Article published online: 2025-03-07

Association of *SLC14A1C/T* Polymorphisms in Patients with Bladder Cancer in Comparison with Healthy Controls

Saziya Bidi[1,2] R.B Nerli[1,2] Shadab Rangrez[3] Shridhar C. Ghagane[1,4]

[1] Urinary Biomarkers Research Centre, KLES Dr. Prabhakar Kore Hospital & Medical Research Centre, Nehru Nagar, Belagavi, Karnataka, India
[2] Department of Urology, JN Medical College, KLE Academy of Higher Education & Research (Deemed-to-be-University), JNMC Campus, Belagavi, Karnataka, India
[3] Department of Biochemistry, JGMM Medical College, Hubballi, Karnataka, India
[4] KAHER's Dr. Prabhakar Kore Basic Science Research Center, KLE Academy of Higher Education & Research, JNMC Campus, Belagavi, Karnataka, India

Address for correspondence R.B. Nerli, MS, M.Ch., PhD, Department of Urology, JN Medical College, KLE Academy of Higher Education & Research (Deemed-to-be-University), JNMC Campus, Belagavi 590010, Karnataka, India (e-mail: rbnerli@gmail.com).

South Asian J Cancer

Abstract


Saziya Bidi


R.B. Nerli

Background Transitional cell carcinoma of the urinary bladder is one of the most common malignancies affecting the urinary tract. Genomic instability is one of the most important common features of urothelial cancers of the bladder. Gene polymorphisms of the *SLC14A1* gene are known to be related to carcinogenesis of the bladder in humans. Similarly, the use of tobacco products including chewing and smoking is an established risk factor for bladder cancer in both men and women. The primary aim of the study was to assess the relationship between bladder cancer and polymorphisms of the *SLC14A1* gene (rs17674580) in our patients with image and histologically confirmed bladder cancer and secondarily to assess if use of tobacco products in these patients further accentuated the risk of bladder cancer.

Patients and Methods All patients aged ≥18 years with images (ultrasonography/computed tomography) and histologically confirmed bladder cancer formed the study group. Age- and gender-matched individuals aged ≥18 years, genetically unrelated, formed the controls. A 2-mL blood sample was collected from patients as well as controls, for genotyping of *SLC14A1C/T* gene polymorphisms. Demographic data were obtained from all the participants, and individuals who smoked once a day for more than 5 years were defined as smokers. Similarly, patients who chewed tobacco for more than 5 years were defined as tobacco users.

Results During the study period, 107 patients (84 males and 23 females) with image and histologically confirmed bladder cancer formed the study group. The mean age of the patients with bladder cancer was 58.47 ± 14.5 years and that of the controls was 60.01 ± 12.5 years. Among patients with bladder cancer, 28 (26.2%) showed no polymorphisms (rs17674580) of the *SLC14A1* gene, whereas 79 (73.8%) patients

Keywords
- gene polymorphisms
- transitional cell carcinoma
- tobacco (smoking/chewing)
- histopathology
- south India

DOI https://doi.org/10.1055/s-0045-1805081 ISSN 2278-330X

How to cite this article: Bidi S, Nerli R.B., Rangrez S, et al. Association of SLC14A1C/T Polymorphisms in Patients with Bladder Cancer in Comparison with Healthy Controls. South Asian J Cancer 2025;00(00):00–00.

Thieme Medical and Scientific Publishers Pvt. Ltd., A-12, 2nd Floor, Sector 2, Noida-201301 UP, India

showed polymorphisms. Heterozygous variations (CT) were noted in 46 (42.9%) patients, whereas homozygous variations were noted in 33 (30.9%) patients with the odds ratio being 2.772 (1.459–3.247) and 3.349 (1.610–6.922), respectively. The use of tobacco (smoking/chewing) was also found to modulate risks of bladder cancer in *SLC14A1* variants.

Conclusion Gene polymorphisms of *SLC14A1C/T* are associated with a high risk of bladder cancer in our group of patients in South India. Moreover, the use of tobacco, be it smoking or chewing, further increases the risk of bladder cancer in these patients.

Introduction

Transitional cell carcinoma of the urinary tract (UT) is one of the most common malignancies seen worldwide.[1] Within the UT, urothelial carcinoma (UC) of the urinary bladder accounts for the majority of them (90–95%), while only approximately 5 to 10% of the cases occur in the upper UT.[2] Comparative genomic hybridization studies have shown that certain chromosomal areas are frequently altered during the progression of bladder cancer (BC).[3–5] This makes it necessary to fully understand the molecular mechanisms of the pathogenesis of BC.

The human gene encoding the type B urea transporter protein, namely, the *SLC14A1*, has been mapped to chromosome 18q12.3, adjacent to another urea transporter, namely, *SLC14A2*, locus (►Fig. 1).[6] This protein is known to facilitate the passive transport of urea, which is responsible for establishing an osmotic gradient within the inner medulla and thereby preventing intracellular toxicity within the extrarenal cells.[7] It is interesting to note that *SLC14A1* gene polymorphisms have been known to be related to carcinogenesis of the bladder in humans.[8–10] Genetic variations in *SLC14A1* could provide new etiological insights into carcinogenesis of the bladder.

Use of tobacco products, chewing and smoking, is an established risk factor for BC in both men and women.[11,12] Previous studies have estimated the risk in smokers to be three times.[13,14] The present study aimed to assess the relationship between BC and *SLC14A1* gene polymorphisms (rs17674580) in patients with image and histologically confirmed BC. Our secondary objective was to assess if the use of tobacco products in these patients further increased the risk of BC.

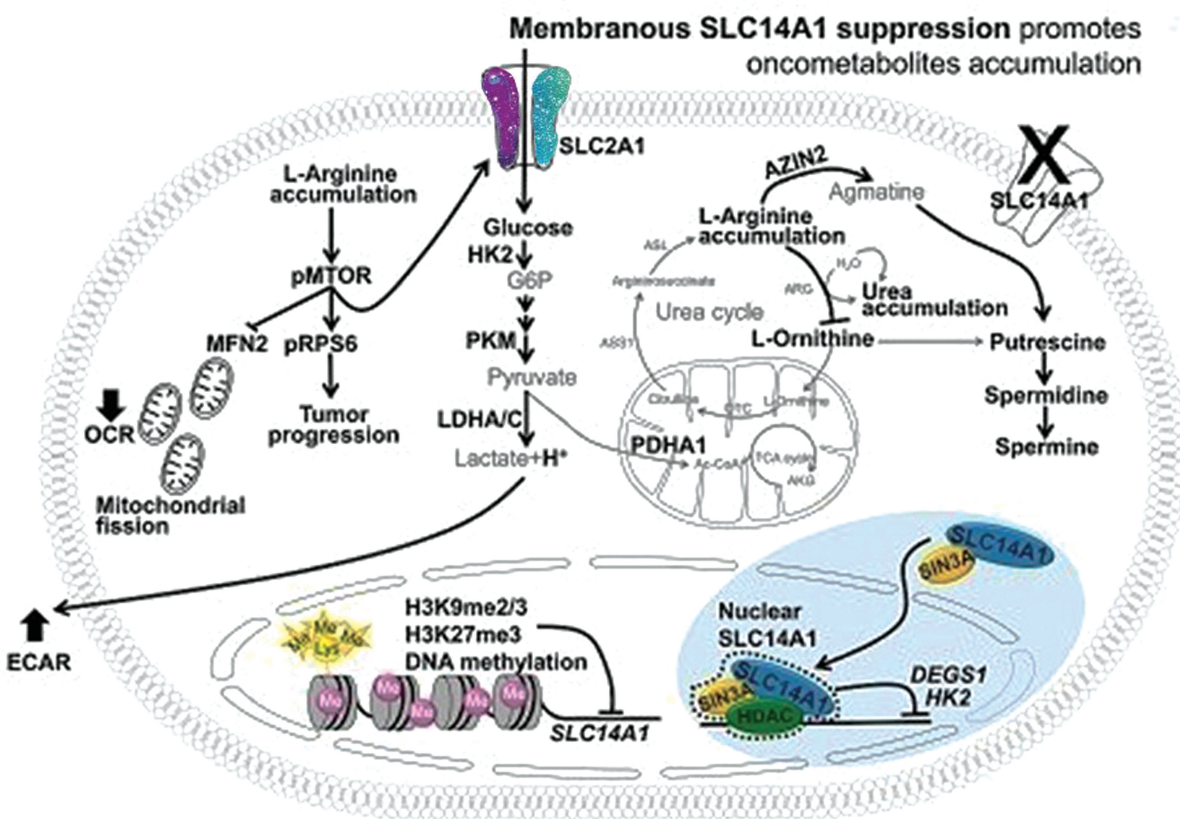

Fig. 1 Mechanism of upregulation and downregulation of the *SLC14A1* gene.

Patients and Methods

This prospective study was conducted with the approval from the university's ethical committee (KAHER/EC/21–22/003). All patients aged ≥18 years with an image (ultrasonography/computed tomography) and histologically confirmed BC formed the study group. Age- and gender-matched controls aged ≥18 years, genetically unrelated, formed the controls. Patients with a previous history of other cancers, cancer metastasized to the bladder from another origin, and a previous history of radiotherapy were excluded from the study. The participating patients signed a written informed consent to be part of the study.

Demographic data were obtained by interviewing each one of the participants. Individuals who smoked once a day for more than 5 years were defined as smokers. Similarly, patients who chewed tobacco for more than 5 years were defined as tobacco users. The individuals who had never smoked in their lifetime were regarded as nonsmokers. After the interview, a 2-mL blood sample was collected into coded ethylenediaminetetraacetic acid (EDTA) vials and stored at –80°C.

Genotyping

Genomic DNA was extracted from peripheral blood lymphocytes by using an Invitrogen (Thermo Fisher Scientific) Kit. For genotyping of *SLC14A1C/T* gene polymorphisms, predesigned TaqMan assays were performed with a Thermal cycler (Bio-Rad CFX96 real-time system). Positive and negative controls were used in each genotyping assay, and 10% of the samples were randomly selected and run in duplicates with 100% concordance. This was done to confirm that the results were reproducible with no discrepancy in genotyping.

Statistical Analysis

Quanto software, version 1.0 (available from: http://hydra.usc.edu/gxe), was used to calculate the power of the study. A binary logistic regression model was used to estimate the risk as the odds ratio (OR) at the 95% confidence interval (CI). Bonferroni correction was applied in case of multiple comparisons using the formula $p_c = p \times n$ (pc represents the corrected value, where n is the number of comparisons performed). The statistical analysis was done using the Statistical Package for Social Sciences software, version 22.0 (SPSS, Chicago, IL), and a p-value less than 0.05 was considered statistically significant.

Results

During the study period, from August 2020 to August 2023, a total of 107 patients (84 males and 23 females) with image and histologically confirmed BC formed the study group. The mean age of the patients with BC was 58.47 ± 4.5 years and that of the controls was 60.01 ± 12.5 years.

In patients with BC, 28 (26.2%) showed no polymorphisms (rs17674580) of the *SLC14A1* gene, whereas 79 (73.8%) patients showed polymorphisms in the gene and the types of mutations. In comparison, 54 (51.5%) of the controls showed no polymorphisms in the gene. The different types of polymorphisms (rs17674580) are seen within the *SLC14A1* gene. Heterozygous polymorphisms (CT) were noted in 46 (42.9%) patients, whereas homozygous polymorphisms were noted in 33 (30.9%) patients. The ORs were 2.772 (1.459–3.247) and 3.349 (1.610–6.922), respectively. The subgroup analysis of the association between polymorphisms of *SLC14A1* in relation to tumor grade and stage is shown in ►**Table 1**. The relation between polymorphisms of *SLC14A1* and the use of tobacco is shown in the graph in ►**Fig. 2**.

Discussion

Single nucleotide polymorphism (SNP) is a variation that occurs in a genetic sequence and affects only one of the basic building blocks—adenine (A), guanine (G), thymine (T), or cytosine (C)—in a segment of a DNA molecule. These variations occur in more than 1% of a population.[14] Roughly 90% of the genetic variation between humans is the result of SNPs. Studies have shown that both the nuclear and membranous *SLC14A1* proteins play the role of tumor suppression through several signaling pathways in both in vitro and in vivo clinical specimens of UC.[10] Another study on meta-analysis of genome-wide association studies of urinary bladder UCs recently reported that *SLC14A1*-rs10775480, a variant at intron 6, is highly associated with susceptibility in BC.[15]

Table 1 Subgroup analysis of the association between *SLC14A1*, tumor grade, and stage

Genotype (stage)	Ta–T1 (39)	T2–T4 (68)	Odds ratio
CC (28)	11 (28.2)	17 (25)	Reference
CT (46)	17 (43.6)	29 (42.6)	1.103 (0.420–2.900)
TT (33)	11 (28.2)	22 (32.4)	1.294 (0.453–3.692)
CT + TT (79)	29 (71.8)	50 (75)	1.115 (0.460–2.705)
Genotype (grade)	**Low (41)**	**High (66)**	**Odds ratio**
CC (28)	11 (26.8)	17 (25.8)	Reference
CT (46)	17 (41.4)	29 (43.9)	1.103 (0.420–2900)
TT (33)	13 (31.8)	20 (30.3)	0.995 (0.355–2.790)
CT + TT (79)	30 (73.2)	49 (74.2)	1.056 (0.436–2.558)

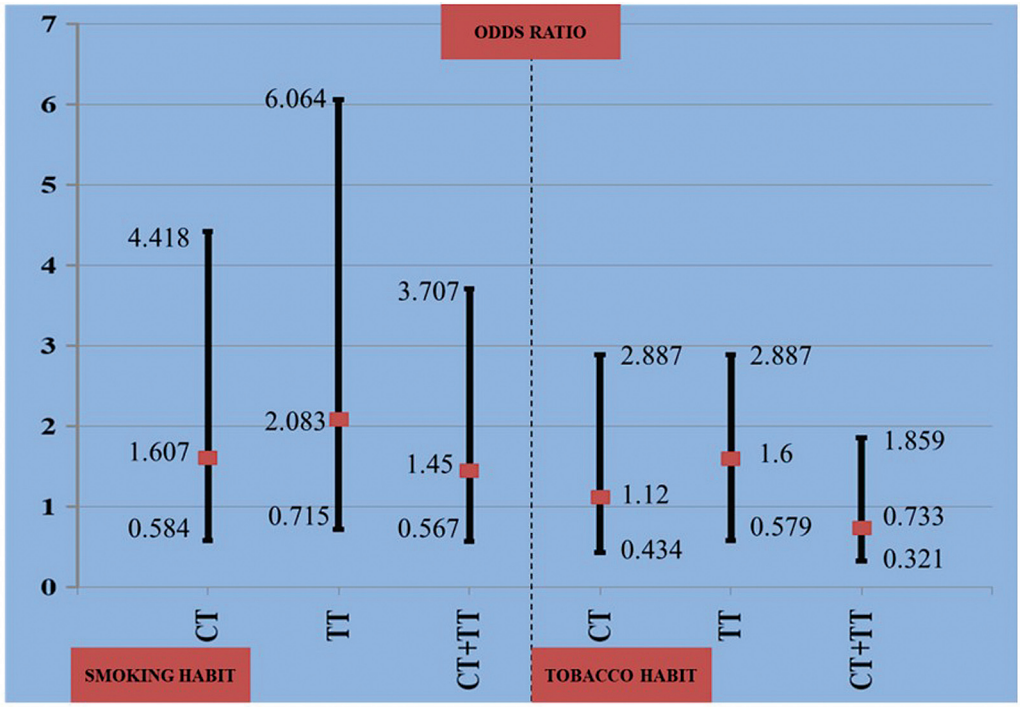

Fig. 2 Association between *SLC14A1* and exposure to smoking and tobacco use.

To determine whether *SLC14A1* gene polymorphisms increase the risk of invasive BC, we compared genotype distribution between tumor grade and stage and discovered significant heterogeneity in the effect of the combined CT/TT genotype for stage-wise comparison was (OR for CT/TT (Cytosine, Thymine/Thymine, Thymine) = 1.115, 95% CI = 0.460–2.705) and for grade-wise distribution (OR for CT/TT = 1.056, 95% CI = 0.436–2.558). When low- and high-risk non–muscle-invasive bladder cancer (NMIBC) patients were compared, the heterozygous genotype (CT) of *SLC14A1C/T* was shown to be linked with BC susceptibility. Significant risks of BC susceptibility were found among North Indians, with a threefold risk of BC susceptibility in the case of variant TT genotype and a 1.5-fold risk at the variant T allele level, which were nearly consistent with our findings. In a Genome wide association study (GWAS) conducted by Rafnar et al,[8] the highest signal was provided by an SNP in intron 3, rs1764580, which demonstrated considerable risk in both the discovery and follow-up groups. Moreover, a study conducted by Chan et al[10] concluded that clinical studies, animal models, and in vitro indicators provided solid evidence that the *SLC14A1* gene was a novel tumor suppressor in UCs.

In India, the use of tobacco is done in several ways. Apart from cigarette smoking, people in rural areas smoke rolled dried tobacco leaves called bidis. Tobacco is also used as a component along with beetle leaves or mouth freshener (locally called as gutka) and chewed. Some also use raw tobacco or mixed with lime paste and kept in the buccal crevices over a long time. The risk of these tobacco products has not been studied in relation to BC.[8] Furthermore, our data showed that the combined CT/TT genotype had a larger impact on tobacco exposure in smokers (OR for CT/TT = 1.45, 95% CI = 0.567–3.707) than in nonsmokers (OR for CT/TT = 0.236, 95% CI = 0.101–0.551; ►Fig. 2). Previous studies have shown more than threefold increased risk of BC in current tobacco smokers and twofold increased risk in former smokers compared with nonsmokers.[16,17] A 2016 meta-analysis[18] of 89 observational studies from the past 50 years reported that smoking was associated with the highest risk of BC compared with any other environmental or occupational risk factor. Studies[19] of patients with NMIBC, which represents approximately 75% of cases, have reported mixed results on the impact of lifetime smoking behavior on cancer outcomes. Some studies have found that current, heavy, and long-term prediagnosis smoking was associated with worse recurrence-free survival, while other studies have found no such association.[20]

Finally, our findings establish the role of *SLC14A1C/T* as major contributing genetic factors in the process of bladder carcinogenesis in North Karnataka, which is supported by significant findings with clinical parameters such as smoking and tumor grade and stage of the patients, which may have important clinical implications. The limitation of this study was a smaller sample size, which lowered the statistical power for discovering the interaction effects. More research with larger sample sizes and various ethnicities is needed to corroborate our findings.

Conclusion

Urothelial BC is a common UT tumor. It is associated with several risk factors including the use of tobacco. *SLC14A1* is a human gene encoding the type B urea transporter protein. Gene polymorphisms associated with this gene are associated with patients with BC. The use of tobacco products further increases the risks in these patients.

Funding
None.

Conflict of Interest
None declared.

References
1 Rosenberg JE, Hoffman-Censits J, Powles T, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet 2016;387(10031):1909–1920
2 Audenet F, Isharwal S, Cha EK, et al. Clonal relatedness and mutational differences between upper tract and bladder urothelial carcinoma. Clin Cancer Res 2019;25(03):967–976
3 Nerli RB, Ghagane SC, Shankar K, et al. Low-grade, multiple, Ta non-muscle-invasive bladder tumors: tumor recurrence and worsening progression. Indian J Surg Oncol 2018;9(02):157–161
4 Nerli RB, Ghagane SC, Rangrez S, Chandra S, Thakur ML, Gomella L. Detection of bladder cancer using voided urine sample and by targeting genomic VPAC receptors. Indian J Urol 2021;37(04):345–349
5 Kallioniemi A, Kallioniemi OP, Citro G, et al. Identification of gains and losses of DNA sequences in primary bladder cancer by comparative genomic hybridization. Genes Chromosomes Cancer 1995;12(03):213–219
6 Puranik SI, Ghagane SC, Nerli RB, Hiremath MB. Incidence of bladder cancer at a tertiary care centre in north Karnataka. Middle East J Cancer 2022;13(02):363–371
7 Shayakul C, Clémençon B, Hediger MA. The urea transporter family (SLC14): physiological, pathological and structural aspects. Mol Aspects Med 2013;34(2–3):313–322
8 Rafnar T, Vermeulen SH, Sulem P, et al. European genome-wide association study identifies SLC14A1 as a new urinary bladder cancer susceptibility gene. Hum Mol Genet 2011;20(21):4268–4281
9 Garcia-Closas M, Ye Y, Rothman N, et al. A genome-wide association study of bladder cancer identifies a new susceptibility locus within SLC14A1, a urea transporter gene on chromosome 18q12.3. Hum Mol Genet 2011;20(21):4282–4289
10 Chan TC, Wu WJ, Li WM, Shiao MS, Shiue YL, Li CF. SLC14A1 prevents oncometabolite accumulation and recruits HDAC1 to transrepress oncometabolite genes in urothelial carcinoma. Theranostics 2020;10(25):11775–11793
11 IARC. Tobacco Smoke and Involuntary Smoking. Lyon, France: IARC; 2004
12 Silverman DT, Devesa SS, Moore LE, Rothman N. Bladder cancer. In: Schottenfeld D, Fraumeni JF Jr, eds. Cancer Epidemiology and Prevention. 3rd ed New York, NY: Oxford University Press; 2006: 1101–1127
13 Hemelt M, Yamamoto H, Cheng KK, Zeegers MP. The effect of smoking on the male excess of bladder cancer: a meta-analysis and geographical analyses. Int J Cancer 2009;124(02):412–419
14 Biggerstaff J. The relationship between the risk of smoking-related disease, inflammatory response and single-nucleotide polymorphisms. Microreviews Cell Molecular Biology 2019;5(01):1–5
15 de Maturana EL, Rava M, Anumudu C, Sáez O, Alonso D, Malats N. Bladder cancer genetic susceptibility. A systematic review. Bladder Cancer 2018;4(02):215–226
16 Zeegers MP, Tan FE, Dorant E, van Den Brandt PA. The impact of characteristics of cigarette smoking on urinary tract cancer risk: a meta-analysis of epidemiologic studies. Cancer 2000;89(03):630–639
17 Shadab R, Nerli RB, Bidi SR, Ghagane SC. Risk factors for bladder cancer: results of a survey of hospital patients. J Cancer Allied Spec 2023;9(01):485
18 van Osch FH, Jochems SH, van Schooten FJ, Bryan RT, Zeegers MP. Quantified relations between exposure to tobacco smoking and bladder cancer risk: a meta-analysis of 89 observational studies. Int J Epidemiol 2016;45(03):857–870
19 Kamat AM, Hahn NM, Efstathiou JA, et al. Bladder cancer. Lancet 2016;388(10061):2796–2810
20 Kwan ML, Haque R, Young-Wolff KC, et al. Smoking behaviors and prognosis in patients with non-muscle-invasive bladder cancer in the be-well study. JAMA Netw Open 2022;5(11):e2244430

