

# *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 (*SIRT1*) 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<sup>+</sup>) 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 (*SIRT1-7*) 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-biotech, 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-biotech, 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	CATTACTCTTAGCTGCTTGCTC	
	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<sup>2</sup>, 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 A		149(62.08)	185(78.75)	1.0	
		91(37.92)	51(21.25)	2.215(1.477-3.321)	<b>0.002</b>

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
		TT	16 (13.3)	2 (1.7)	9.529 (2.116-42.918)	<b>0.003</b>
	Dominant	CC	68 (56.7)	68 (56.7)	1.00	
		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	
TT		16 (13.3)	2 (1.7)	8.923 (2.004-39.737)	<b>0.002</b>	
C T			172 (71.7)	197 (83)	1.00	
			68 (28.3)	39 (17)	1.997 (1.282-3.112)	<b>0.003</b>

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- $\kappa$ B 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 *SIRT1* 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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## References

- Ploeg M, Aben KK, Kiemeny LA. The present and future burden of urinary bladder cancer in the world. *World J Urol.* 2009;27(3):289-93.
- Salehi A, Khezri A-a, Malekmakan L, Aminsharifi A. Epidemiologic status of bladder cancer in Shiraz, southern Iran. *Asian Pac J Cancer Prev.* 2011;12(5):1323-7.
- EL-Arabey AA. New insight for metformin against bladder cancer. *Gene Environ.* 2017;39(1):13.
- Zaitu M, Nakamura F, Toyokawa S, Tonooka A, Takeuchi T, Homma Y, et al. Risk of alcohol consumption in bladder cancer: case-control study from a nationwide inpatient database in Japan. *Tohoku J Exp Med.* 2016;239(1):9-15.
- Zeng Y, Jiang H-Y, Wei L, Xu W-D, Wang Y-J, Wang Y-D, et al. Association between the CYP1A2 rs762551 Polymorphism and Bladder Cancer Susceptibility: a Meta-Analysis Based on Case-Control Studies. *Asian Pac J f Cancer Prev.* 2015;16(16):7249-54.
- North BJ, Verdin E. Sirtuins: Sir2-related NAD-dependent protein deacetylases. *Genome Biol.* 2004;5(5):224.
- Rajendran R, Garva R, Krstic-Demonacos M, Demonacos C. Sirtuins: molecular traffic lights in the crossroad of oxidative stress, chromatin remodeling, and transcription. *BioMed res Int.* 2011;2011.
- Nakahata Y, Sahar S, Astarita G, Kaluzova M, Sassone-Corsi P. Circadian control of the NAD<sup>+</sup> salvage pathway by CLOCK-SIRT1. *Science.* 2009;324(5927):654-7.
- Lim J-H, Lee Y-M, Chun Y-S, Chen J, Kim J-E, Park J-W. Sirtuin 1 modulates cellular responses to hypoxia by deacetylating hypoxia-inducible factor 1 $\alpha$ . *Mol cell.* 2010;38(6):864-78.
- Zschoernig B, Mahlkecht U. SIRTUIN 1: regulating the regulator. *Biochem Biophys Res Commun.* 2008;376(2):251-5.
- Fagerberg L, Hallström BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, et al. Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. *Mol Cell Proteomics.* 2014;13(2):397-406.
- Yi J, Luo J. SIRT1 and p53, effect on cancer, senescence and beyond. *Biochim Biophys Acta Proteins Proteom.* 2010;1804(8):1684-9.
- Rizk SM, Shahin NN, Shaker OG. Association between SIRT1 gene polymorphisms and breast cancer in Egyptians. *PLoS One.* 2016;11(3):e0151901.
- Zhang W-G, Bai X-J, Chen X-M. SIRT1 variants are associated with aging in a healthy Han Chinese population. *Clin Chim Acta.* 2010;411(21-22):1679-83.
- Byles V, Zhu L, Lovaas J, Chmielewski L, Wang J, Faller D, et al. SIRT1 induces EMT by cooperating with EMT transcription factors and enhances prostate cancer cell migration and metastasis. *Oncogene.* 2012;31(43):4619.
- Shen Z-l, Wang B, Jiang K-w, Ye C-x, Cheng C, Yan Y-c, et al. Downregulation of miR-199b is associated with distant metastasis in colorectal cancer via activation of SIRT1 and inhibition of CREB/KISS1 signaling. *Oncotarget.* 2016;7(23):35092.
- Hu Q, Wang G, Peng J, Qian G, Jiang W, Xie C, et al. Knockdown of SIRT1 suppresses bladder cancer cell proliferation and migration and induces cell cycle arrest and antioxidant response through FOXO3a-mediated pathways. *BioMed Res Int.* 2017;2017.
- Kilic U, Gok O, Bacaksiz A, Izmirli M, Elibol-Can B, Uysal O. SIRT1 gene polymorphisms affect the protein expression in cardiovascular diseases. *PLoS One.* 2014;9(2):e90428.
- Clark SJ, Falchi M, Olsson B, Jacobson P, Cauchi S, Balkau B, et al. Association of sirtuin 1 (SIRT1) gene SNPs and transcript expression levels with severe obesity. *Obesity.* 2012;20(1):178-85.
- Mohtavinejad N, Nakhaee A, Harati H, Poodineh J, Afzali M. SIRT1 gene is associated with cardiovascular disease in the Iranian population. *Egypt J Med Hum Genet.* 2015;16(2):117-22.
- Galavi H, Noorzehi N, Saravani R, Sargazi S, Mollashahee-Kohkan F, Shahraki H. Association study of SREBF-2 gene polymorphisms and the risk of type 2 diabetes in a sample of Iranian population. *Gene.* 2018;660:145-50.

22. ou FM, Huo N, Gu YQ, Luo M-c, Ma Y, Hane D, et al. BatchPrimer3: a high throughput web application for PCR and sequencing primer design. *BMC bioinformatics*. 2008;9(1):253.
23. Sanyal S, Festa F, Sakano S, Zhang Z, Steineck G, Norming U, et al. Polymorphisms in DNA repair and metabolic genes in bladder cancer. *Carcinogenesis*. 2004;25(5):729-34.
24. Kurylowicz A. In Search of New Therapeutic Targets in Obesity Treatment: Sirtuins. *Int J Mol Sci*. 2016;17(4):572.
25. Ramkaran P, Moodley D, Chuturgoon AA, Phulukdaree A, Khan S. sirtuin 1 rs1467568 and rs7895833 in south african indians with early-onset coronary artery disease. *Cardiovasc J Afr*. 2016 Jul;27(4):213.
26. Chae H-D, Broxmeyer HE. SIRT1 deficiency downregulates PTEN/JNK/FOXO1 pathway to block reactive oxygen species-induced apoptosis in mouse embryonic stem cells. *Stem cells and development*. 2010;20(7):1277-85.
27. McBurney MW, Clark-Knowles KV, Caron AZ, Gray DA. SIRT1 is a highly networked protein that mediates the adaptation to chronic physiological stress. *Genes cancer*. 2013;4(3-4):125-34.
28. al. CMe. Candidate gene association study conditioning on individual ancestry in patients with type 2 diabetes and metabolic syndrome from Mexico city. *Diabetes Metab Res Rev*. 2010;26(4):261-70.
29. Consiglio CR, Da Silveira SJ, Monticelo OA, Xavier RM, Brenol JCT, Chies JAB. SIRT1 promoter polymorphisms as clinical modifiers on systemic lupus erythematosus. *Mol Biol Rep*. 2014;41(7):4233-9.
30. Shaker OG, Wadie MS, Ali RMM, Yosry A. SIRT1 gene polymorphisms and its protein level in colorectal cancer. *Gene Reports*. 2017;7:164-8.