Association of SIRT1 single gene nucleotide polymorphisms and serum SIRT1 levels with laryngeal squamous cell carcinoma patient survival rate

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Received 11 May 2021
Accepted 30 September 2021

Abstract.
BACKGROUND: SIRT1 is a multifunctional protein, possibly essential in tumorigenesis pathways, which can act both as a tumor promoter and tumor suppressor depending on the oncogenes, specific to particular tumors. Pathogenesis of laryngeal cancer is multifactorial and the association of SIRT1 expression with the clinical characteristics and prognosis of LSCC has not been fully identified.

OBJECTIVES: The study aimed to evaluate associations between single gene nucleotide polymorphisms (SNPs) of SIRT1 (rs3818292, rs3758391, and rs7895833), serum SIRT1 levels, and 5-year survival rate in patients with laryngeal squamous cell carcinoma (LSCC).

METHODS: The study involved 302 patients with LSCC and 409 healthy control subjects. The genotyping of SNPs was performed using RT-PCR, and serum SIRT1 levels were determined by the ELISA method.

RESULTS: Our study found significant differences in genotype distributions of SIRT1 rs3758391 polymorphisms between the study groups. SIRT1 rs3758391 T/T genotype was associated with the increased LSCC development odds (OR = 1.960 95% CI = 1.028–3.737; p = 0.041). Carriers of SIRT1 rs3758391 T/T genotype had statistically significantly increased odds of LSCC development into advanced stages under the codominant and recessive genetic models (OR = 2.387 95% CI = 1.091–5.222; p = 0.029 and OR = 2.287 95% CI = 1.070–4.888; p = 0.033, respectively). There were no statistically significant differences in serum SIRT1 levels between the LSCC and control groups. However, LSCC patients with SIRT1 rs3818292 AG genotype demonstrated a tendency to significantly lower SIRT1 serum levels than controls (p = 0.034). No statistically significant associations between SIRT1 (rs3818292, rs3758391, and rs7895833) SNPs and the 5-year survival rate of LSCC patients were found.

CONCLUSION: The present study indicated a statistically significant association between the SIRT1 rs3758391 T/T genotype and increased LSCC development odds. LSCC patients with SIRT1 rs3818292 AG genotype showed a tendency to manifest with lower SIRT1 serum levels. No associations between SIRT1 SNPs and the 5-year survival rate of LSCC patients were detected.

Keywords: SIRT1, laryngeal cancer, five-year survival rate, SIRT serum level

1. Introduction

Laryngeal squamous cell carcinoma (LSCC) is one of the most common malignancies of the upper aerodigestive tract, with high mortality and poor prognosis for patients. In 2020, 184615 new cases of LSCC were
diagnosed, and 99840 patients died from this cancer worldwide [1]. World Health Organization has estimated that the incidence and mortality of LSCC will have increased from 52.4% to 64.5%, respectively, by 2040 [2]. The most significant increment is projected in countries with a low Human Development Index [2]. This cancer occurs much more frequently in men than in women. According to The Global Cancer Observatory, the age-standardized incidence rate of laryngeal cancer is 3.6/100,000 people among males and 0.49/1,00,000 people among females per year [1]. This can be explained by the fact that men are more likely to have harmful habits. Laryngeal cancer is a multifactorial disease; however, it is generally accepted that tobacco and high-intensity alcohol consumption are the main risk factors for LSCC [3,4,6,7]. Epidemiological studies have consistently linked pro-inflammatory dietary intake, human papillomavirus infection, environmental and occupational exposures with increased risk of LSCC [8–14]. A rising focus on identifying genetic and epigenetic factors to develop new diagnostic tools and treatment for laryngeal cancer can be seen [15]. Several studies have shown an essential role of gene variants, especially in combination with toxins, such as alcohol and cigarette smoke, in the etiopathology and the overall risk/survival rate of LSCC [15].

Despite the rather evident symptoms (hoarseness, dysphonia, dyspnea, and swallowing dysfunction) that may onset quite early in patients with laryngeal malignancies, in many cases, cancer already is at the advanced stage (III or IV) of the disease when diagnosed. Unfortunately, modern treatment strategies do not achieve improvement in therapeutic outcomes and survival [16,17]. Therefore, to implement effective strategies for the prevention and early diagnosis, LSCC needs to be determined from epidemiological and clinical data and modern diagnostic methods based on genetic discoveries.

A silent information regulator two homolog 1 (SIRT1) is a nicotinamide adenine dinucleotide (NAD+)-dependent enzyme which plays a critical role in a wide variety of cellular processes, such as genomic stability, glucose, and lipid metabolism, gene transcription, oxidative stress, and aging process via autophagy [18–26]. Dysregulation of SIRT1 is involved in the pathogenesis of diabetes, neurodegenerations, depression, anorexia nervosa, and cardiovascular diseases and has been proposed as a therapeutic target [18,19,21,25,27]. Moreover, SIRT1 is a multifunctional protein, possibly essential in tumorigenesis pathways, which can act both as a tumor promoter and tumor suppressor depending on the oncogenes specific to particular tumors [28]. Meta-analyses carried out by Wang et al. in 2017 and Sun et al. in 2019 revealed that overexpression of SIRT1 indicates a poor prognosis for patients with various cancers [29,30]. Ye et al. in their study, have demonstrated the inhibition of proliferation, invasion, and migration of lung cancer through downregulation of SIRT1 expression and activation of cell autophagy [31]. Several studies have established an association between SIRT1 expression and a reasonable prognosis of head and neck tumors [32,33]. The pathogenesis of laryngeal cancer is multifactorial, and the association of SIRT1 expression with the clinical characteristics and prognosis of LSCC has not been fully identified.

Genetic variants may cause different SIRT1 expressions, and it could play an essential role in laryngeal carcinogenesis. However, just one study has investigated the involvement of SIRT1 single nucleotide polymorphism (SNP) in laryngeal cancer and has not found any associations between LSCC and SIRT1 rs12778366 gene polymorphism [34].

The study aimed to evaluate associations between SNPs of SIRT1 (rs3818292, rs3758391, and rs7895833), serum SIRT1 levels, and the 5-year survival rate of patients with LSCC.

2. Materials and methods

2.1. Ethics statement

This case-control study was conducted at the Department of Otorhinolaryngology, Lithuanian University of Health Sciences (LUHS), Kaunas, Lithuania. The research protocol was approved by the Kaunas Regional Ethics Committee for Biomedical Research (authorization number BE-2-37). All procedures performed in the study followed the institution’s ethical standards, the Helsinki declaration, and its later amendments or similar ethical standards. Informed consent was obtained from all individual participants included in the study.

2.2. Study protocol/design

Study population. A total study group consisted of 711 subjects: 302 patients with LSCC and 409 healthy individuals as a control group. The LSCC patient group included 292 (96.7%) males and 10 (3.3%) females. The control group included 393 (96.1%) males and 16 (3.9%) females. The LSCC patient and control groups...
Table 1
Demographic characteristics of the study

| Characteristic                  | LSCC \(n = 302\) | Control group \(n = 409\) | \(p\) value |
|--------------------------------|------------------|---------------------------|-------------|
| Male, \(n (%)\)                | 292 (96.7)       | 393 (96.1)                | 0.673*      |
| Female \(n (%)\)               | 10 (3.3)         | 16 (3.9)                  |             |
| Age years; mean (SD)           | 63.2 (8.1)       | 63.3 (11.6)               | 0.146**     |
| Smoking \(***\), \(n\)         | < 0.001          |                           |             |
| Yes                            | 124 (41.1)       | 33 (8.1)                  |             |
| No                             | 4 (1.3)          | 156 (38.1)                |             |
| Alcohol consumption\(***\), \(n\) | < 0.001          |                           |             |
| Yes                            | 101 (33.4)       | 102 (24.9)                |             |
| No                             | 27 (8.9)         | 87 (21.3)                 |             |
| Stage, \(n (%)\)              |                  |                           |             |
| I                              | 110 (36.4)       |                           |             |
| II                             | 66 (21.9)        |                           |             |
| III                            | 53 (17.5)        |                           |             |
| IV                             | 73 (24.2)        |                           |             |
| T, \(n (%)\)                  |                  |                           |             |
| 1                              | 113 (37.4)       |                           |             |
| 2                              | 63 (20.9)        |                           |             |
| 3                              | 59 (19.5)        |                           |             |
| 4                              | 67 (22.2)        |                           |             |
| N, \(n (%)\)                  |                  |                           |             |
| 0                              | 252 (83.4)       |                           |             |
| 1                              | 17 (5.6)         |                           |             |
| 2                              | 33 (10.9)        |                           |             |
| M, \(n (%)\)                  |                  |                           |             |
| 0                              | 299 (99.0)       |                           |             |
| 1                              | 2 (0.7)          |                           |             |
| 2                              | 1 (0.3)          |                           |             |
| G, \(n (%)\)                  |                  |                           |             |
| 0                              | 1 (0.3)          |                           |             |
| 1                              | 89 (29.5)        |                           |             |
| 2                              | 187 (61.9)       |                           |             |
| 3                              | 25 (8.3)         |                           |             |

*Pearson Chi-Square, **Student’s \(t\) test SD – standard deviation, T – tumor size, M – metastasis, N – metastasis to the neck lymph nodes, G – tumor differentiation grade, \(***\)Data about smoking and alcohol consumption were collected from 128 LSCC patients and 189 control group subjects.

were adjusted by sex and age. Demographic characteristics of the study groups are presented in Table 1.

**LSCC group.** A detailed otolaryngological examination, including flexible endoscopy and/or video laryngostroboscopy, and neck palpation was performed for all LSCC patients at the Out-patient Office in the Department of Otolaryngology, LUHS. Peripheral venous blood samples were collected before preparation for general anesthesia and direct microlaryngoscopy with biopsy. The diagnosis of LSCC was histologically confirmed at the Department of Pathology, LUHS. The final diagnosis of LSCC was based on clinical data and the results of histological examination and laryngeal and neck CT or MRI data. The staging of LSCC was accomplished as described by the American Joint Committee on Cancer [35].

**Healthy controls.** Subjects who were consulted at the Out-patient Office of the Department of Otorhinolaryngology, LUHS, and selected for surgical treatment (tympanoplasty, ossiculoplasty, tympanostomy, nasal bones reposition, septoplasty, rhinoseptoplasty, uvulopalatopharyngoplasty, or radiofrequency thermoablation of soft palate) for disorders not associated with the development of LSCC or other cancerogenic disease were enrolled in the present study as a healthy control group. The peripheral venous blood samples from these patients were collected from the same catheter that was used to induce general anesthesia. Individuals with no diagnosed oncological diseases who attended the family doctor’s consultation for general health check-ups and had a peripheral blood sample collection were also included in this study as healthy controls. All patients with the diagnosed another type and localization of cancer, acute or chronic infectious disease, individuals using psychomotor suppressants and anti-epileptic drugs, and persons younger than 18 years old were excluded from this study.

**Survival rate.** Data on the LSCC group mortality rate, including the survival period after diagnosis of LSCC and the cause of death, were collected from the Lithuanian State Register of Death Cases and Their Causes.

2.3. **DNA extraction, genotyping, and enzyme-linked immunosorbent assay**

We extracted DNA samples from peripheral venous blood using the DNA salting-out method. The genotyping of all three SNPs was performed using TaqMan® Genotyping assays (Applied Biosystems Foster City, CA, USA): SIRT1 (rs3818292, rs3758391, and rs7895833) according to manufacturer’s instructions using the real-time polymerase chain reaction (PCR) method. SIRT1 serum levels were assessed in 15 control subjects and 24 LSCC patients. The SIRT1 level in serum of LSCC patients was determined using the commercial enzyme-linked immunoassay (ELISA) kit for human SIRT1 (Human SIRT1 ELISA Kit, Abcam, Cambridge, United Kingdom), according to the manufacturer’s instructions, and the optical density was immediately measured at 450 nm wavelength using a microplate reader (Multiskan FC microplate photometer, Thermo Scientific, Waltham, MA)). The SIRT1 level was calculated according to the standard curve; standard curve sensibility range: 0.63–40 ng/mL, sensitivity 132 pg/mL.
2.4. Quality control of genotyping

The repetitive analysis of 5% randomly chosen samples was performed for all SNPs to confirm the same rate of genotypes from initial and repetitive genotyping.

2.5. Statistical analysis

Statistical analysis was performed applying the SPSS/W 20.0 software (Statistical Package for the Social Sciences for Windows, Inc., Chicago, Illinois, USA).

The data of subjects’ age were presented as the mean with standard deviation (SD) and median with interquartile range (IQR). The Student t-test was performed to compare the average age of the study groups and the Mann Whitney U test was used to compare the serum SIRT1 levels between study groups. We used the sample size formula to calculate the minimum number in each group of serum SIRT1 levels. Estimation of sample size power for comparing two means was used to calculate the study power setting. Hardy-Weinberg equilibrium analysis was performed to compare the observed and expected frequencies of SIRT1 rs3818292, rs3758391, and rs7895833. The frequencies of genotypes and alleles, gender, age, distribution of LSCC stage, tumor size, neck lymph nodes, metastasis, and tumor differentiation grade are described using absolute numbers with percentages. The distributions of the genotypes and alleles of SIRT1 rs3818292, rs3758391, and rs7895833 in the LSCC and control groups were compared using the $\chi^2$ test. Binomial logistic regression analysis was performed to estimate the impact of the genotypes on LSCC development. Our results revealed that SIRT1 rs3758391T/T genotype carriers had statistically significant increased LSCC development odds under the codominant model (OR = 1.960 95% CI = 1.028–3.737; $p = 0.041$) (Table 4).

We analyzed the distributions of frequencies of the following genotypes and alleles of SIRT1 rs3818292, rs3758391, and rs7895833 in the LSCC and control groups. There was no statistically significant difference between these two groups (Table 3). Binomial logistic regression was performed to evaluate the impact of SIRT1 rs3818292, rs3758391, and rs7895833 on LSCC development. Our results revealed that SIRT1 rs3758391T/T genotype carriers had statistically significantly increased LSCC development odds under the codominant model (OR = 1.960 95% CI = 1.028–3.737; $p = 0.041$) (Table 4).

We analyzed the distributions of SIRT1 rs3818292, rs3758391, and rs7895833 genotype and allele frequencies between the control and patient with LSCC (in early- and advanced-stages) groups. The patient group matched the Hardy-Weinberg equilibrium (HWE) ($p > 0.001$) (Table 2).

### Table 2

| SNP          | Allele frequencies | Genotype distribution | $p$ value |
|--------------|------------------|-----------------------|-----------|
| SIRT1 rs3818292 | A/A | 255 (84.4) | 357 (87.3) | 0.133 |
|              | A/G | 47 (15.6)  | 49 (12.0)  |         |
|              | G/G  | 0 (0)   | 3 (7.0)   |          |
|              | Total | 302 (100) | 409 (100) |          |
| Allele       | A | 557 (92.2) | 763 (93.3) | 0.445 |
|              | G | 47 (7.8) | 55 (6.7) |          |

### Table 3

| Polymorphism | LSCC n (%) | Control group n (%) | $p$ value |
|--------------|------------|---------------------|-----------|
| rs3818292    | A/A | 255 (84.4) | 357 (87.3) | 0.133 |
|              | A/G | 47 (15.6)  | 49 (12.0)  |         |
|              | G/G  | 0 (0)   | 3 (7.0)   |          |
|              | Total | 302 (100) | 409 (100) |          |
| Allele       | A | 557 (92.2) | 763 (93.3) | 0.445 |
|              | G | 47 (7.8) | 55 (6.7) |          |

### Table 4

| SNP          | Allele frequencies | Genotype distribution | $p$ value |
|--------------|------------------|-----------------------|-----------|
| SIRT1 rs3758391 | C/C | 151 (50.0) | 222 (54.3) | 0.115 |
|              | C/T | 127 (42.1) | 169 (41.3) |         |
|              | T/T  | 24 (7.9) | 18 (4.4) |          |
|              | Total | 302 (100) | 409 (100) |          |
| Allele       | C | 429 (71.0) | 613 (75.3) | 0.099 |
|              | T | 175 (28.0) | 205 (24.7) |         |

### Results

The distributions of the analyzed SNPs (SIRT1 rs3818292, rs3758391, and rs7895833) in the control group matched the Hardy-Weinberg equilibrium (HWE) ($p > 0.001$) (Table 2).

We analyzed the distributions of frequencies of the following genotypes and alleles of SIRT1 rs3818292, rs3758391, and rs7895833 in the LSCC and control groups. There was no statistically significant difference between these two groups (Table 3). Binomial logistic regression was performed to evaluate the impact of SIRT1 rs3818292, rs3758391, and rs7895833 on LSCC development. Our results revealed that SIRT1 rs3758391T/T genotype carriers had statistically significantly increased LSCC development odds under the codominant model (OR = 1.960 95% CI = 1.028–3.737; $p = 0.041$) (Table 4).

We analyzed the distributions of SIRT1 rs3818292, rs3758391, and rs7895833 genotype and allele frequencies between the control and patient with LSCC (in early- and advanced-stages) groups. The patient group matched the Hardy-Weinberg equilibrium (HWE) ($p > 0.001$) (Table 2).

We analyzed the distributions of frequencies of the following genotypes and alleles of SIRT1 rs3818292, rs3758391, and rs7895833 in the LSCC and control groups. There was no statistically significant difference between these two groups (Table 3). Binomial logistic regression was performed to evaluate the impact of SIRT1 rs3818292, rs3758391, and rs7895833 on LSCC development. Our results revealed that SIRT1 rs3758391T/T genotype carriers had statistically significantly increased LSCC development odds under the codominant model (OR = 1.960 95% CI = 1.028–3.737; $p = 0.041$) (Table 4).
revealed that the SIRT1 group and control groups (A/A, A/G, and G/G) distributions between the T4 subgroup and control groups (p = 0.030). Further analysis revealed that the SIRT1 rs7895833 G/G genotype was more frequent in the T4 subgroup than in controls (6.0% vs. 1.2%, p = 0.008) (Table 7). The binomial logistic regression revealed that SIRT1 rs3818292 genotype A/G and rs7895833 genotype A/G carriers had a statistically significantly increased odds of LSCC development into T3 under the codominant and recessive genetic models (OR = 2.059 95% CI = 1.039–4.081, p = 0.039 and OR = 2.076 95% CI = (1.048–4.115), p = 0.036; OR = 1.796 95% CI = (1.018–3.170), p = 0.043 and OR = 1.826 95% CI = (1.035–3.223), p = 0.038, respectively) (Table 8). The SIRT1 rs3758391T/T genotype and SIRT1 rs7895833 G/G genotype increased odds of LSCC development into T4 under codominant and recessive genetic models (OR = 3.083 95% CI = 1.239–7.672, p = 0.015 and OR = 2.945 95% CI = (1.226–7.077), p = 0.016; OR = 5.183 95% CI = (1.342–20.010), p = 0.017 and OR = 5.130 95% CI = (1.342–19.618), p = 0.017 respectively) (Table 9).

We also performed a comparative analysis of the distribution of the SIRT1 rs3818292, rs3758391, and rs7895833 genotypes and alleles in the LSCC patient subgroups with metastasis to the neck lymph nodes and without metastases to the neck lymph nodes, and the control group. We found that the rs3758391 TT genotype was statistically significantly more frequent in patients without metastases to the neck lymph nodes than in healthy controls (8.3% vs. 4.4%, p = 0.037) (Table 10). However, these differences can be consid-
Frequencies of genotypes and alleles of SIRT1 rs3818292, rs3758391, and rs7895833 in the control group and early and advanced stage LSCC subgroups

| Genotype/allele | Control group % n = 409 | Early-stage (I + II) % n = 176 | p value | Advanced stage (III + IV) % n = 126 | p value | p value* |
|----------------|--------------------------|---------------------------------|---------|-----------------------------------|---------|---------|
| rs3818292      |                          |                                 |         |                                   |         |         |
| A/A            | 357 (87.3)               | 152 (86.4)                      | 0.455   | 103 (81.7)                        | 0.130   | 0.275   |
| A/G            | 49 (12.0)                | 24 (13.6)                       |         | 23 (18.3)                         |         |         |
| G/G            | 3 (0.7)                  | 0 (0.0)                         |         | 0 (0.0)                           |         |         |
| Total          | 409 (100)                | 176 (100)                       |         | 126 (100)                         |         |         |
| Allele         |                          |                                 |         |                                   |         |         |
| A              | 763 (93.3)               | 328 (93.2)                      | 0.953   | 229 (90.9)                        | 0.199   | 0.296   |
| G              | 55 (6.7)                 | 24 (8.6)                        |         | 23 (9.1)                          |         |         |
| rs3758391      |                          |                                 |         |                                   |         |         |
| C/C            | 222 (54.3)               | 89 (50.6)                       | 0.413   | 62 (49.2)                         | 0.083   | 0.692   |
| C/T            | 169 (41.3)               | 75 (42.6)                       |         | 52 (41.3)                         |         |         |
| T/T            | 18 (4.4)                 | 12 (6.8)                        |         | 12 (9.5)                          |         |         |
| Total          | 409 (100)                | 176 (100)                       |         | 126 (100)                         |         |         |
| Allele         |                          |                                 |         |                                   |         |         |
| C              | 613 (74.9)               | 253 (71.9)                      | 0.273   | 176 (69.8)                        | 0.108   | 0.587   |
| T              | 205 (25.1)               | 99 (28.1)                       |         | 76 (30.2)                         |         |         |
| rs7895833      |                          |                                 |         |                                   |         |         |
| A/A            | 298 (72.9)               | 132 (75.0)                      | 0.706   | 83 (65.9)                         | 0.154   | 0.081   |
| A/G            | 106 (25.9)               | 43 (24.4)                       |         | 39 (31.0)                         |         |         |
| G/G            | 5 (1.2)                  | 1 (0.6)                         |         | 4 (3.2)                           |         |         |
| Total          | 409 (100)                | 176 (100)                       |         | 126 (100)                         |         |         |
| Allele         |                          |                                 |         |                                   |         |         |
| A              | 702 (85.8)               | 307 (87.2)                      | 0.525   | 205 (81.3)                        | 0.084   | 0.048   |
| G              | 116 (14.2)               | 45 (12.8)                       |         | 47 (18.7)                         |         |         |

*p value*: Early stage vs. advanced stage; 1SIRT1 rs3758391 TT genotype was statistically significantly more frequent in the advanced LSCC stage subgroup than the control group, p = 0.029.

| Model          | Genotype/allele | OR (95% CI) | p value | AIC    |
|----------------|-----------------|-------------|---------|--------|
| SIRT1 rs3758391| Codominant      | C/T vs. C/C | 1.102 (0.724–1.676) | 0.651 | 583.597 |
|                |                 | T/T vs. C/C | 2.387 (1.091–5.222) | 0.029 |         |
|                | Dominant        | C/T + T/T vs. C/C | 1.225 (0.822–1.828) | 0.319 | 585.070 |
|                | Recessive       | T/T vs. C/C + C/T | 2.287 (1.070–4.888) | 0.033 | 581.802 |
|                | Overdominant    | C/T vs. C/C + T/T | 0.998 (0.665–1.497) | 0.992 | 586.063 |
|                | Additive        | T            | 1.317 (0.951–1.823) | 0.097 | 583.341 |

OR: odds ratio; CI: confidence interval; p value: significance level (alpha = 0.05); AIC: Akaike Information Criterion.

The LSCC patient group was divided into three subgroups according to the tumor differentiation grade G1, G2, and G3. Analysis of SIRT1 rs3818292, rs3758391, and rs7895833 genotypes and allele frequencies between the control group and patient subgroups was performed. The SIRT1 rs3758391 TT genotype, as well as T allele, were more frequent in the G1 subgroup than in controls (10.0% vs. 4.4%, p = 0.034; 32.2% vs. 25.1%, p = 0.048, respectively). In the overall group, rs3758391 genotype (C/C, C/T, and T/T) distributions between the G3 subgroup and controls were statistically significantly different (p = 0.003), and the further analysis showed that SIRT1 rs3758391 TT genotype was more frequent in the G3 subgroup than in controls and the G2 subgroup (20.0% vs. 4.4%, p < 0.001; 20.0% vs. 5.3%, p < 0.001, respectively) (Table 11). Moreover, we found statistically significant differences in SIRT1 rs7895833 genotype (A/A, A/G, and G/G) distributions between the G3 subgroup and the control group (p < 0.001). Further analysis revealed that the SIRT1 rs7895833 G/G genotype was more frequent in the G3 subgroup than in controls, and the G1 and G2 subgroups (12.0% vs. 1.2%, p < 0.001; 12.0% vs. 1.1%, p = 0.009 and 12.0% vs. 0.5%, p < 0.001, re-
Table 7
Frequencies of genotypes and alleles of SIRT1 rs3818292, rs3758391, and rs7895833 in the healthy control group and LSCC patients with different tumor size

| Genotype/allele | Control group % | T1 % | p value | T2 % | p value | T3 % | p value | T4 % | p value |
|----------------|----------------|------|---------|------|---------|------|---------|------|---------|
| rs3818292      |                |      |         |      |         |      |         |      |         |
| A/A            | 357 (87.3)     | 95 (84.1) | 0.366 | 57 (90.5) | 0.668 | 46 (78.0) | 0.087 | 57 (85.1) | 0.629 |
| A/G            | 49 (12.0)      | 18 (15.9) | 6 (9.5) | 13 (22.0) | 10 (14.9) |      |        |      |         |
| G/G            | 3 (0.7)        | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |      |        |      |         |
| Total          | 409 (100)      | 113 (100) | 63 (100) | 59 (100) | 67 (100) |      |        |      |         |

| Allele         |                |      |         |      |         |      |         |      |         |
|----------------|----------------|------|---------|------|---------|------|---------|------|---------|
| A              | 763 (93.3)     | 208 (92.0) | 0.517 | 120 (95.2) | 0.404 | 105 (89.0) | 0.093 | 124 (92.5) | 0.753 |
| G              | 55 (6.7)       | 18 (8.0) | 6 (4.8) | 13 (11.0) | 10 (7.5) |      |        |      |         |

| rs3758391      |                |      |         |      |         |      |         |      |         |
| C/C            | 222 (54.3)     | 54 (47.8) | 0.217 | 35 (55.6) | 0.967 | 30 (50.8) | 0.688 | 32 (47.8) | 0.039 |
| C/T            | 169 (41.3)     | 50 (44.2) | 25 (39.7) | 25 (42.4) | 27 (40.3) |      |        |      |         |
| T/T            | 18 (4.4)       | 9 (8.0) | 3 (4.8) | 4 (6.8) | 8 (11.9) |      |        |      |         |
| Total          | 409 (100)      | 113 (100) | 63 (100) | 59 (100) | 67 (100) |      |        |      |         |

| Allele         |                |      |         |      |         |      |         |      |         |
|----------------|----------------|------|---------|------|---------|------|---------|------|---------|
| C              | 613 (74.9)     | 158 (69.9) | 0.128 | 95 (75.4) | 0.912 | 85 (72.0) | 0.459 | 91 (67.9) | 0.086 |
| T              | 205 (25.1)     | 68 (30.1) | 31 (24.6) | 33 (28.0) | 43 (32.1) |      |        |      |         |

| rs7895833      |                |      |         |      |         |      |         |      |         |
| A/A            | 298 (72.9)     | 84 (74.3) | 0.494 | 49 (77.8) | 0.657 | 36 (61.0) | 0.085 | 46 (68.7) | 0.030 |
| A/G            | 106 (25.9)     | 29 (25.7) | 13 (20.6) | 23 (39.0) | 17 (25.4) |      |        |      |         |
| G/G            | 5 (1.2)²       | 0 (0.0) | 0 (0.0) | 0 (0.0) | 4 (6.0)² |      |        |      |         |
| Total          | 409 (100)      | 113 (100) | 188 (100) | 59 (100) | 67 (100) |      |        |      |         |

| Allele         |                |      |         |      |         |      |         |      |         |
|----------------|----------------|------|---------|------|---------|------|---------|------|---------|
| A              | 702 (85.8)     | 197 (87.2) | 0.604 | 111 (88.1) | 0.491 | 95 (80.5) | 0.129 | 109 (81.3) | 0.176 |
| G              | 116 (14.2)     | 29 (12.8) | 15 (11.9) | 21 (19.5) | 25 (18.7) |      |        |      |         |

OR: odds ratio; CI: confidence interval; p value: significance level (alpha = 0.05); AIC: Akaike information criterion.

T – tumor size; ¹SIRT1 rs3758391 T/T genotype was statistically significantly more frequent in T4 patients’ subgroup than in the control group (11.9% vs. 4.4%, p = 0.012); ²SIRT1 rs7895833 G/G genotype was statistically significantly more frequent in T4 patients’ subgroup than in the control group (6.0% vs. 1.2%, p = 0.008).

Table 8
Binomial logistic regression analysis of SIRT1 rs3818292, rs7895833 in healthy control and patients with LSCC T3 groups

| Model          | Genotype/allele | OR (95% CI) | P     | AIC  |
|----------------|-----------------|-------------|-------|-----|
| SIRT1 rs3818292| Codominant      | A/G vs. A/A | 2.059 (1.039–4.081) | 0.039 | 353.882 |
|                |                  | G/G vs. A/A | 0.000 (0.000–0.000) | 0.999 |       |
|                | Dominant         | A/G vs. A/A | 0.000 (0.000–0.000) | 0.999 |       |
|                |                  | G/G vs. A/A | 1.940 (0.982–3.833) | 0.056 | 353.257 |
|                | Recessive        | G/G vs. A/A | 0.000 (0.000–0.000) | 0.999 | 355.787 |
|                |                  | A/G vs. A/A | 2.076 (1.048–4.115) | 0.036 | 352.606 |
|                | Overdominant     | G/G vs. A/A | 1.703 (0.903–3.214) | 0.100 | 354.123 |
|                | Additive         | G            | 1.531 (0.902–2.597) | 0.114 | 354.208 |
| SIRT1 rs7895833| Codominant      | A/G vs. A/A | 2.059 (1.039–4.081) | 0.039 | 353.311 |
|                |                  | G/G vs. A/A | 0.000 (0.000–0.000) | 0.999 |       |
|                | Dominant         | A/G vs. A/A | 0.000 (0.000–0.000) | 0.999 |       |
|                |                  | G/G vs. A/A | 1.706 (0.973–3.023) | 0.056 | 353.234 |
|                | Recessive        | G/G vs. A/A | 0.000 (0.000–0.000) | 0.999 | 355.243 |
|                |                  | A/G vs. A/A | 1.826 (1.035–3.223) | 0.038 | 352.442 |
|                | Overdominant     | G/G vs. A/A | 1.531 (0.902–2.597) | 0.114 | 354.208 |

OR: odds ratio; CI: confidence interval; p value: significance level (alpha = 0.05); AIC: Akaike information criterion.

Spectively) (Table 11). Also, the G allele at rs7895833 was statistically significantly more frequent in G3 subgroup patients than in controls (26% vs. 14.2%, p = 0.023) (Table 11). The binomial logistic regression was then applied to evaluate the impact of SIRT1 rs3818292, rs7895833, and rs3758391 on the development of LSCC with different tumor differentiation grades. No statistically significant impact was found.

SIRT1 serum levels were measured in 24 samples from the control group and 29 from the LSCC group. We did not find any statistically significant differences in SIRT1 serum levels between the LSCC and control group.
Table 9

Binomial logistic regression analysis of SIRT1 rs3758391, rs7895833 in healthy control and patients with LSCC T4 groups

| Model               | Genotype/allele | OR (95% CI)      | P      | AIC    |
|---------------------|-----------------|------------------|--------|--------|
| SIRT1 rs3758391     |                 |                  |        |        |
| Codominant          | C/T vs. C/C     | 1.108 (0.640–1.921) | 0.714  | 385.607|
|                     | T/T vs. C/C     | 3.083 (1.239–7.672) | 0.015  | 357.850|
| Dominant            | C/T + T/T vs. C/C | 1.298 (0.774–2.178) | 0.322  | 383.741|
| Recessive           | T/T vs. C/C + C/T | 2.945 (1.226–7.077) | 0.016  | 388.805|
| Overdominant        | C/T vs. C/C + T/T | 0.959 (0.5661.623) | 0.875  | 385.760|
| Additive            | T               | 1.454 (0.961–2.201) | 0.077  | 387.051|
| SIRT1 rs7895833     |                 |                  |        |        |
| Codominant          | A/G vs. A/A     | 1.039 (0.571–1.891) | 0.90   | 385.843|
|                     | G/G vs. A/A     | 5.183 (1.342–20.10) | 0.017  | 383.333|
| Dominant            | A/G + G/G vs. A/A | 1.226 (0.700–2.146) | 0.477  | 383.859|
| Recessive           | G/G vs. A/A + A/G | 5.130 (1.342–19.61) | 0.017  | 388.281|
| Overdominant        | A/G vs. A/A + G/G | 0.972 (0.537–1.759) | 0.925  | 388.821|
| Additive            | G               | 1.401 (0.862–2.277) | 0.173  | 387.051|

OR: odds ratio; CI: confidence interval; p value: significance level (alpha = 0.05); AIC: Akaike information criterion.

Table 10

Frequencies of genotypes and alleles of SIRT1 rs3818292, rs3758391, and rs7895833 in healthy controls and LSCC patients with and without metastases to the neck lymph nodes

| Genotype/allele | Control group % | Not spread to lymph nodes % | p value | Spread to lymph nodes % | p value | p value* |
|-----------------|-----------------|-----------------------------|---------|-------------------------|---------|---------|
|                 | n = 409         | n = 252                     |         | n = 50                  |         |         |
| rs3818292       |                 |                             |         |                         |         |         |
| A/A             | 357 (87.3)      | 210 (83.3)                  | 0.099   | 45 (90)                 | 0.759   | 0.235   |
| A/G             | 49 (12.0)       | 42 (16.7)                   |         | 5 (10.0)                | 0.017   | 0.017   |
| G/G             | 3 (0.7)         | 0 (0.0)                     |         | 0 (0.0)                 |         |         |
| Total           | 409 (100)       | 252 (100)                   |         | 50 (100)                |         |         |
| Allele          |                 |                             |         |                         |         |         |
| A               | 763 (93.3)      | 462 (91.7)                  | 0.276   | 95 (95.0)               | 0.510   | 0.256   |
| G               | 55 (6.7)        | 42 (8.3)                    |         | 5 (5.0)                 |         |         |
| rs3758391       |                 |                             |         |                         |         |         |
| C/C             | 222 (54.3)      | 124 (49.2)                  | 0.086   | 27 (54.0)               | 0.874   | 0.764   |
| C/T             | 169 (41.3)      | 107 (42.5)                  |         | 20 (40.0)               |         |         |
| T/T             | 18 (4.4)        | 21 (8.3)                    |         | 3 (6.0)                 |         |         |
| Total           | 409 (100)       | 252 (100)                   |         | 50 (100)                |         |         |
| Allele          |                 |                             |         |                         |         |         |
| C               | 613 (74.9)      | 355 (70.4)                  | 0.073   | 74 (74.0)               | 0.838   | 0.473   |
| T               | 205 (25.1)      | 149 (29.6)                  |         | 26 (26.0)               |         |         |
| rs7895833       |                 |                             |         |                         |         |         |
| A/A             | 298 (72.9)      | 178 (70.6)                  | 0.817   | 37 (74.0)               | 0.282   | 0.267   |
| A/G             | 106 (25.9)      | 71 (28.2)                   |         | 11 (22.0)               |         |         |
| G/G             | 5 (1.2)         | 3 (1.2)                     |         | 2 (4.0)                 |         |         |
| Total           | 409 (100)       | 252 (100)                   |         | 57 (100)                |         |         |
| Allele          |                 |                             |         |                         |         |         |
| A               | 702 (85.8)      | 427 (84.7)                  | 0.583   | 85 (85.0)               | 0.825   | 0.944   |
| G               | 116 (14.2)      | 77 (15.3)                   |         | 15 (15.0)               |         |         |

*Metastases to the neck lymph nodes vs. no metastases; \(^\text{1}\) SIRT1 rs3758391 TT genotype was statistically significantly more frequent in patients without metastases to the neck lymph nodes than in the control group, \(p = 0.037\).

Groups (0.395 (0.714) ng/ml vs. 0.417 (0.596) ng/ml, \(p = 0.957\)) (Fig. 1). Also, we performed a comparison of the genotype distribution and serum levels of SIRT1 between the control and LSCC groups. LSCC patients with SIRT1 rs3818292 AG genotype had statistically significantly lower SIRT1 serum levels than controls (\(p = 0.034\)) (Table 12). However, the power setting of this study sample was rather low and reached 37%.

Finally, the 5-year survival rate of LSCC patients was determined. The 5-year overall survival rate of LSCC patients was 62.7% and the LSCC-specific survival rate of five years was 72.6%.
Table 11

| Genotype/allele | Control group % n = 409 | G1 % n = 90 | p value | G2 % n = 188 | p value | G3 % n = 25 | p value |
|----------------|-------------------------|-------------|---------|--------------|---------|-------------|---------|
| rs3818292      |                         |             |         |              |         |             |         |
| A/A            | 357 (87.3)              | 73 (81.1)   | 0.161   | 162 (86.2)   | 0.417   | 21 (84.0)   | 0.769   |
| A/G            | 49 (12.0)               | 17 (18.9)   |         | 26 (13.8)    |         | 4 (16.0)    |         |
| G/G            | 3 (0.7)                 | 0 (0.0)     |         | 0 (0.0)      |         | 0 (0.0)     |         |
| Total          | 409 (100)               | 90 (100)    |         | 188 (100)    |         | 25 (100)    |         |
| Allele         |                         |             |         |              |         |             |         |
| A              | 763 (93.3)              | 163 (90.6)  | 0.201   | 350 (93.1)   | 0.903   | 46 (92.0)   | 0.728   |
| G              | 55 (6.7)                | 17 (9.4)    |         | 26 (6.9)     |         | 4 (8.0)     |         |

rs3758391

| Genotype/allele | Control group % n = 409 | G1 % n = 90 | p value | G2 % n = 188 | p value | G3 % n = 25 | p value |
|----------------|-------------------------|-------------|---------|--------------|---------|-------------|---------|
| C/C            | 222 (54.3)              | 41 (45.6)   | 0.064   | 99 (52.7)    | 0.857   | 12 (48.0)   | 0.003   |
| C/T            | 169 (41.3)              | 40 (44.4)   |         | 79 (42.0)    |         | 8 (32.0)    |         |
| T/T            | 18 (4.4)                | 9 (10.0)    |         | 10 (5.3)     |         | 5 (20.0)    |         |
| Total          | 409 (100)               | 90 (100)    |         | 188 (100)    |         | 25 (100)    |         |
| Allele         |                         |             |         |              |         |             |         |
| C              | 613 (74.9)              | 122 (67.8)  | 0.048   | 277 (73.7)   | 0.64    | 32 (64.0)   | 0.086   |
| T              | 205 (25.1)              | 58 (32.2)   |         | 99 (26.3)    |         | 18 (36.0)   |         |

rs7895833

| Genotype/allele | Control group % n = 409 | G1 % n = 90 | p value | G2 % n = 188 | p value | G3 % n = 25 | p value |
|----------------|-------------------------|-------------|---------|--------------|---------|-------------|---------|
| A/A            | 298 (72.9)              | 61 (67.8)   | 0.602   | 140 (74.5)   | 0.705   | 15 (60.0)   | < 0.001 |
| A/G            | 106 (25.9)              | 28 (31.1)   |         | 47 (25.0)    |         | 7 (28.0)    |         |
| G/G            | 5 (1.2)                 | 1 (1.1)     |         | 1 (0.5)      |         | 3 (12.0)    |         |
| Total          | 409 (100)               | 90 (100)    |         | 188 (100)    |         | 3 (100)     |         |
| Allele         |                         |             |         |              |         |             |         |
| A              | 702 (85.8)              | 150 (83.3)  | 0.393   | 327 (87.0)   | 0.493   | 37 (74.0)   | 0.023   |
| G              | 116 (14.2)              | 30 (16.7)   |         | 49 (13.0)    |         | 13 (26.0)   |         |

G-Tumor differentiation grade: 1SIRT1 rs3758391T/T genotype was statistically significantly more frequent in the G1 subgroup than in the control group, p = 0.034; 2SIRT1 rs3758391T/T genotype was statistically significantly more frequent in the G3 subgroup than in the control group, p < 0.001; 3SIRT1 rs3758391T/T genotype was statistically significantly more frequent in the G3 subgroup than in the G2 subgroup, p < 0.001; 4SIRT1 rs7895833 G/G genotype was statistically significantly more frequent in the G3 subgroup than in the control group, p = 0.009; 5SIRT1 rs7895833 G/G genotype was statistically significantly more frequent in the G3 subgroup than in the G1 subgroup, p = 0.009; 6SIRT1 rs7895833 G/G genotype was statistically significantly more frequent in the G2 subgroup, p < 0.001.

G-Tumor differentiation grade: 1SIRT1 rs3758391T/T genotype was statistically significantly more frequent in the G1 subgroup than in the control group, p = 0.034; 2SIRT1 rs3758391T/T genotype was statistically significantly more frequent in the G3 subgroup than in the control group, p < 0.001; 3SIRT1 rs3758391T/T genotype was statistically significantly more frequent in the G3 subgroup than in the G2 subgroup, p < 0.001; 4SIRT1 rs7895833 G/G genotype was statistically significantly more frequent in the G3 subgroup than in the control group, p = 0.009; 5SIRT1 rs7895833 G/G genotype was statistically significantly more frequent in the G3 subgroup than in the G1 subgroup, p = 0.009; 6SIRT1 rs7895833 G/G genotype was statistically significantly more frequent in the G2 subgroup, p < 0.001.

Table 12

| SNP/genotype | LSCC level (pg/mL) | Controls (IQR) | P-value |
|--------------|--------------------|----------------|---------|
| SIRT1 rs3818292 |                   |                |         |
| A/A          | 0.48 (0.86)       | 0.42 (0.62)   | 0.396*  |
| A/G          | 0.20 (0.30)       | 0.57 (—)      | 0.034*  |
| G/G          | —                  | —              | —       |
| SIRT1 rs3758391 |                   |                |         |
| C/C          | 0.39 (0.57)       | 0.36 (0.42)   | 0.661*  |
| C/T          | 0.56 (1.33)       | 0.65 (2.01)   | 0.622*  |
| T/T          | —                  | —              | —       |
| SIRT1 rs7895833 |                   |                |         |
| A/A          | 0.40 (0.76)       | 0.41 (0.52)   | 0.587*  |
| A/G          | 0.39 (4.92)       | 0.65 (14.18)  | 0.338*  |
| G/G          | —                  | —              | —       |

* Obtained using the Mann-Whitney U test.

4. Discussion

The SIRT1 is located on the long arm of chromosome 10 (10q21.3) [36]. Evidence from many studies has revealed that SIRT1 participates in the regulation of diverse cellular processes, including carcinogene-
In cancer development, the role of \textit{SIRT1} is dual because it can function as both a tumor promoter and a tumor suppressor [28,37–39]. The \textit{SIRT1} acts as a tumor suppressor in holding genomic stability and negatively regulates anti-apoptotic genes, resulting in increased anticancer ability [37]. On the other hand, to meet the tumor’s high-energy needs and promote its development, \textit{SIRT1} not only induces angiogenesis but also improves HIF1\(\alpha\)/HIF2\(\alpha\) (regulation of the oxygen-sensitive genes controlling angiogenesis, glycolysis, and cellular apoptosis) and suppresses p53 (interference of p53-dependent apoptosis in response to DNA damage signals) [37].

Epigenetic modifications are critical for LSCC carcinogenesis [8]. Gene variants may account for different \textit{SIRT1} expressions, determining individuals’ susceptibility to specific pathologies [19,24,26,40–45]. However, little is known about the effect of polymorphism of the \textit{SIRT1} in both LSCC carcinogenesis and disease progression. Therefore, the focus of the present study was to estimate the associations between \textit{SIRT1} SNPs (rs3818292, rs3758391, rs7895833), \textit{SIRT1} serum levels, and LSCC.

In the present study, we found that \textit{SIRT1} rs3758391 T/T genotype carriers had statistically significantly increased odds of LSCC. Moreover, the current study results revealed that patients with this genotype had 2.387 odds of LSCC development into advanced stages. To the best of our knowledge, this is the first report that associates SNP in the \textit{SIRT1} gene (rs3758391) with LSCC in a pure and homogenous LSCC patients’ cohort.

According to the literature, the \textit{SRT1} rs3758391 polymorphism has been associated with healthy aging in the Chinese population and better cognitive function in older individuals [46–48]. Also, several studies have shown the role of this \textit{SRT1} SNP in several diseases, including type 2 diabetes mellitus [49], cardiovascular diseases [50], systemic lupus erythematosus [51], and major depressive disorder [52]. Also, the accumulating evidence has suggested the importance of the \textit{SRT1} rs3758391 in cancer development [43,45,53,54]. Furthermore, Shafieian et al. found that the \textit{SRT1} rs3758391 TT genotype increased the risk of urinary bladder cancer in the Iranian population [45]. Shaker et al. study showed that carriers of the \textit{SRT1} rs3758391 CC genotype were more often in the colorectal cancer group than in the control group [54]. Rizk et al. study revealed that the \textit{SRT1} rs3758391 polymorphism (TT genotype) was associated with the increased breast cancer risk and that the \textit{SRT1} rs3758391T allele was a potential risk factor for breast cancer [43]. Kan et al. conducted a large-scale case-control study on diffuse large B cell lymphoma and compared results of patients with the healthy control group. They found that the \textit{SRT1} rs3758391T allele presented an increased risk of diffuse large B cell lymphoma. Also, they noticed \textit{SRT1} mRNA expression upregulation in diffuse large B cell lymphoma patients with TT genotype. Moreover, a worse survival rate was revealed in patients with TT genotypes. The authors concluded that the \textit{SRT1} rs3758391T might be used as a biomarker for the prognosis of susceptibility and survival of diffuse large B cell
lymphoma [53]. We did not find a significant impact of SIRT1 SNPs on LSCC patients’ 5-year survival rate in the present study. Lv et al. did not find any association between SIRT1 gene polymorphisms, including rs3758391, and lung cancer [55].

The results of the present study revealed that two SNPs of SIRT1 (rs3818292 and rs7895833) are associated with the increased odds of LSCC development into T3 and T4. No associations between the aforementioned SIRT1 SNPs and any cancer were described in the literature, so far. However, these polymorphisms of SIRT1 have been investigated mainly in the context of metabolism. They have been associated with the increased risk of visceral (rs3818292) and general (rs7895833) obesity in adults and decreased risk (a protective effect) of obesity in children (rs7895833) [56–58]. Associations of SIRT1 rs3818292 with diabetic nephropathy and non-diabetic type 1 cardiovascular syndrome have also been found [59,60]. Kilic et al., in their study, found that SIRT1 rs7895833 might affect longevity. They observed a significant increase in serum SIRT1 level in older people and carriers of SIRT1 rs7895833, suggesting an association between SIRT1 rs7895833 SNP and longevity [61].

In the present study, we measured the serum concentration of SIRT1 in patients with LSCC and compared it with the results of the control group. We did not find any statistically significant differences between these groups. However, comparing the genotype distribution and serum levels of SIRT1 between the control and LSCC groups, we found that only LSCC patients with SIRT1 rs3818292 AG genotype demonstrated a clear tendency to significantly lower SIRT1 serum levels than controls. These findings are in some contradiction with the data of the literature. For instance, Shaker et al. in their study found that patients with colorectal cancer had a significant increment in the mean level of serum SIRT1 as compared to the control group and the mean level of serum SIRT1 was higher in patients with tumor size $\geq$ 5.0 cm compared to the size < 5.0 cm [54]. Rizk et al. conducted a study on breast cancer and found significantly higher serum SIRT1 levels in breast cancer patients than in the control group. However, no significant relationship was observed between the SIRT1 levels and clinicopathological factors (types of breast cancer, tumor size, lymph node involvement, metastasis, receptor status), except the tumor differentiation grades (higher SIRT1 level in Grade 2 compared to Grade 1 patients, and in Grade 3 compared to Grade 2 patients) [43]. Thus, it can be presumed that increased SIRT1 serum levels were associated with some particu-lar and rather aggressive tumors (colorectal and breast cancers) and tumor differentiation grades. The LSCC, on the contrary, is a relatively less aggressive tumor considering rather low local spreading and low metastatic rate [62,63]. Therefore, LSCC possibly does not cause SIRT1 serum level increment. Consequently, the role of SIRT1 serum levels as an indicator of the prognosis of LSCC remains doubtful.

On the other hand, Noguchi et al. assessed the SIRT1 expression immunohistochemically using surgical samples from 437 consecutive head and neck squamous cell carcinoma (HNSCC) patients. Results of the study showed that the SIRT1 expression acted as a tumor suppressor and served as an indicator for a good prognosis [33]. Yu et al. investigated SIRT1 expression in laryngeal and hypopharyngeal squamous cell carcinoma (HSCC) tissue samples using immunohistochemical staining and its correlations with clinical parameters. They found a significant correlation between the decreased SIRT1 expression and clinical tumor stage, metastasis to the neck lymph nodes, and the patient’s survival rate. The authors concluded that SIRT1 acts as a tumor suppressor in LSCCs and HSCC [64]. However, those authors pulled their LSCC and HNSCC patients into one group; therefore, these results cannot be directly compared to the cohort of pure LSCC patients used in the present study. On the other hand, the results of the current study may suggest that in cases of LSCC, the SIRT1 serum levels remain unchanged despite the increment of SIRT1 expression directly in the tumor tissue. This phenomenon may be caused by a relatively low tumor suppressor activity of SIRT1 related to the less aggressive nature of LSCC.

The strength of the present study is a relatively large and homogenous group of pure LSCC patients (302 subjects) and age and sex-matched controls (409 subjects). This specific feature ensured a comprehensive analysis of associations between SIRT1 serum levels and selected SIRT1 SNPs and the development of a particular tumor in one anatomical region of the head and neck, i.e., LSCC.

Several limitations of the present study must be considered. The sample size for analysis of serum concentration levels of SIRT1 was limited and too small to reach the desired power setting. Therefore, the results obtained in the present study regarding the associations between the SIRT1 serum concentration levels and LSCC should be considered as the tendency. Further investigation with a large enough sample size is foreseen to confirm the possible role of serum concentration levels of SIRT1 in LSCC development. The data about
smoking and alcohol consumption was available and analyzed in a limited cohort of study participants that did not match the patient and control groups. Therefore, the impact of smoking and alcohol consumption habits was not completely investigated. However, this is the target task for future investigation.

5. Conclusion

The present study showed a statistically significant association between SIRT1 rs3758391 TT/TT genotype and increased odds of LSCC development, as well as increased LSCC development into the advanced stages and T4 odds. Also, SIRT1 rs3818292 and rs7895833 SNPs revealed increased odds of LSCC development into T3 and T4. LSCC patients with SIRT1 rs3818292 AG genotype showed a tendency to manifest with lower SIRT1 serum levels. No associations between SIRT1 (rs3818292, rs3758391, and rs7895833) SNPs and the 5-year survival rate of LSCC patients were found in the present study. Therefore, the role of SIRT1 in cancer, including LSCC, remains incompletely understood. Further research is needed to investigate possible mechanisms of the associations found, before selecting candidates for prognostic factors or a future therapeutic target.

Author contributions

Conception: R.L., V.U.
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Preparation of the manuscript: P.V., V.U.
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