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Genetic Insights into Bladder Carcinogenesis: Impact of VEGFA -460T>C (rs833061) Polymorphism

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ABSTRACT

Background: Vascular endothelial growth factor A (VEGFA) plays a critical role in tumor angiogenesis and cancer progression. Polymorphisms in the VEGFA gene, particularly rs833061 (-460T>C), have been implicated in cancer susceptibility and clinical outcomes. However, the association between VEGFA rs833061 and bladder cancer remains underexplored, particularly in the North Indian population.

Objective: The objective of the present investigation was to evaluate the potential relationship between the VEGFA rs833061 genetic polymorphism and susceptibility to bladder cancer, along with its influence on clinical outcomes, in a North Indian cohort.

Methods: A case-control study was conducted involving 60 bladder cancer patients and 60 healthy individuals (Control group). Genotypic frequencies for the VEGFA rs833061 polymorphism were determined using Amplification refractory mutation system PCR analysis. To determine the magnitude of the association between different genotypes and bladder cancer susceptibility, odds ratios (ORs) along with 95% confidence intervals (CIs) were computed.

Results: A markedly elevated frequency of the T allele was observed in bladder cancer patients (0.56) compared with the control group (0.17) ($p < 0.001$). Analysis under dominant, recessive, and co-dominant models indicated an enhanced cancer risk for the combined CT+TT genotypes. Notably, individuals carrying the TT genotype exhibited a significantly higher likelihood of developing bladder cancer (OR = 1.89, $p = 0.002$). In the dominant model, the combined CT + TT genotype conferred a higher cancer risk (OR = 0.051, $p = 6.67 \times 10^{-12}$). The recessive model also showed a moderate but significant association (OR = 0.273, $p = 0.0257$). Clinically, the TT genotype correlated with higher tumor grade ($p = 0.0024$) and advanced disease stage ($p = 0.015$), indicating a potential role of VEGFA polymorphisms in disease aggressiveness.

Conclusion: Present research demonstrated a significant association between the VEGFA rs833061 polymorphism and bladder cancer risk and progression in studied population. The T allele and TT genotype emerged as potential genetic markers for bladder cancer susceptibility and poor clinical outcomes. The findings indicate that VEGFA rs833061 could function as a predictive biomarker as well as a promising therapeutic target for anti-angiogenic strategies in bladder cancer. Nonetheless, confirmation through larger cohort studies and functional investigations is essential to substantiate these results and elucidate the underlying molecular mechanisms.

Keywords: *VEGF-A; Angiogenesis; Bladder cancer; Gene polymorphism; Genetic susceptibility;*

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1. INTRODUCTION

Bladder cancer, one of the most frequently diagnosed malignancies of the urinary tract, originates from the epithelial lining of the urinary bladder. Among its histological variants, urothelial carcinoma predominates, representing more than 90% of all cases reported in industrialized countries (Kalita et al., 2024).Squamous cell carcinoma, a less common variant, is more prevalent in Africa and parts of the Middle East, often linked to schistosomiasis infections (Majithia et al., 2023).

According to the latest GLOBOCAN 2024 data, 573,278 new cases of bladder cancer are expected globally, making it the 7th most common cancer worldwide and the 4th most common among men. The disease accounts for ~3.1% of all new cancer diagnoses, with an estimated 212,536 deaths globally (Su et al., 2025). The highest incidence rates are reported in North America, Western Europe, and the Middle East, with Greece having the highest rate among men and Lebanon leading in female incidence (Safiri et al., 2021).

Bladder cancer remains a predominantly age-related disease, with 90% of cases occurring in individuals over 55 and an average age of diagnosis around 73 years in the U.S. (Mailankody et al., 2024). Notably, India reports approximately 20,000 new cases annually, with 11,000 deaths per year, and urban regions such as Delhi, Mumbai, and Chennai recording the highest incidence rates (Malhotra et al., 2024). Five-year survival rates vary globally, with high-income countries reaching 77%, while India's survival rate remains lower at ~60% due to late-stage detection and limited access to advanced treatments (Singh et al., 2024).

The key risk factors influencing bladder cancer include tobacco use, which is linked to ~50% of cases, occupational exposure to industrial chemicals, and arsenic-contaminated drinking water in regions like West Bengal and Bihar (Mishra &Balasubramaniam, 2021). The disease's incidence is expected to increase by 12-15% by 2040, emphasizing the need for enhanced screening programs, early detection strategies, and improved treatment accessibility globally (Khanna et al., 2024).

Aberrant angiogenesis remains a**hallmark of cancer progression,** playing a pivotal role in tumor growth, invasion, and metastasis. Recent research highlights that**tumor-induced neovascularization is driven by hypoxia-mediated signaling pathways,** with **VEGF**as a regulator.

The vascular endothelial growth factor (VEGF) gene, located on chromosome 6p12-p21, encodes a family of proangiogenic proteins through alternative splicing of eight exons and seven introns (Choi et al., 2025). VEGF plays a pivotal role in tumor angiogenesis, regulating endothelial cell proliferation, survival, and vascular permeability. Overexpression of VEGF and its receptors (VEGFR-1, VEGFR-2) has been strongly correlated with tumor aggressiveness, metastasis, and resistance to therapy (Nowacka et al., 2025).

Recent studies confirm that the **VEGFA gene** exhibits **more than 30 single-nucleotide polymorphisms (SNPs),** many of which are localized in regulatory regions that **modulate VEGF expression** (Barus et al., 2024). These single nucleotide polymorphisms (SNPs) are distributed across different regulatory regions of the gene, including the promoter region (rs699947, rs1570360, rs833061), the 5′-untranslated region (UTR) (rs2010963, rs25648), and the 3′-UTR (rs3025039, rs10434). They influence **VEGF** transcription, mRNA stability, and subsequent angiogenicsignaling pathways (Moustakli et al., 2025). Among these, **rs699947 (-2578C>A), rs2010963 (-634G>C), rs833061 (-460C>T), and rs3025039 (+936C>T)** have been widely studied for their regulatory effects on **VEGF-A expression in different cancers and vascular diseases**(Founini et al., 2025). Therefore, Genetic variability within the **VEGFA gene** plays a crucial role in modulating **bladder carcinogenesis** by influencing angiogenesis. Among these variations, the **rs833061 polymorphism** in the promoter region has been identified as a key regulator of **VEGF transcriptional activity,**potentially affecting tumor progression. **Elevated VEGF expression** is linked to increased **tumor vasculature,** which may enable**improved** efficient delivery of chemotherapy**agents**to tumor tissues while simultaneously mitigating **radiation-induced hypoxia,** a major factor contributing to tumorradioresistance. This dynamic interaction between **chemotherapy and radiotherapy** may lead to a **synergistic therapeutic effect,** improving treatment outcomes. However, **previous studies have yielded conflicting and inconclusive findings,** highlighting the need for further research.

The current study has investigated the **genetic impact of the VEGFA (-460T>C) polymorphism**on**bladder cancer susceptibility** and its potential role in **disease progression.**

2. MATERIALS METHODS

The study received ethical clearance from the Institutional Human Ethics Committee (IHEC) of Maharshi Dayanand University, Rohtak (Approval No. HEC/2023/34, dated 23/05/2023). Written informed consent was obtained from all participants before their inclusion, and the research was conducted in full compliance with the ethical standards outlined

in the Declaration of Helsinki.

Patients from Pt. B. D. Sharma University of Health Sciences, belonging to population groups with bladder cancer disorder were chosen for this study. Similar numbers of age matched healthy individuals were recruited as control groupIn this study, analyses were performed on 60 patients with histologically confirmed bladder cancer and 60 age-matched healthy controls who had no prior history of malignancy.

Inclusion criteria: Patients with reported cases of bladder cancer of any stage were included. Patients with recurrent bladder cancer were included.

Exclusion criteria: Patients reported to have any other disease like diabetes; cardiac disorder, arthritis etc. were excluded. Co-morbid disorders were excluded. Patients undergoing chemotherapy, radiotherapy were excluded.

Clinical Data

Clinical history for both patients and healthy controls was collected through face-to-face interviews conducted one day prior to sample collection.A specialised and structured questionnaire was used to record factors such as age, family history of bladder cancer, occupation of patients, life style, nutrition etc.

Collection of Sample

Peripheral blood samples were obtained from both bladder cancer patients and age-matched healthy controls by a trained laboratory technician.Following clinical and pathological assessment, a 3 mL peripheral blood was obtained via venipuncture and transferred into EDTA-coated collection tubes. Bladder cancer Patients with a prior history of bladder cancer recurrence were also included in the study.

DNA isolation from blood of bladder cancer patients and healthy individuals

Bladder cancer patient's blood samples were collected from Department of urology PGIMS, Rohtak in 3ml blood vacutainer (EDTA coated and clot activator). Age matched Healthy individuals were taken as control group. Blood sample (EDTA coated) for DNA extraction was processed using Nucleo-pore DNASure Blood Mini Kit (cat.# NP - 61105) as per instructions. The isolated DNA was dissolved in elution buffer and preserved at −20 °C for subsequent analyses. Its concentration and integrity were evaluated using a NanoDrop spectrophotometer (Nano-400A Micro-Spectrophotometer).

SNP in VEGF-A

Genotyping of (VEGF-A) (−460T>C) SNP were done byARMS-PCR method. This approach utilizes allele-specific sequence (primers) that only allow the amplification when corresponding allele is present in DNA. The detection or non-detection of an amplified productafter the ARMS reaction indicated the respective genotype. VEGF-A (−460T>C) specificprimers were designed using Primer-BLAST software (Table 1).

Table 1 Amplification-Refractory Mutation System (ARMS)-PCR Primers for the VEGF-A rs833061 (−460T>C) Promoter Polymorphism

Gene	Sequence of primer	Size of product	AT
Forward outer VEGF-A	5-caaagcccattccctcttta-3	**414**	**56**
Reverse outer VEGF-A	5-cacagcctgaaaattaccca-3		
Forward Inner C VEGF-A	5-cgtgtggggttgagtgc-3	**264**	
Reverse Inner T VEGF-A	5-ctccccgctccacca-3	**181**	

The ARMS-PCR assay was carried out in a total reaction volume of 25 µL. Each reaction contained 80 ng of template DNA, 0.60 µL each of forward outer (FO), reverse outer (RO), and reverse inner (RI) primers (20 pmol of each), along with 12.5 µL of GeNei™ PCR master mix (Table 2). The final volume was adjusted to 25 µL using nuclease-free water. For each PCR reaction, 3 µL of DNA was used from both patient and control group samples.

Table 2 PCR Reaction Setup

	1X
PCR master mix	12.5ul
Nuclease free water	8.10ul
Forward outer	0.60 ul
Reverse outer	0.60 ul
Forward inner T	0.60 ul

Reverse inner C	0.60 ul
DNA	3 ul
Total volume	**25** ul

Thermocycling Conditions

PCR amplification began with denaturation at 95 °C for 10 minutes, which was followed by denaturation at 94 °C for 35 seconds for 40 cycles, anneals at 56 °C for 40 sec., and extension at 72 °C for 45 sec. The reaction concluded with a terminal extension phase carried out at 72 °C for 10 minutes. Optimization was carried out using gradient PCR, with temperatures ranging from 56°C to 64°C to determine the most suitable annealing condition. The optimized protocol employed 43 cycles, which markedly improved the yield of all three PCR products. These modifications improved the amplification efficiency of the mutant allele while minimizing competitive amplification fromcontrol allele(Figure 1). The temperature of annealing was optimized by lowering it from 60 °C to 56 °C.

Gel Electrophoresis

The amplified products were visualised on a 2% agarose gel by using a gel documentation system.

3. RESULTS

Study Population

This population-based case–control study included a total of 120 participants, comprising 60 bladder cancer patients and 60 healthy Individualswith same age without any previous data of malignancy.

Demographic Characteristics of Bladder Cancer Patients

The clinical and pathological analysis of patients of bladder cancer showed that 71.66% were older than 56 years. Regarding tumor stage, 81.66% of the cases were classified as early stage (I and II), while 18.33% were in progressive stages (III and IV). Histological grading revealed that 71.66% of tumors were low grade, whereas 28.33% were high grade (Table 3).

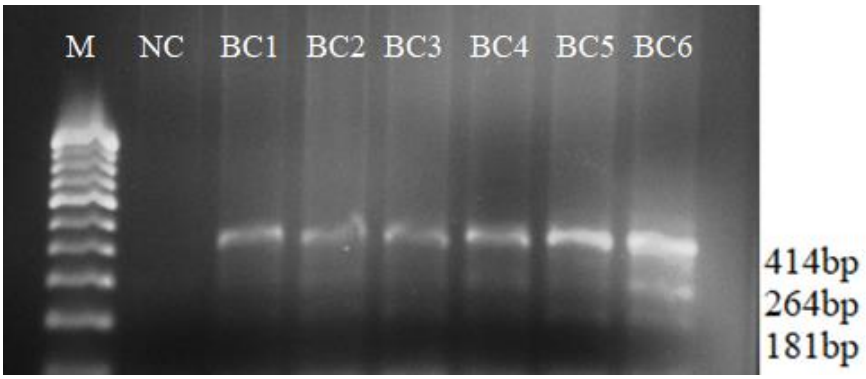
Table 3. Distribution of clinical and pathological Features in Bladder Cancer Patients

Parameters	N=60	Frequency percentage
AGE		
AGE<55	17	28.33%
AGE>56	43	71.66%
STAGE		
T1	23	38.33%
T2	26	43.33%
T3	8	13.33%
T4	3	5%
GRADING		
LOW GRADE	43	71.66%
HIGH GRADE	17	28.33%

The Forward outer and Reverse outer primers were synthesized to amplify the exon region of the VEGFA gene, producing a 414 bp fragment that functioned as an internal control for evaluating both DNA quality and quantity. The wild-type allele (C allele) was amplified using primers Fwt and RO, resulting in a 264 bp fragment, whereas the mutant allele (T allele) was amplified with primers FO and Rmt, producing a 181 bp fragment (Figure 1).

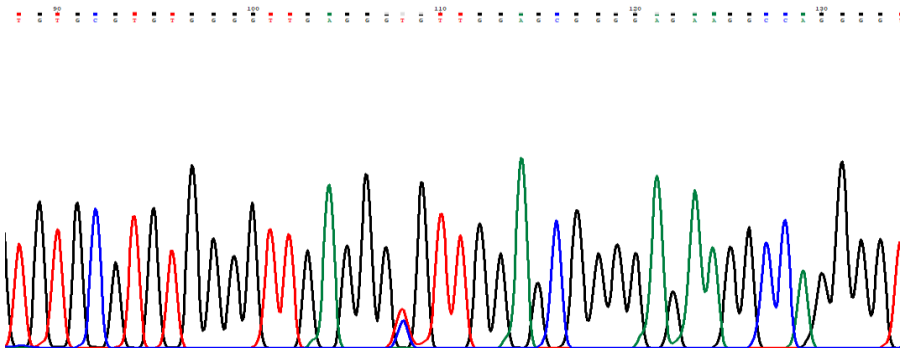
Assessment of Hardy–Weinberg Equilibrium (HWE)

Among the bladder cancer group, the frequencies of the CC, CT, and TT genotypes were 13.33%, 61.67%, and 25%, respective. In contrast, healthy individuals exhibited genotype frequencies of 75% for CC, 16.67% for CT, and 8.33% for TT. These marked differences in genotype distribution suggest a strong connection between the T allele and increased bladder cancer risk. The distribution of genotypes and allele frequencies for the VEGFA rs833061 (-460T>C) polymorphism revealed a significant deviation from Hardy-Weinberg equilibrium (HWE) in both bladder cancer patients and healthy control groups ($\chi^2 = 12.88$, $p = 0.0015$), indicating a potential genetic association.

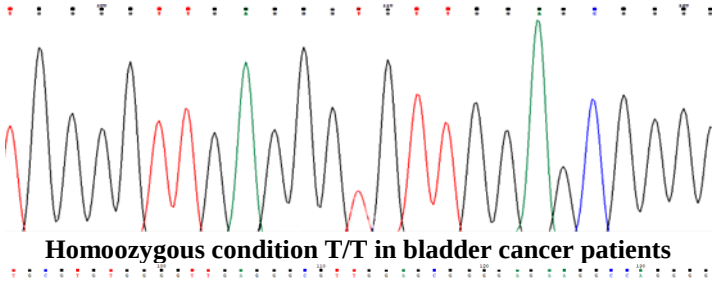


Control band 414 bp;C Allele 264 bp; T Allele 181bp

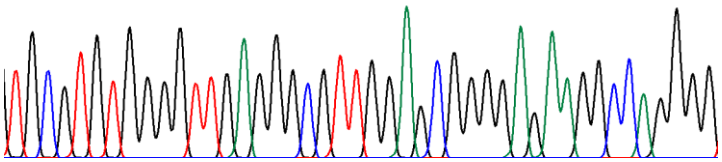
Figure 1. Detection of VEGF-A (−460T>C) Polymorphism in Bladder Cancer Patients by ARMS-PCR



Heterozygous condition C/T in bladder cancer patients



Homoozygous condition T/T in bladder cancer patients



Homoozygous condition C/C in Healthy Individuals

4. STATISTICAL ANALYSIS

The relationship between VEGF-A (−460T>C) genotypes and susceptibility to bladder cancer was assessed by calculating odds ratios (ORs), risk ratios (RRs), and risk differences (RDs), each presented with corresponding 95% confidence intervals (CIs).To further investigate the genetic influence, three inheritance models were tested. Under the dominant model (CT + TT vs. CC), individuals carrying at least one T allele showed a markedly elevated risk of bladder cancer, with an OR of 0.051 and a *p*-value of 6.67×10^{-12}, indicating a strong genetic effect even among heterozygotes. In the **recessive model** (TT vs. CT + CC), the TT genotype conferred a moderate but statistically significant risk (OR = **0.273**, p = **0.0257**), suggesting a weaker impact when both alleles are mutated. The **codominant model**, which examined each genotype independently against the CC reference, revealed ORs of **3.670** for TT and **8.040** for CT, both highly significant, suggesting that even a single T allele significantly increases disease risk. Analysis of the variations in the allele and genotype distributions of VEGF-A (−460T>C) was conducted using the Chi-square test to compare the cases and control groups. For group comparisons, **Student's two-sample *t*-test** was applied to continuous variables, while categorical variables were analyzed using the Chi-square test.

Comparative Analysis of the VEGF-A rs833061 (−460T>C) Polymorphism between Cases and Control Group

Among patients, the CT genotype was most prevalent (61.67%), followed by TT (25%) and CC (13.33%). In contrast, the majority of healthy individuals exhibited the CC genotype (75%), with much lower frequencies of CT (16.67%) and TT (8.33%). Allelic analysis further revealed that the T allele was substantially frequent among patients (56%) than in healthy individuals (17%), while the C allele was more common in the control group (83%) than in the patient group (44%). This difference was highly significant ($\chi^2 = 24.40$, $p = 0.0001$), suggesting a strong association between the presence of the T allele and increased susceptibility to bladder cancer. These findings imply that the VEGFA rs833061 polymorphism, particularly the T variant, may serve as a potential genetic marker for identifying individuals at elevated risk for bladder cancer. The distribution of VEGFA rs833061 (-460T>C) genotype exhibited a statistically significant variation between bladder cancer patients and healthy control su, indicating a potential genetic predisposition(Table 4).

Table 4Comparative Analysis of the VEGF-A rs833061 (−460T>C) Polymorphism between Cases and Control Group

Subjects	N	CC	TT	CT	C	T	X^2	P VALUE
BLADDER CANCER	60	8 (13.33%)	15 (25%)	37 (61.67%)	.44	.56	24.40	0.0001
HEALTHY INDIVIDUALS	60	45 (75%)	5 (8.33%)	10 (16.67)	.83	.17	6.70	0.0351
TOTAL	120	53	20	47				

Co-relation of VEGF-A (-460T>C) with clinical and pathological features

The present study explored the relationship between the **VEGF-A rs833061 (−460T>C) polymorphism** and the **clinicopathological features of bladder cancer patients**. revealing significant correlations that underscore the polymorphism's potential prognostic value. Genotypic analysis among 60 patients demonstrated that the CT genotype was consistently the most prevalent across all examined variables. Stratification by age showed that in both younger (<56 years) and older (≥56 years) patient groups, the CT genotype dominated (64.7% and 65.11%, respectively), with significant associations identified (p = 0.0199 and p = 0.0001), indicating a potential age-dependent modulation of genotype distribution. Tumor staging further highlighted this trend: CT genotype was most frequent in stages T1 (60.86%), T2 (65.38%), and T3 (75%), with corresponding p-values of 0.0150, 0.0024, and 0.0312, respectively, while no significant association was found in the limited T4 cohort (p = 0.3679). Notably, tumor grade analysis revealed a marked enrichment of the CT genotype in high-grade tumors (76.47%) compared to low-grade tumors (58.13%), with strong statistical significance (p = 0.0008 for high grade and p = 0.0024 for low grade)(Table 5).

Table 5. Association of VEGF-A rs833061 (-460T>C) Polymorphism with Clinicopathological Features of Bladder Cancer Patients

Variables	N=60	CC	TT	CT	X^2	DF	P value
AGE							
AGE<56	17 (28.33%)	4 (23.52%)	2 (11.76%)	11 (64.70%)	7.836	2	0.0199
AGE>56	43 (71.66%)	6 (13.95%)	9 (20.93%)	28 (65.11%)	19.84	2	0.0001
STAGE							
T1	23 (38.33%)	3 (13.04%)	6 (26.08%)	14 (60.86%)	8.396	2	0.0150
T2	26 (43.33%)	5 (19.23%)	4 (15.38%)	17 (65.38%)	12.03	2	0.0024
T3	8 (13.33%)	2 (25%)	0	6 (75%)	6.937	2	0.0312
T4	3 (5%)	0	2 (66.66%)	1 (33.33%)	2.000	2	0.3679
GRADING							
LOW GRADE	43 (71.66%)	8 (18.60%)	10 (23.25%)	25 (58.13%)	12.03	2	0.0024
HIGH GRADE	17 (28.33%)	2 (11.76%)	2 (11.76%)	13 (76.47%)	14.17	2	0.0008

Odds Ratios and Relative Risks of VEGF-A Genotypes Under Codominant, Dominant, and Recessive Models

The multivariate analysis conducted to assess the association between **VEGF-A gene polymorphism** and the risk of bladder cancer revealed a statistically robust and biologically relevant relationship between specific genotypes and disease susceptibility. Under the **codominant model**, significant genotypic disparities were observed between bladder cancer patients and healthy individuals. The **CC genotype**, considered the wild-type homozygous variant, was markedly more frequent among healthy controls than among bladder cancer cases, yielding an **odds ratio (OR) of 0.051** and a **relative risk (RR) of 0.178**, with a **highly significant p-value ($p \approx 6.67\times10^{-6}$)**, suggesting a strong protective effect of the CC genotype against bladder cancer development. In contrast, the **CT genotype** appeared in 37 cancer patients but only 10 controls, resulting in a dramatically increased **OR of 8.040** and **RR of 3.700** ($p \approx 6.89\times10^{-7}$), indicating that heterozygous carriers of this polymorphism possess a significantly elevated risk of developing bladder cancer. Additionally, individuals with**TT genotype** (n = 15 in patients, n = 5 in controls) were also found to be at increased risk, with an **OR of 3.670** and **RR of 3.000** ($p \approx 2.60\times10^{-2}$), though the magnitude of risk was lower than that observed in CT carriers**(Table 6)**.

The analysis of genetic models further reinforced these associations. In the **dominant model**, where individuals carrying at least one T allele (i.e., CT or TT) genotypes were grouped and compared to those with the CC genotype, there was a pronounced risk elevation with an **OR of 0.051** and **RR of 0.178**, accompanied by an exceptionally significant **p-value ($p \approx 6.67\times10^{-12}$)**. This model underscores the cumulative risk imparted by the presence of the T allele, even in heterozygous form. Meanwhile, the **recessive model**, which contrasts TT homozygotes against the combined CC and CT genotypes, revealed a more modest association with cancer risk, reflected in an **OR of 0.273** and **RR of 0.818** ($p \approx 2.60\times10^{-2}$)**(Table 6)**.

Allelic frequency analysis further supported these findings. The **T allele** was substantially more frequent among bladder cancer patients (**frequency = 0.56**) compared to healthy controls (**frequency = 0.17**), while the **C allele** was more prevalent among controls (**frequency = 0.83**) than in cancer cases (**frequency = 0.44**). This clear shift in allelic distribution highlights the **T allele as a risk allele** for bladder cancer, while the **C allele appears to confer a protective effect (Table 6)**. Collectively, these findings suggest that the VEGF-A rs833061 polymorphism, particularly the presence of the T allele—either in CT or TT genotypic forms—plays a **significant role in modulating genetic susceptibility** to bladder cancer, offering potential utility as a **biomarker for genetic risk assessment and early detection**.

Table 6.Multivariate Analysis of VEGF-A Polymorphism and Its Association with Bladder Cancer Susceptibility

GENOTYPES	BLADDER CANCER	HEALTHY INDIVIDUALS	OR	RR	P- VALUE
CO-DOMINANT					
VEGFA- CC	**8**	**45**	0.051	0.178	$6.67x10^{6}$
VEGFA- TT	**15**	**5**	3.670	3.000	$2.60x\ 10^{2}$
VEGFA- CT	**37**	**10**	8.040	3.700	$6.89x\ 10^{7}$
DOMINANT					
VEGFA- CC	**8**	**45**			
VEGFA- (CT+TT)	**52**	**15**	0.051	0.178	$6.67x\ 10^{12}$
RECESSIVE					
VEGFA-(CC+CT)	**45**	**55**			
VEGFA- TT	**15**	**5**	0.273	0.818	$2.60x10^{2}$
ALLELE					
VEGFA- C	**.44**	**.83**			
VEGFA- T	**.56**	**.17**			

5. DISCUSSION

Bladder cancer is a genetically complex disease influenced by environmental exposures and inherited susceptibilitiesRecent genomic investigations have identified several genetic pathways implicated in tumor initiation and progression, including those regulating cell cycle control, DNA repair mechanisms, chromatin remodeling, and angiogenesis (Robertson et al., 2017). Within the angiogenic pathway, VEGF-A serves as a key mediator of tumor-associated neovascularization, a process critical for tumor growth, invasion, and metastasis. Genetic polymorphisms in the **VEGF-A** gene, particularly in its promoter region, have been shown to modify transcriptional activity and thereby influence individual susceptibility to different types of cancer (Shahbazi et al., 2002; Renner et al., 2000).

The present case–control study explored the association of **VEGF-A rs833061 (-460T>C) polymorphism** with bladder cancer susceptibility in an Indian population. A significant variation from Hardy-Weinberg equilibrium in both cases and controls group ($p = 0.0015$) suggested a non-random distribution of this polymorphism, likely driven by selective pressure or linkage disequilibrium in cancer risk. Notably, the **CT genotype** was the most common among bladder cancer patients (61.67%), followed by **TT (25%)** and **CC (13.33%)**, whereas the **CC genotype predominated** among healthy controls (75%). Allelic analysis confirmed that the **T allele frequency was significantly higher** in cancer cases (56%) compared to controls (17%) ($p = 0.0001$), indicating a Significance between the **T allele** and increased risk of bladder cancer.Multivariate analysis supported these observations across all genetic models. In the **codominant model**, individuals with the CT and TT genotypes showed markedly elevated odds of developing bladder cancer, with **ORs of 8.04 and 3.67**, respectively, while the **CC genotype appeared protective** (OR = 0.051). The **dominant model (CT+TT vs. CC)** exhibited the strongest risk signal (OR = 0.051; $p \approx 6.67\times10^{-12}$), affirming the role of the T allele as a potent risk factor. The **recessive model** (TT vs. CC+CT) showed a moderate association (OR = 0.273; $p \approx 2.60\times10^{-2}$), suggesting that even heterozygous carriers face significant risk. These findings are in line with earlier studies on VEGF-A polymorphisms in colorectal, breast, and gastric cancers, where the **-460T allele has been linked to increased transcriptional activity and VEGF protein expression** (Lu et al., 2005; Stevens et al., 2003).

Clinically, the VEGF-A rs833061 polymorphism was also significantly associated with **tumor stage and histological grade**. The **CT genotype was prevalent in early (T1, T2) and intermediate (T3) stages**, with statistical significance in all but T4 cases, likely due to small sample size. Importantly, **high-grade tumors were markedly enriched for the CT genotype (76.47%)**, further implicating this polymorphism in disease aggressiveness. Additionally, the age-based stratification revealed a significantly higher prevalence of the CT genotype in patients aged over 56 years ($p = 0.0001$), suggesting possible interactions between genetic risk and age-related biological factors.

These data highlight the potential of VEGF-A rs833061 (-460T>C) as a **molecular biomarker** for both bladder cancer susceptibility and clinical progression. Given VEGF-A's role in angiogenesis, this polymorphism could also have predictive value for anti-angiogenic therapies such as **bevacizumab or tyrosine kinase inhibitors**, which are being explored in urothelial carcinomas. However, further functional studies are needed to establish the mechanistic link between the -460T allele and VEGF expression in bladder tissue.

6. CONCLUSION

This study provides compelling evidence for a significant association between the **VEGFA rs833061 (-460T>C) polymorphism** and both the **susceptibility** and **clinicopathological progression** of bladder cancer in a North Indian population. The **T allele**, particularly in the **CT heterozygous and TT homozygous genotypes**, was found to be markedly more prevalent among bladder cancer patients compared to healthy controls. These genotypes were significantly associated with **higher tumor grade**, **advanced disease stages**, and **older age**, suggesting a contributory role of this polymorphism in both **carcinogenesis and tumor aggressiveness**.

Multivariate analysis under codominant, dominant, and recessive genetic models revealed strong and statistically significant correlations between the T allele and increased cancer risk, with the **CT genotype conferring the highest odds ratio**. Furthermore, the allelic frequency shift—favoring the T allele among patients—underscores its potential utility as a **genetic risk biomarker**.

Given VEGFA's established role in angiogenesis and its overexpression in solid tumors, the rs833061 polymorphism emerges as a biologically plausible candidate for **risk stratification**, **prognosis prediction**, and potentially even **therapeutic targeting**, especially in the context of anti-angiogenic therapies such as bevacizumab. Importantly, the association with clinical parameters like tumor stage and grade highlights its relevance not only in susceptibility but also in disease behavior.

While the study provides important insights, further research involving **larger, multi-centric cohorts** and **functional validation studies** is warranted to confirm these associations and to explore the mechanistic pathways by which rs833061 may influence VEGFA expression and angiogenic potential in bladder tissues.

In conclusion, the **VEGFA rs833061 (-460T>C) polymorphism** represents a promising candidate for genetic screening and prognostic evaluation in bladder cancer, especially in populations with similar genetic and environmental backgrounds.

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