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ORIGINAL ARTICLE

Genetic Variation in *CD166* Gene and Its Association with Bladder Cancer Risk in North Indian Population

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Abstract Adhesion molecules play a key role in cancer progression and tumorigenesis. Genetic polymorphism of adhesion molecules may alter the normal functioning thereby leading to bladder cancer susceptibility. Hence we aimed to evaluate three SNPs of *CD166* gene *(CD166rs6437585 C/T, CD166rs10511244 C/T,* and *CD166rs1157 A/G)* in bladder cancer patients and normal controls of North Indian population. A total of 270 healthy controls and 240 confirmed bladder cancer patients were recruited for this study. Three SNPs of *CD166* gene viz. *CD166rs6437585 C/T, CD166rs10511244 C/T,* and *CD166rs1157 A/G* were selected for this study. *CD166rs6437585 C/T* and *CD166rs10511244 C/T* were genotyped by Taqman allelic discrimination assay and *CD166rs1157 A/G* was genotyped by PCR–RFLP. The statistical analysis was done using the SPSS software, version 16.0 (SPSS, Chicago, IL), and $p < 0.05$ was considered statistically significant. Haplotypic analysis was done by using SNP analyzer version 1.2A. *CD166rs6437585 C/T* and *CD166rs10511244 C/T* showed significant association with reduced risk in bladder cancer while *CD166rs1157 A/G* showed significant high risk along with association at genotypic and allelic levels. Haplotypic analysis showed 1.8-folds risk in CCG combination, whereas CTA and TCG showed significant association with reduced risk. Further stratification on the basis of smoking, tumor grade/stage and BGC therapy revealed no association of these three polymorphic sites of *CD166*. Our study suggests that *CD166rs6437585 C/T* and *CD166rs10511244 C/T* are predictive for the reduced risk of bladder cancer, whereas *CD166rs1157 A/G* had shown significant association with high risk of bladder cancer in North Indians. This somehow suggests that *CD166rs1157 A/G* can be used as a marker for risk prediction of bladder cancer.

Keywords *CD166* gene (*ALCAM*) · Bladder cancer · PCR–RFLP · BCG immunotherapy

Introduction

Bladder cancer is the 9th most common cancer worldwide, with an estimated 74,000 new cases expected to occur every year [1]. Bladder cancer incidence is about 4 times higher in men than in women. Bladder cancer incidence rates decreased from 2007 to 2011 by 1.6 % per year in men and by 1.1 % per year in women. An estimated 16,000 deaths will occur in 2015, 72 % of which will be in men [1]. In males, it is the fourth most common cancer (4 % of male total), whilst it is the 13th most common cancer in females (2 % of female total) [2]. As a general prevalence, in India, out of 1,00,000 people 3.0 male and 1 female develop BC each year [3].

Cancer stem cell is a recent theory in cancer study which is being established and extensively studied [4, 5]. Cancer stem cells are the population of TICs (Tumor Initiating Cells) which have the potential of generating a whole new tumor. Studies suggest their involvement in tumor initiation, progression, relapse and metastasis [6]. One of the best ways of using CSCs in novel treatment modality is to target their surface markers. For which the surface marker needs to be extensively studied. Out of various CSC

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markers, CD166 is identified as a purported stem cell marker in various cancers [7–10].

CD166 gene, also known as activated leukocyte cell adhesion molecule (ALCAM) is located on chromosome 3(3q13.1q13.2) containing 16 exons. CD166 is a 110-kDa multi-domain type 1 transmembrane glycoprotein of the immunoglobulin super family and is highly conserved. Hanahan and Weinberg [11], described various hallmarks of cancer like limitless replicative potential, uninterrupted angiogenesis, evasion of apoptosis, self-sufficiency with respect to growth signals, resistance to anti-growth signals, tissue invasion and metastasis [11]. Adhesion molecules are involved in the hallmark processes of cancer development. They also found to play role in various physiological functions like development of different tissues during embryogenesis, it is also expressed in varied class of malignancies such as melanoma and esophageal, gynecologic, prostate, and pancreatic cancers, and its expression is associated with diverse outcomes in different tumors [12]. Therefore, modulation of the function of adhesion molecules must be studied in various cancers.

Bladder cancer is the second most common among all forms of Urogenital cancer The prevalence of BC worldwide is estimated at over a million and is increasing steadily [13]. Despite its low incidence in western countries, it is still a major problem in India. The prevalence of BC is 3:1 in men: women [14]. More than 90 % of all newly diagnosed BC cases are transitional cell carcinomas. Zhou and group reported *CD166 rs6437585* to be associated with increased risk of breast cancer among Chinese population [15]. *CD166rs10511244* is very least reported, it showed no association with gall bladder cancer risk among North Indians [16]. *CD166rs1157* is widely reported in many cancers viz. positive association with bladder cancer risk in Swedish population [17], No association with bladder cancer in Polish population [17], significant association with colon cancer risk [18] etc. Zhang et al. [19] in their review article have compiled various studies of genetic variants to be significantly associated with colon cancer risk and *CD166rs1157* is among them. The presented case–control study was performed on North Indian population and focused on the effects of putative functionally relevant SNPs in candidate gene with a strong probability to be involved in BC risk. We investigated the effects of SNPs in *CD166* gene and their contribution to BC susceptibility, tumour stage/grade and outcome after BCG immunotherapy with the aim to identify possible clinical markers. Here, we present the study of three unique genetic variants of *CD166* gene i.e. *CD166rs6437585 C/T, CD166rs 10511244 C/T,* and *CD166rs1157 A/G* in a case–control study from North India in BC.

Materials and Methods

Study Subjects

A total of 240 confirmed bladder cancer patients and 270 healthy controls were recruited in the present study. All subjects in this study were of similar ethnicity, North India. All the patients enrolled in this study were histologically confirmed bladder cancer. Those with a previous history of other cancer, cancer metastasized to other site of body from another origin and previous radiotherapy was excluded. At the same time, 270 healthy controls (Mean age = 54.5 years, M:F = 249:21) were recruited from volunteers who came to the hospital for their routine checkups, unrelated to patients and to each other were also age and ethnicity matched. The criteria for selecting controls included no evidence of any personal history of cancer or other malignant conditions or any other chronic diseases. 240 confirmed bladder cancer patients were employed in this study. The ratio of male: female among 240 patients is 211:29, with the mean age of 56.9 years. The patients were subjected to detailed demographic, clinical and pathological investigations, which contained the details of age, stage, disease history, family history and other relevant details such as smoking history, occupational history and other lifestyle factors. 5-ml blood sample was drawn into coded tubes from every subject. Informed and written consent were taken from all subjects when interviewing for the demographic details and blood sample collection. The Ethical Review Board of the Institute approved the study.

Epidemiology

An epidemiological questionnaire was designed for the participants of this study to obtain data of demographic characteristics such as occupation, smoking and other lifestyles. Individuals who smoked once a day for more than 5 years were defined as smokers. The individuals who had never smoked in their lifetime were regarded as non smokers. At the conclusion of the interview, 5 ml of blood sample was drawn into coded EDTA vials. The demographic and clinical characteristics of the patients are demonstrated in Table 1.

Clinical Data Collection

The clinical information about tumor stage and grade, intravesical therapy and dates of recurrence, radical cystectomy and pathological findings at cystectomy were provided by the urologists in our department. The classification tumor stages were as per the American Joint

Table 1 Baseline demographic and clinical characteristics of bladder cancer patients and healthy controls

Variables	Cases n = 240 n (%)	Controls n = 270 n (%)	Chi square *p* value[#]
Sex			
Female	29 (12.1)	21 (7.8)	0.105
Male	211 (87.9)	249 (92.2)	
Age (years)			
Mean age ± SD	56.96 ± 13.86	54.50 ± 10.23	0.138
Smoking[a]			
Non smokers	48 (29.6)	214 (79.3)	**<0.001**
Smokers	116 (70.4)	56 (20.7)	
Tumor grade stage			
TaG1	48 (20.0)	–	–
TaG2-3 + T1G1-3	128 (53.3)	–	
T2+	64 (26.7)	–	
Intravesical therapy			
Non treated	83 (47.7)	–	–
BCG induction (BCG i + m)	86 (52.3)	–	
Event			
Recurrence	74 (43.9)	–	–
Non-recurrence	95 (56.1)	–	

BCG i + m, Bacillus calmette-guerin induction + maintenance

Statistically significant values are shown in bold

[a] The sum could not add up to the total due to some missing values

[#] Student t-test was used to determine the *p* value

Committee on Cancer's TNM staging system [20]. Of the 240 total patients enrolled in the study, 180 patients had non muscle invasive bladder cancer (NMIBC) while the rest 60 had muscle invasive bladder cancer (MIBC). Patients with NMIBC at high risk (high grade, multiple and large tumor) were treated with intravesical *Bacillus* Calmette-Guerin (BCG) (n = 94). The patients with NMI cancer of low risk (low grade and single small tumor) were kept on cystoscopic surveillance and considered as non-BCG patients. Subsequently, all the patients were examined by cystoscopy after every 3 months in first and second years and later at 6 monthly intervals as long as there was no tumor recurrence. BCG treatment consisted of 6 weekly instillation induction BCG (n = 94). Since the number of patients receiving maintenance BCG was too low, we did not categorize the patients according to BCG regime for statistical analysis. The end point of study included tumor recurrence, defined as a newly found bladder tumor following a previous negative follow-up cystoscopy, or end of study time (60 months). Patients with invasive BC (n = 60) were treated with radical cystectomy with or without adjuvant chemotherapy, which included cisplatin, gemcitabine followed by periodical cystoscopy.

Candidate SNPs

SNP selection was based on previous studies on association between *CD166* gene and cancer in different populations [4–7, 9–13] as well as on the basis of functional properties of the gene. The functional polymorphisms within the *CD166* gene were selected by using the HapMap Project database (www.hapmap.org) based on the GIH population data of hapmap. We used certain criteria for the candidate gene polymorphisms viz., a minor allele frequency (MAF) greater than 10 % in Caucasian population; situated in the 3′UTR, 5′UTR, intronic and exonic regions of the tested genes which shows some biological significance according to the location within the gene. The LD Plot with SNPs is furnished in Fig. 1.

Three SNPs of *CD166* viz. *CD166rs6437585 C/T, CD166rs10511244 C/T,* and *CD166rs1157 A/G* were selected for the presented study. rs6437585 is present in 5′-UTR of *CD166 gene* and is found to be potentially functional in tumor progression. rs10511244 is present in intronic region of *CD166* gene and it also contributes in cancer progression. Third candidate SNP rs1157 is present in 3′-UTR and is also associated with enhancement of cancer.

Fig. 1 Linkage disequilibrium (LD) plot of *CD166* gene in Hapmap-GIH population

Genotyping

Genomic DNA was extracted from venous blood by following standard salting out method [21]. The isolated DNA was qualified and quantified by using Nanodrop spectrophotometer (Thermo Fisher Scientific/Nanodrop Products, Wilmington, Delaware, USA). Genotyping of *CD166 rs6437585 C/T* and *CD166 rs10511244 C/T* was done by using Taqman allelic discrimination assay. For the assay primers and probes were provided as pre-designed assays by Applied Biosystems (Foster City, CA). Genotyping was performed with ABI 7500HT Fast Sequence Detection System (Applied Biosystems, Foster City, CA) using 96-well plates. Positive and negative controls were used in each genotyping assay plate, and 10 % of the samples were randomly selected and run in duplicates with 100 % concordance. The results were reproducible with no discrepancy in genotyping. *CD166 rs1157 A/G* was genotyped by PCR-based restriction fragment length polymorphism (PCR–RFLP) analysis. The primer sequence used for *CD166rs1157 A/G* were adopted from a previous study [18]. Genotyping was done on 10 % Poly-Acrylamide Gel using molecular weight markers and visualized after staining with ethidium bromide. 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. The results were reproducible with no discrepancy in genotyping. About 5 % of the randomly selected samples were validated by sequencing.

Statistical Analysis

Hardy–Weinberg equilibrium (HWE) test of SNP was performed using Michael H. Court's (2005–2008) online calculator (http://www.tufts.edu/~mcourt01/Documents/Court%20lab%20-%20HW%20calculator.xls). Tests in bladder cancer patients and healthy unrelated controls did not show any significant deviation from HWE for any of the SNPs.

The power of the study was calculated using Quanto software, version 1.0 (available from: http://hydra.usc.edu/gxe). The present study achieved 80 % of the statistical power. The goodness-of-fit Chi square test was used to analyze any deviation from the Hardy–Weinberg equilibrium in controls. A binary logistic regression model was used to estimate the risk as the OR at the 95 % confidence interval. The statistical analysis was done using the Statistical Package for Social Sciences software, version 16.0 (SPSS, Chicago, IL), and $p < 0.05$ was considered statistically significant. Haplotypic analysis was done by using SNP analyzer version 1.2A.

Results

Demographic Characteristics of Study Subject

Of the 510 samples (patients and controls), there was no significant difference between the patients and controls in age ($p = 0.138$), sex ($p = 0.105$). The cases had significantly higher percentage of smokers (70.4 %) than the controls (20.7 %) ($p < 0.001$). The demographic details of the study subjects and clinical characteristics of the patients are described in Table 1.

Frequency Distribution of *CD166* Gene Polymorphism (*CD166rs6437585 C/T, CD166rs10511244 C/T,* and *CD166rs1157 A/G*)

The genotypic distributions of *CD166* gene polymorphisms in controls were in Hardy–Weinberg equilibrium. The genotypic and allelic frequencies of *CD166rs6437585 C/T, CD166rs10511244 C/T,* and *CD166rs1157 A/G* gene polymorphism and their association with BC risk is demonstrated in Table 2.

A significant association with reduced risk was found in additive model (TT; $p = 0.001$: Adjusted OR = 0.198, 95 % CI = 0.074–0.530), dominant model (CT + TT; $p = 0.007$: Adjusted OR = 0.596, 95 % CI = 0.410–0.867) and allelic model (T allele; $p < 0.001$: Adjusted OR = 0.553: 95 % CI = 0.401–0.762) of *CD166rs6437585 C/T*. Similar kind of results were seen in *CD166rs10511244 C/T,* at genotypic level in additive (TT; $p = 0.021$) as well as dominant model (CT + TT; $p = 0.028$), at allelic level, the variant allele, T showed reduced risk for BC ($p = 0.009$). Whereas, in *CD166rs1157 A/G* we found significant association with risk in heterozygous genotype, AG ($p = 0.029$, Adjusted OR = 1.517, 95 % CI = 1.043–2.206) and a marginal

association in variant genotype, GG of the additive model ($p = 0.050$, Adjusted OR = 1.751, 95 % CI = 1.000–3.065). In the dominant model of *CD166rs1157 A/G,* AG + GG, significant risk for BC was seen ($p = 0.031$, Adjusted OR = 1.566, 95 % CI = 1.100–2.230). Also, at allelic level the variant allele, G showed reduced risk to BC ($p = 0.013$, Adjusted OR = 1.388, 95 % CI = 1.071–1.799) (Table 2).

Association of *CD166* Gene Polymorphisms *CD166rs6437585 C/T, CD166rs10511244 C/T,* and *CD166rs1157 A/G* at Genotypic Level with Smoking

We correlated *CD166 gene* polymorphism (*CD166rs6437585 C/T, CD166rs10511244 C/T,* and *CD166rs1157 A/G*) with smoking habits among patients. For this we stratified patients in two groups: Smokers and non-smokers. This was analyzed by using Fischer's exact test. No association was seen in any variants of *CD166 gene* with respect to smoking (Table 3).

Association of *CD166* Gene Variants *CD166rs6437585 C/T, CD166rs10511244 C/T,* and *CD166rs1157 A/G* Genotypes with Tumor Stage/Grade of BC Patients

For this study the BC patients were stratified into three groups based on their tumor stage/grade [TaG1 (low risk NMIBC), TaG_{2-3} + $T1G_{1-3}$ (High risk NMIBC) and T2+ (muscle invasive)]. TaG_1 was taken as a reference. The patients with similar stage but with different grades respond to treatment differently. However, no association was found in any of CD166 gene variants *CD166rs6437585 C/T, CD166rs10511244 C/T,* and *CD166rs1157 A/G* with any of the tumor stage/grade of BC patients (Table 4).

Association of *CD166* Gene Variants *CD166rs6437585 C/T, CD166rs10511244 C/T,* and *CD166rs1157 A/G* Haplotypes with Bladder Cancer Risk

Haplotypic analysis could be more manifesting in predicting risk and finding the association of disease as

Table 2 Frequency distribution of *CD166* gene variants *CD166rs6437585 C/T, CD166rs10511244 C/T,* and *CD166rs1157 A/G* among bladder cancer patients and controls

Genetic model	Genotypes	Controls n = 270 n (%)	Patients n = 240 n (%)	*p* value	OR[a] (95 % CI)
CD166rs6437585 C/T					
Additive	CC	165 (61.1)	174 (72.5)	Ref	Ref
	CT	81 (30.0)	61 (25.4)	0.095	0.714 (0.481–1.060)
	TT	24 (8.9)	5 (2.1)	**0.001**	0.198 (0.074–0.530)
Dominant	CC	165 (61.1)	174 (72.5)	Ref	Ref
	CT + TT	105 (38.9)	66 (27.5)	**0.007**	0.596 (0.410–0.867)
Multiple	C	411 (76.1)	409 (85.2)	Ref	Ref
	T	129 (23.9)	71 (14.8)	**<0.001**	0.553 (0.401–0.762)
CD166rs10511244 C/T					
Additive	CC	129 (47.8)	138 (57.5)	Ref	Ref
	CT	109 (40.4)	86 (35.8)	0.108	0.738 (0.509–1.069)
	TT	32 (11.9)	16 (6.7)	**0.021**	0.467 (0.245–0.892)
Dominant	CC	129 (47.8)	138 (57.5)	Ref	Ref
	CT + TT	141 (52.2)	102 (42.5)	**0.028**	0.676 (0.477–0.960)
Multiple	C	367 (68.0)	362 (75.4)	Ref	Ref
	T	173 (32.0)	118 (24.6)	**0.009**	0.692 (0.525–0.911)
CD166rs1157 A/G					
Additive	AA	132 (48.9)	91 (37.9)	Ref	Ref
	AG	109 (40.4)	114 (47.5)	**0.029**	1.517 (1.043–2.206)
	GG	29 (10.7)	35 (14.6)	**0.050**	1.751 (1.000–3.065)
Dominant	AA	132 (48.9)	91 (37.9)	Ref	Ref
	AG + GG	138 (51.1)	149 (62.1)	**0.013**	1.566 (1.100–2.230)
Multiple	A	373 (69.0)	296 (61.7)	Ref	Ref
	G	167 (30.9)	184 (38.3)	**0.013**	1.388 (1.071–1.799)

Statistically significant values are shown in bold

[a] OR (95 % CI) age, gender adjusted odds ratio and 95 % confidence interval

Table 3 Association of *CD166* gene polymorphisms on the basis of smoking

Genotype	Patients non smokers n = 48 n (%)	Patients smoker n = 116 n (%)	*p* value	OR[a] (95 % CI)
CD166rs6437585C/T				
CC	33 (68.8)	80 (69.0)	Ref	Ref
CT	14 (29.2)	33 (28.4)	0.941	0.972 (0.462–1.048)
TT	1 (2.1)	3 (2.6)	0.856	1.237 (0.124–1.833)
CD166rs10511244C/T				
CC	25 (52.1)	64 (55.2)	Ref	Ref
CT	19 (39.6)	46 (39.7)	0.877	0.946 (0.467–1.217)
TT	4 (8.3)	6 (5.2)	0.437	0.586 (0.152–0.953)
CD166rs1157A/G				
AA	17 (35.4)	41 (35.3)	Ref	Ref
AG	28 (58.3)	55 (47.4)	0.579	0.814 (0.394–1.183)
GG	3 (6.3)	20 (17.2)	0.137	2.764 (0.725–3.043)

[a] OR (95 % CI) age, gender adjusted odds ratio and 95 % confidence interval

compared to single nucleotide polymorphism analysis. Keeping this in mind, we examined the effects of *CD166* gene variants by constructing haplotype sets, taking CCA as a reference as these three alleles are wild alleles from all three candidate SNPs.

Significant association was seen in three out of eight haplotypic combinations among these three sets one set i.e. CCG revealed significant association with about two fold risk for BC (CTA $p = \mathbf{0.034}$, OR = 0.653, 95 % CI = 0.440–0.967; CCG $p < \mathbf{0.001}$, OR = 1.844, 95 % CI = 1.308–2.599; and TCG $p = \mathbf{0.002}$, OR = 0.296, 95 % CI = 0.138–0.634), after applying Bonferroni correction (CCG pc = **0.008** and TCG pc = **0.016**) (Fig. 2).

Modulation of *CD166* Gene Variants, *CD166rs6437585 C/T, CD166rs10511244 C/T,* and *CD166rs1157 A/G* Genotypes and Outcome After BCG Immunotherapy

To analyze the association of *CD166rs6437585 C/T, CD166rs10511244 C/T,* and *CD166rs1157 A/G* gene variants and the risk of recurrence in NMIBC patients, further analysis was trammeled only to NMIBC patients (n = 180). The median follow-up of NMIBC patients was 14 months. We analyzed the association of genotypes and risk of recurrence after BCG immunotherapy. We grouped patients into BCG treated (n = 94) and non-treated (n = 86) as these were patients of low grade tumors and did not require BCG immunotherapy. None of the polymorphisms were associated with risk of recurrence free survival (Data not shown).

Discussion

Adhesion molecules play an important role in the behavior of both malignant and benign cells. These molecules are thought to be involved in tumor growth and metastases, and are therefore important to prognosis. As a result, they may be targets for novel treatment modalities [22].

In this hospital based case–control study we found significant low risk to BC in case of *CD166rs6437585 C/T* polymorphism in the CD166 promoter. Also a reduced risk was seen in case of *CD166rs10511244 C/T* polymorphism of *CD166* gene. However, we worked out a third variant of *CD166* gene, *CD166rs1157 A/G,* and found significantly high risk at genotypic as well as allelic level.

CD166 is a member of the cell surface immunoglobulin super family which is involved in cell-to-cell interactions. In a previous study, which used dot blot analysis, it was found that CD166 transcript levels were higher than normal in the tissues of five out of eight breast cancers [23]. IHC based expression on human melanoma cells revealed CD166 expression with correlation to melanoma progression [24]. CD166 expression was observed in 68 % of esophageal squamous cell carcinoma at RNA and protein level which indicates that CD166 correlates with tumor invasion and metastasis [25]. Another IHC based expression study revealed strong association of CD166 with prognosis in high grade cervical cancer [26]. CD166 was shown to be upregulated in 86 % of prostate cancer patients, suggesting its role as a good prognostic as well as diagnostic marker for prostatic cancer [27].

Our study suggested significant association of *CD166rs6437585* C/T and T/T of CD166 gene. *CD166rs6437585* C/T hetero and variant genotype were found to have OR = 1.38 for developing bladder cancer

Table 4 Association of *CD166* gene variants *CD166rs6437585 C/T, CD166rs10511244 C/T,* and *CD166rs1157 A/G* genotypes with tumor stage/grade of BC patients

Genotypes	(a) n = 48 n (%)	(b) n = 128 n (%)	(c) n = 64 n (%)	*p* value (a–b)	OR[a] (95 % CI)	*p* value (a–c)	OR[a] (95 % CI)	*p* value (b–c)	OR[a] (95 % CI)
CD166rs6437585C/T	TaG1	TaG2-3,T1G1-3	T2+						
CC	38 (79.2)	93 (72.7)	43 (67.2)	Reference		Reference		Reference	
CT	10 (20.8)	32 (25.0)	19 (29.3)	0.513	1.308 (0.585–1.922)	0.249	1.679 (0.696–2.053)	0.466	1.284 (0.655–2.517)
TT	NA	3 (2.3)	2 (3.1)	NA	NA	NA	NA	0.694	1.442 (0.232–1.947)
CD166rs10511244C/T	TaG1	TaG2-3,T1G1-3	T2+						
CC	29 (60.4)	78 (60.9)	31 (48.4)	Reference		Reference		Reference	
CT	16 (33.3)	42 (32.8)	28 (43.8)	0.947	0.976 (0.477–1.198)	0.225	1.635 (0.739–2.029)	0.110	1.677 (0.890–2.162)
TT	3 (6.3)	8 (6.3)	5 (7.8)	0.990	0.991 (0.246–1.395)	0.566	1.559 (0.342–2.116)	0.457	1.573 (0.477–2.181)
CD166rs1157A/G	TaG1	TaG2-3,T1G1-3	T2+						
AA	26 (54.2)	41 (32.0)	24 (37.5)	Reference		Reference		Reference	
AG	17 (35.4)	66 (51.6)	31 (48.4)	0.081	2.462 (1.192–3.083)	0.100	1.975 (0.878–2.445)	0.513	0.802 (0.415–1.253)
GG	5 (10.4)	21 (16.4)	9 (14.1)	0.079	2.663 (0.893–3.438)	0.286	1.950 (0.572–2.444)	0.511	0.732 (0.289–1.354)

[a] OR (95 % CI) age, gender adjusted odds ratio and 95 % confidence interval

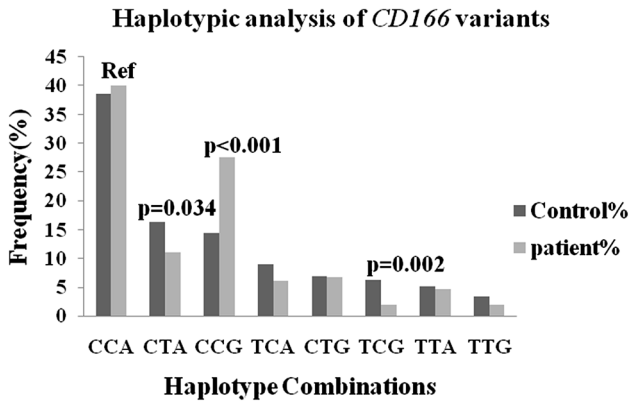

Fig. 2 Haplotype analysis of *CD166* gene variants

as compared to the wild genotype. Expression studies showed that the variant allele i.e. T allele was associated with high transcriptional activity of *CD166* gene in breast cancer [15]. Whereas Jiang et al. showed *CD166rs6437585 C/T* to be associated with increased risk of breast cancer in Chinese population. They also checked *CD166rs6437585 C/T* in various in vitro assays and found its variant allele to be involved in enhanced transcription rates of *CD166* gene. Their study also suggested involvement of *CD166rs6437585 T,* variant allele in tumor progression.

CD166rs10511244 C/T, in our study showed reduced risk for bladder cancer. Although the association and risk factors vary from ethnicity to ethnicity as well as in case of different diseases. The third SNP of *CD166* gene *CD166rs1157 A/G,* demonstrated significant high risk for bladder cancer in the present study. Supporting our study, rs1157 had shown a hazard ratio of 3.42 in Swedish population for breast cancer [17]. Being located in the 3′UTR region of *CD166* gene and having a functional role in miRNA binding [17] any aberration in rs1157 can be said to be involved in a discrepancy in normal functioning of the cell, which may lead to cancer. CD166 is a ligand for CD6, it is a cell surface scavenger receptor involved in T cell activation and proliferation, as well as in thymocyte differentiation. Chappell and group reported the binding sites on CD6 and CD166 and showed that a SNP in CD6 causes glycosylation that hinders the CD6/CD166 interaction and also suggested that how the interactions of consecutive domains can be perturbed by SNPS in ligands as well as receptors [28].

In our study, we showed that genetic variant in *CD166* gene may be of significance for the prognosis of bladder cancer. Further analyses with explanation at functional consequences of these variants on mRNA and protein expression are warranted with more number of cases and in wide ethnicity variation.

Conclusion

Our study suggests that *CD166rs6437585 C/T* and *CD166rs10511244 C/T* are predictive for the reduced risk of bladder cancer, whereas *CD166rs1157 A/G* had shown significant association with high risk of bladder cancer in North Indians. This somehow suggests that *CD166rs1157 A/G* can be used as a marker for risk prediction of bladder cancer although some more studies with larger sample size, varied ethnicity and with more advanced techniques are suggested in support of this study. Our result suggests the importance of testing large sample sizes and of performing expression studies to validate genetic associations of CD166 in bladder cancer.

To the best of our knowledge, present study is the first to report a group of three SNPs of *CD166* gene variants with bladder cancer risk in North Indian population.

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Compliance with Ethical Standards

Conflict of interest Authors have no conflicts of interest in this work.

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