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# *SIRT1* Gene Polymorphisms Are Associated with Urinary Bladder Cancer in an Iranian Population

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## Abstract

***Background:*** The present study was undertaken to evaluate the possible association between silent information regulator of transcription 1 gene (*SIRT 1*) polymorphisms and risk of urinary bladder cancer (UBC) in an Iranian population.

***Methods:*** The *SIRT1* polymorphisms rs3758391 T/C and rs369274325 G/A were evaluated in 120 Iranian bladder cancer patients and 118 healthy individuals as the control group. The *SIRT1* rs369274325 G/A and rs3758391 T/C polymorphisms were genotyped using tetra-primer ARMS PCR and PCR-RFLP methods, respectively.

***Results:*** The *SIRT1* rs3758391 TT genotype occurred significantly more frequently in the UBC patients than in the controls (13.3 vs. 1.7%) in both the additive and recessive models due to a significant difference in either of additive (TT vs. CC; OR= 9.529, P = 0.003) or recessive models (TT vs. CC + CT genotype; OR= 8.923, P = 0.002). Also, for rs369274325, the AG genotype was found in a significantly greater percentage of UBC patients than in controls (75.8 vs. 43.2%, respectively, P < 0.0001.

***Conclusions:*** Our preliminary study suggests that *SIRT1* rs3758391 T/C and rs369274325 G/A polymorphisms may confer an increased risk of bladder cancer in our patients.

**Keywords:** Gene polymorphism, *SIRT1*, Urinary Bladder Neoplasms.

## Introduction

Cancer is a complicated disease caused by interactions between environmental and genetic factors. Urinary bladder cancer (UBC), the second most common tumor of the genitourinary tract in the Iranian population, affects almost 2.7 million people worldwide, with incidence being the highest in more developed communities (1, 2). Relative and attributable risk factors for the onset of UBC include smoking, exposure to amines and polycyclic aromatic hydrocarbons in the chemical industry, contact with carcinogenic agents, industrial colors, papillomavirus infection, pelvic radiotherapy, diabetes mellitus (DM), obesity, and alcohol abuse (3-5).

Sirtuins are a class of enzymes with histone deacetylase (HDAC) activity that require nicotinamide adenine dinucleotide (NAD+) for their function (6). Sirtuins are involved in a wide range of cellular processes including aging, transcription, apoptosis, and oxidative stress induction (7). Seven types of sirtuins (SIRT 1-7) have been identified in mammals. Silent information regulator of transcription 1 (SIRT1), also known as NAD-dependent deacetylase SIRT1, is a nuclear protein that deacetylates histones H3 and H4 to directly modify chromatin, silence transcription, and modulate the meiotic checkpoint (8-10). In humans, the *SIRT1* gene contains 11 exons and spans 33.7 kb on chromosome 10 (10q21.3), displaying ubiquitous expression in adrenal, testis, and other tissues (11). Studies showed that activation of *SIRT1* may be beneficial in the prevention of aging and all types of malignancies, including breast and bladder cancers (12-14). Other studies have reported *SIRT1* up-

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regulation in prostate and colorectal cancers (15, 16). Moreover, it has been indicated that knockdown of *SIRT1* expression suppresses bladder cancer cell proliferation and migration by inducing cell cycle arrest (17). Polymorphic variants of *SIRT1* have been associated with susceptibility to myocardial infarction and severe obesity (18, 19). Two *SIRT1* polymorphisms, rs3758391 T/C and rs369274325 G/T, have been located in the *SIRT1* promoter region, and may account for differential *SIRT1* expression and susceptibility to certain disorders (20).

To our knowledge, no study has yet evaluated the impact of these two genetic variants on the risk for UBC. The aim of this study was to analyze a possible correlation between these two polymorphisms located in the *SIRT1* 5' region and susceptibility to this type of urinary-tract cancer.

## Materials and methods

### *Patients*

This case-controlled study was conducted on 120 pathologically-confirmed UBC patients referring to a Labafinejad Hospital in Tehran and 118 healthy subjects as the control group. The study protocol was approved by the local ethics committee of the Zahedan University of Medical Sciences, and permission was received from all participants with the signature of written informed consent according to the Code of Ethics of the World Medical Association Declaration of Helsinki for human experiments.

### *DNA preparation and genotyping*

Blood samples were collected from participants into EDTA-containing tubes and stored at -20 °C until DNA extraction. Genomic DNA of was extracted from peripheral blood cells by the salting-out method (21). The *SIRT1* rs369274325 G/A polymorphism was genotyped by the tetra-primer amplification refractory mutation system-polymerase chain reaction (T-ARMS-PCR) method. Two sets of primers were designed using BatchPrimer3 v1.0 software (22) and synthesized by Pishgam (Pishgam-biotec, Tehran, Iran) (Table 1). For PCR, 1 ng of template DNA, 1 µL of each primer (10 µM), 8 µL of Master Mix (Ampliqon, Denmark), and 6 µL of DNase-free water (Pishgam-biotec, Tehran, Iran) were added for a final volume of 20 µL. The cycling conditions for the *SIRT1* rs369274325 G/A polymorphism were as follows: a primary denaturation at 95 ˚C for 5 minutes, 30 cycles at 95 ˚C for 30 sec, annealing at 56 ˚C for 30 sec and at 72 ˚C for 30 sec, and a final extension at 72 ˚C for 5 min. The *SIRT1* rs3758391 T/C polymorphism was determined by PCR-RFLP using specific primers also shown in Table 1. The PCR mixture and conditions for the rs3758391 T/C polymorphism were the same as with the SIRT1 rs369274325 G/A polymorphism, except the annealing temperature was 60 °C. PCR products were electrophoresed on 2% agarose gels containing 3 µg/mL Green Viewer (Pishgam Biotech, Tehran, Iran) and DNA bands visualized using a UV transilluminator. The *SIRT1* rs369274325 G/A PCR products were 229 bp for the G allele and 152 bp for the A allele. The control product using the two outer primers was 336 bp. Digestion of the 241 bp rs3758391 C/T PCR product with HindIII resulted in fragments of 146 and 95 bp for T allele while the C allele remained undigested.

**Table 1.** Primers used to detect single-nucleotide polymorphisms in *SIRT1* rs369274325 G/A and rs3758391 C/T

| Gene polymorphism | Primers | Sequence (5′ to 3′) | Annealing Temp. |
|---|---|---|---|
| **rs369274325 G/A** | FO | TAGGTTCCATACCCCATGAAG | 56°C |
| | RO | CATTACTCTTAGCTGCTTGGTC | |
| | FI (G allele) | GAATTGTGTCATAGGTTAGGAGG | |
| | RI (A allele) | ACAGCAAAGTTTGGCATATTGAT | |
| **rs3758391 C/T** | Forward | GTCACGCAGGTAATTGATGCAG | 60°C |
| | Reverse | GGCTTAGTGGAAAGCCCTTC | |

FO: Forward outer, RO: Reverse outer, FI: Forward inner, RI: Reverse inner

### *Statistical analysis*

Statistics were analyzed using the SPSS program for Windows version 16.0 (SPSS, Chicago, IL, USA) using Student's t, $X^2$, or Fisher probability tests. Statistics of continuous variables of demographic data and genotype/allele frequencies were analyzed. Odds ratios (ORs) and 95% confidence intervals

(CIs) were calculated using binary logistic regression analysis. Adjusted ORs were stratified by age, hypertension, DM, smoking status, and alcohol consumption. A two-sided P value less than 0.05 was considered statistically significant.

## Results

The demographic characteristics of UBC patients and healthy controls were not significantly different for age, alcohol consumption, smoking, or DM status; however, the percentage of UVC patients who smoked was significantly greater than that of the healthy controls (Table 2). The *SIRT1* rs3758391 C/T and rs369274325 G/A genotypes and allele frequencies were compared between the two groups. For *SIRT1* SNP rs369274325, the A allele frequency and GA genotype were significantly greater in the UBC patients than in the control group (P = 0.002 and P < 0.0001, respectively, Table 3). The A allele was found in a significantly greater percentage of the UBC patients than in the controls (37.92 vs. 21.25%, P = 0.002). For rs369274325, the AG genotype was found in a significantly greater percentage of UBC patients than in controls (75.8 vs. 43.2%, respectively, P < 0.0001, Table 3).

Significant differences were found between the two groups regarding genotype distribution of *SIRT1* at position rs3758391 C/T in both the additive and recessive models. In both models the TT genotype was significantly frequent in the UBC patients than in the controls (13.3 vs. 1.7%, P = 0.003 and 0.002, respectively). At the allelic level, the T allele was significantly more prevalent in the UBC patients than in the controls (28.3 vs. 17%, P = 0.003, Table 4)).

**Table 2**. Clinical and biochemical data of UBC patients and healthy subjects

| | UBC (n=120) (n ± SD) | Controls (n=118) (n ± SD) | P value |
|---|---|---|---|
| **Age(year)** | 63.4±12.0 | 62.7±7.5 | 0.603 |
| **Alcohol consumption, n (%)** | 6 (5) | 5 (4.2) | 0.932 |
| **Current smoker, n (%)** | 65 (54.2) | 5 (4.2) | **<0.0001** |
| **Diabetes mellitus, n (%)** | 12 (10) | 6 (5.1) | 0.253 |

**Table 3.** Genotype and allele frequencies of *SIRT1* rs369274325 G/A gene polymorphism in UBC patients

| SNP/Alleles | Type | UBC (%) | Control (%) | *OR (95%CI)* | *P value* |
|---|---|---|---|---|---|
| **rs369274325 G/A** | GG | 29 (24.2) | 67 (56.8) | 1.00 | |
| | GA | 91 (75.8) | 51 (43.2) | 0.243 (0.139-0.422) | **<0.0001** |
| **G** | | 149(62.08) | 185(78.75) | 1.0 | |
| **A** | | 91(37.92) | 51(21.25) | 2.215(1.477-3.321) | **0.002** |

SNP, single nucleotide polymorphism; UBC, urinary bladder cancer; *OR*, odds ratio; *CI*, confidence interval. *P<0.05* was considered statistically significant.

**Table 4.** Genotype and allele frequencies of the *SIRT1* rs3758391 C/T gene polymorphism in UBC patients

| SNP/Alleles | Model | Type | UBC (%) | Control (%) | *OR (95% CI)* | *P value* |
|---|---|---|---|---|---|---|
| **rs3758391 C/T** | Additive | CC | 68 (56.7) | 81 (68.6) | 1.00 | |
| | Additive | CT | 36 (30.0) | 35 (29.7) | 1.225 (0.696-2.158) | 0.482 |
| | Additive | TT | 16 (13.3) | 2 (1.7) | 9.529 (2.116-42.918) | **0.003** |
| | Dominant | CC | 68 (56.7) | 68 (56.7) | 1.00 | |
| | Dominant | CT+TT | 52 (43.3) | 37 (31.4) | 1.674 (0.984-2.846) | 0.076 |
| | Recessive | CC+CT | 104 (86.7) | 116 (98.3) | 1.00 | |
| | Recessive | TT | 16 (13.3) | 2 (1.7) | 8.923 (2.004-39.737) | **0.002** |
| **C** | | | 172 (71.7) | 197 (83) | 1.00 | |
| **T** | | | 68 (28.3) | 39 (17) | 1.997 (1.282-3.112) | **0.003** |

SNP, single nucleotide polymorphism; UBC, urinary bladder cancer; *OR*, odds ratio; *CI*, confidence interval. *P<0.05* was considered statistically significant.

## Discussion

The genetic polymorphisms in a large number of proteins and metabolic enzymes have been reported as modulators of UBC risk (23). SIRT1, SIRT6, and SIRT7 are HDACs found primarily in the nucleus, where they participate in regulating energy metabolism, stress and inflammatory responses, DNA repair (SIRT1 and SIRT6), and rDNA transcription (SIRT7) (24). SIRT1 plays an important role in cytoplasmic processes as this protein functions as an anti-inflammatory agent by decreasing the mRNA levels of various pro-inflammatory cytokines involved in the NF-κB pathway. Accordingly, SIRT1 effectively prohibits

the expression of lectin-like oxidized low-density lipoprotein receptor-1 (Lox-1), leading to foam cell formation (25). It is also been proposed that SIRT1 could interfere with the FOXO signaling pathway to affect oxidative stress resistance, cell cycle regulation, and energy metabolism in UBC tissues (17). SIRT1 plays a substantial role in oxidative stress-triggered apoptotic cell death in mouse embryonic stem cells by down-regulating the reactive oxygen species-induced FoxO and p53 pathways (26). Considering that UBC is a complex urinary disorder triggered by a multitude of genetic and environmental factors, the main underlying mechanisms are currently ill-defined. However, the controversial findings might be the result of ethnic variations and/or different sample sizes. The conflicting observations might also be due to the fact that SIRT1 is essentially a highly-networked protein that mediates adaptation to non-acute physiological stress (27).

In this work, we found a significant association between UBC and two *SIRT1* polymorphisms: rs3758391 T/C and rs369274325 A/G. We also found that the rs3758391 T allele and the rs369274325 A allele polymorphisms are potential risk factors for UBC. We also discovered that the rs3758391 TT genotype was a risk factor for UBC in both additive and recessive models, suggesting that the TT genotype carriers are at higher risk for UBC than those with the CC genotype. This genetic variant, as well as the rs3758391 polymorphism, has been associated with aging (14) and several diseases including type 2 DM (28), systemic lupus erythematosus, and morbidity (29). In contrast to our findings indicating that patients with GA genotype might be more susceptible to UBC, Mohtavinejad et al. found no significant difference between cardiovascular disease patients and healthy subjects regarding the *SIRT1* rs369274325 genotypes (20). Shaker et al. showed that SIRT1 serum levels were higher in colorectal cancer patients than in healthy subjects, and carriers of the *SIRT1* rs3758391 C allele polymorphism were more susceptible to colorectal cancer risk than controls (30). In agreement with our findings, Rizk et al. found that the *SIRT* rs3758391 or C>T polymorphism was associated with increased breast cancer risk (13). To date, few studies have focused on the possible correlation between these two *SIRT1* variants and the risk of UBC. Examining other *SIRT1* variants, *SIRT1* mRNA levels, and conducting the present study on a larger population might be pursued in future investigations.

The *SIRT1* rs3758391 TT genotype may confer an increased risk for UBC in both additive and recessive models in this Iranian population. Also, the *SIRT1* rs369274325 A allele polymorphism is considered to be a risk factor for UBC. Additional studies on larger populations are warranted to validate our results.

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