investigated whether SIRT1 activators reduce oxidative stress and promote mitochondrial function in neuronal cells. Furthermore, the results suggest that SIRT1 activators may mediate neuroprotective effects during optic neuritis and have the potential to preserve neurons in other neurodegenerative diseases associated with oxidative stress (10). In another study, Guo J et al. note that patients with multiple sclerosis often accompany ON, leading to RGC death and even vision loss. Herein, experimental autoimmune encephalomyelitis was generated by immunizing female C57BL/6 mice with MOG35-55 peptide. To investigate the effect of NAD + on the prevention and treatment of ON, EAE mice received 250 mg/kg NAD + daily by intraperitoneal injection after immunization or onset of EAE. EX 527 (10 mg/kg, SIRT1 inhibitor) was injected intraperitoneally every other day to investigate the role of SIRT1 in NAD +-induced therapeutic effect on EAE. NAD + The intervention attenuated the severity of EAE in mice. NAD + The intervention decreased inflammatory infiltration and infiltration of CD3 + and CD4 + cells and decreased the number and activation of microglia and astrocytes in the optic nerve. NADThe + intervention decreased pro-inflammatory cytokine mRNA and pro-apoptotic protein expression, enhanced anti-inflammatory cytokine mRNA expression and SIRT1 signaling in the optic nerve and retina, and regulated Th1/Th17/Tregs immune response in the spleen. In addition, EX -527 abrogated the therapeutic effect of NAD + on EAE, suggesting that NAD + prevents MS -triggered ON by activating the SIRT1 pathway. This study demonstrates the potential of NAD + as a drug to prevent and treat MS-related ON (20). Another experimental study conducted by the same group of scientists affected experimental autoimmune encephalomyelitis, an experimental model for MS, and it was induced in female C57BL/6 mice by immunization with MOG35-55 peptide and treatment with pertussis toxin. The mice were injected intraperitoneally with 20 mg/kg and 40 mg/kg PT once daily for 25 days, 24 hours after immunization. They found that PT attenuated the severity of EAE and delayed the onset of EAE. Specifically, EX -527, a SIRT1 inhibitor, reversed the effect of the high dose of PT on the optic nerves and retina, suggesting that PT exerts a protective effect via activation of SIRT1 signaling. This study provides a potential candidate treatment for MS (21). Other investigators examined the potential neuroprotective effects of SRT647 and SRT501, two structurally and mechanistically distinct activators of SIRT1, an enzyme involved in cellular stress resistance and survival, in optic neuritis. They used experimental EAE, an animal model of MS, induced by immunization with proteolipid protein peptide in SJL/J mice. Optic neuritis developed in two-thirds of the eyes, with significant loss of retinal ganglion cells (RGCs) 14 days after immunization. The RGCs were retrogradely labeled with fluorogold by injection into the superior colliculi. Optic neuritis was detected by infiltration of the optic nerve with inflammatory cells. Intravitreal injection of SIRT1 activators 0, 3, 7, and 11 days after immunization significantly reduced RGC loss in a dose-dependent manner. This neuroprotective effect was blocked by sirtinol, a SIRT1 inhibitor. Treatment with either SIRT1 activator did not prevent EAE or optic nerve inflammation. A single administration of SRT501 on day 11 was sufficient to limit RGC loss and preserve axon function (14). Another experimental study showed that optic nerve crush was induced in wild-type C57BL/6 mice, in mice overexpressing SIRT1, and in mice with conditional deletion of SIRT1 in neurons. Wild-type mice were treated daily with vehicle or 250 mg/kg resveratrol, a naturally occurring polyphenol that activates SIRT1. RGC function was assessed by pupillometry and optokinetic response (OKR), and RGC survival was measured. Superoxide levels were measured to assess oxidative stress. This study showed that SIRT1 delayed the loss of RGCs after traumatic injury. The

effects are associated with decreased oxidative stress. The results suggest that SIRT1-activating drugs may play a specific role in preventing traumatic optic nerve damage and suggest a broader role for this strategy in treating various optic nerve diseases that may involve an oxidative stress component (15).

Balaiya S and others investigated the role of SIRT1 in maintaining RGC viability in an in vitro model of hypoxia. Staurosphorin-differentiated RGCs (RGC-5) received different hypoxic concentrations (100–500 μM) of cobalt chloride (CoCl2) for 24 hours. The WST-1 assay determined Hypoxia-induced cell viability. The role of SIRT1 in promoting viability was determined indirectly via sirtinol (SIRT1 inhibitor). Hypoxia-induced apoptosis was assessed by measuring stress-activated protein kinase/c-jun N-terminal kinase (SAPK/JNK) and caspase 3 activity. The researchers demonstrated that SIRT1 significantly affected the viability of RGCs. The effect of sirtinol reflects the interaction that SIRT1 has with apoptotic signaling proteins. This study demonstrated that SIRT1 is important in preventing the effects of hypoxia-induced apoptosis (27). Fonseca-Kelly Z et al. investigate the potential neuroprotective and immunomodulatory effects of resveratrol in chronic experimental autoimmune encephalomyelitis induced by immunization with myelin oligodendroglial glycoprotein peptide in C57/Bl6 mice. The effects of two different formulations of resveratrol administered orally daily were compared. Resveratrol delayed the onset of EAE compared with vehicle-treated EAE mice but did not prevent or alter the phenotype of inflammation in the spinal cord or optic nerves. Significant neuroprotective effects were observed, with higher numbers of retinal ganglion cells found in the eyes of resveratrol-treated EAE mice with optic neuritis. The results indicate that resveratrol prevents neuronal cell loss in this chronic demyelinating disease model, similar to recurrent EAE. Differences in immunosuppression compared with previous studies suggest that immunomodulatory effects may be limited and dependent on specific immunization parameters or the timing of treatment. Importantly, neuroprotective effects may occur even in the absence of immunosuppression, suggesting a potential additional benefit of resveratrol combined with anti-inflammatory therapies for MS (7).

Our study found that the *SIRT1* polymorphisms rs3758391 and rs7895833 are associated with ON and ON during MS development, in contrast to other experimental animal models showing that *SIRT1* is a potential candidate for the treatment of MS. Therefore, the investigation of these polymorphisms should be repeated in further studies to understand better their role in ON and MS and animal models.

Declarations

Ethics approval and consent to participate: Kaunas Regional Biomedical Research Ethics Committee approved the study (No. BE-2-13).

Consent for publication: All study subjects provided written informed consent in accordance with the Declaration of Helsinki. The study was conducted in the Department of Ophthalmology, Hospital of LUHS.

Data availability statement: The data used to support the findings of this study are available from the corresponding author upon request.

Conflict of interests: none of the authors has any proprietary interests or conflicts of interest related to this submission. This submission has not been previously published anywhere, and it is not simultaneously being considered for any other publication.

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