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Original Article

PD1.5 variant on *PDCD1* gene, regulator of T lymphocyte activity, influences non-muscle-invasive bladder cancer risk

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Abstract: Background: Since failure in recognition of abnormal cells by the immune system has an important role in bladder cancer development and progression, this study aimed to evaluate whether PD1 (c.627+252C>T) and PD1.5 (c.804C>T) single-nucleotide variants (SNVs) in *PDCD1* gene, enrolled in modulation of T lymphocyte activity, influence risk, clinicopathological aspects, and outcome of non-muscle-invasive bladder cancer (NMIBC) patients. Material and methods: DNA genotyping by real-time polymerase chain reaction was offered to 160 non muscle invasive bladder cancer (NMIBC) patients and 250 controls. One hundred and twenty-seven patients treated with bladder transurethral resection and intravesical bacillus Calmette-Guérin were enrolled in survival analyses. Results: Individuals with PD1.5 CC genotype had 2.3-fold increased risk of developing NMIBC. Similar genotype and haplotype frequencies were seen in patients stratified by clinicopathological aspects. Patients with T allele, CT or TT plus CT or TT genotype and TT haplotype of PD1 and PD1.5 SNVs had up to 4.0-times greater chances of presenting NMIBC relapse and death by any cause than the remaining patients, but analysis of NMIBC specific survival was not possible in study due to the small number of patients evolving to death during follow up. Conclusions: Our data presented for the first time, preliminary evidence that inherited abnormality in regulation of T lymphocyte activity alters NMIBC risk.

Keywords: Bladder cancer, *PDCD1* gene, single-nucleotide variant, risk, prognosis

Introduction

Programmed death 1 (PD1) is a transmembrane receptor that belongs to the immunoglobulin superfamily and is a potent inhibitor of T lymphocytes in the presence of ligands. This receptor participates in the process of immune response regulation [1, 2]. PD1 has two ligands, PDL-1 expressed on macrophages, dendritic cells, and mesenchymal stem cells, and PDL-2 expressed on macrophages, dendritic cells, and peritoneal cells [1-3]. PDL-1 and PDL-2 are also expressed on tumor cells [4, 5]. When PDL-1 or PDL-2 binds to PD1 occurs inhibition of proliferation and apoptosis activation of T lymphocytes, having the survival and proliferation of tumor cells as consequence [1-3] (**Figure 1**).

The regulation of T-lymphocyte activity by PD1 pathway is variable in humans, since PD1 is encoded by *PDCD1* polymorphic gene [6]. PD1 (c.627+252C>T) single-nucleotide variant (SNV) is characterized by a C>T substitution in intron 4 of *PDCD1*, which provides a lower affinity for NF-kB and RUNX1 transcription factors [7].

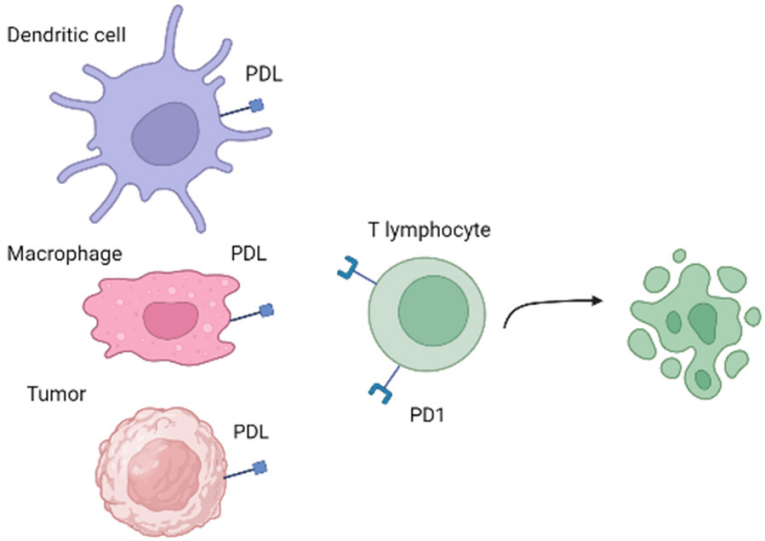

Figure 1. PD1 receptor on T lymphocytes has PDL-1 or PDL-2 ligands and this mechanism regulates lymphocyte activity. PDL is expressed in dendritic cells, macrophages, or tumor cells. The contact of receptor and linkers inhibits proliferation and activates apoptosis of T lymphocytes, and tumor cells grow and proliferate due to escape from the immune system.

PD1.5 (c.804C>T) SNV is characterized by a C>T substitution in exon 5 of *PDCD1*, which produces a silent mutation (alanine by alanine) at position 268 of PD1 protein and seems to reduce protein expression through linkage disequilibrium with other SNVs in the same gene [8]. PD1.5 allele C was associated with decreased *PDCD1* expression in lymphocytes and PD1 expression in $TCD4^+$ lymphocytes in a study conducted by our group, but similar protein expressions were seen in carriers of distinct alleles of PD1 SNV [9].

Recently, we described associations of PD1 and PD1.5 SNVs with risk of melanoma and survival of melanoma patients [9], and PD1.5 with risk of Hodgkin lymphoma [10]. Since bladder cancer [11], melanoma [4], and Hodgkin lymphoma [12] are characterized by high PDL expression, this study aimed to verify whether PD1 and PD1.5 SNVs alter bladder cancer origin, clinicopathological manifestations and prognosis of patients with bladder cancer.

Materials and methods

Study population

This prospective study included 160 non-muscle invasive bladder cancer (NMIBC) patients at diagnosis and 250 controls (blood donors) attended from April 1996 to May 2015 for analysis of tumor risk and clinicopathological aspects. Inclusion criteria were age ≥ 18 years with histopathological diagnosis of NMIBC according to the WHO classification [13]. Patients with upper tract urothelial carcinoma and those who did not accept to participate in the study were excluded from the analysis. For survival analysis, 127 patients with NMIBC treated with bladder transurethral resection (TUR) and intravesical bacillus Calmette-Guérin (BCG) [14] were followed for a median of 60 months. The study was approved by the Ethics Committee of University Hospitals (processes #90803618.2.0000.5404, #90803618.2.3002.5440, #90803618.2.3001.0082).

Candidate SNV selection

The genetic polymorphism data of the entire sequence of *PDCD1* was obtained from the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/). First, all SNVs on *PDCD1* were filtered according to the minor allele frequency ≥ 0.05 in the HapMap global population. Subsequently, two SNVs located in potential functional regions and with described association with risk and/or survival of cancer patients were selected for the study: PD1 (c.627+252C>T, rs41386349) and PD1.5 (c.804C>T, rs2227981).

Genotyping

PD1 (rs41386349) and PD1.5 (rs2227981) genotypes were analyzed by real-time polymerase chain reaction (RT-PCR) in genomic DNA obtained from leukocytes of peripheral blood of patients and controls using SNV Genotyping Assays from Applied Biosystems®. In this assay, we used first probe-labeled with VIC fluorescent dye for detection of the first allele sequence and the second probe-labeled with FAM fluorescent dye for detection of the second allele. In this way, we identify if the sample is homozygous for allele 1, homozygous for allele 2 or heterozygous. PCR was performed with 15 ng of DNA, 0.5 µl of SNP Genotyping Assay Mix 1X (two pairs of probes and two

detector fluorochromes: FAM® and VIC®) and 5 µl of TaqMan® Universal PCR Master Mix 1X, which contains AmpliTaqGold® DNA polymerase, deoxynucleotide (dNTPs) and buffer with reaction optimizers and H_2O for a final volume of 10 µl. Amplification conditions consisted of initial activation of AmpliTaq Gold® at 95°C for 10 minutes, followed by 50 cycles of incubation at 92°C for 15 seconds and at 60°C for 1 minute. The amount of 10% of genotype determinations were replicated in independent experiments with 100% of concordance.

Statistical analysis

Genotype frequencies were checked for the Hardy-Weinberg equilibrium (HWE). Multivariate analysis using logistic regression model was performed to obtain age, gender and smoking habit adjusted odds ratios (OR) with a 95% confidence interval (95% CI) in patients and controls comparisons. To ensure the stability of model, bootstrapping (N = 1000) based on repeatedly random sampling was used.

Recurrence-free survival (RFS) and overall survival (OS) were estimated with Kaplan-Meier method, the log-rank test and Cox proportional hazards regression model. All reported *p* values < 0.05 indicated statistical significance.

All tests were performed using SPSS 21.0 software (SPSS Incorporation, Chicago, IL, USA).

Results

The median age of NMIBC patients was 65 years, 124 were males, 133 were smokers, 51 had a low-grade tumor, and 102 had a high-grade tumor. The median age of controls was 47 years, 134 were males, and 26 were smokers. Compared to controls, NMIBC patients were older, predominantly males and smokers (Table S1).

Controls and patients' samples were in HWE at PD1 (X^2 = 3.79, P = 0.05; X^2 = 0.77, P = 0.38) and PD1.5 (X^2 = 0.70, P = 0.40; X^2 = 0.01, P = 0.92) loci, respectively. The analyses revealed a strong LD between PD1 and PD1.5 SNVs (D' = 0.93).

PD1.5 CC genotype was more common in patients than in controls; CC genotype carriers were 2.3-fold increased risk of developing NMIBC than others (**Table 1**). Similar genotype and haplotype frequencies were seen in patients stratified by clinicopathological aspects (Table S2).

The median follow-up time of 127 available patients was 60 months. At the end of the study, 106 patients were alive (105 without BC, 1 patient with BC), and 21 died (5 of BC, 2 of lung cancer, 1 of esophagus cancer, 1 of prostate cancer, 1 of gastric cancer, and 11 of unrelated causes). At 60 months of follow-up, no differences in RFS were seen in patients stratified by clinicopathological aspects and alleles, genotypes, and haplotypes of PD1 and PD1.5 SNVs. Lower OS was observed in older patients (70.0 vs. 94.5%, P = 0.002), males (76.9 vs. 96.2%, P = 0.01), and patients with high-grade tumors (74.6 vs. 95.1%, P = 0.009), PD1 allele T (57.2 vs. 83.6%, P = 0.003), PD1.5 allele T (77.6 vs. 84.0%, P = 0.04), PD1 CT or CT plus PD1.5 CT or TT combined genotypes (63.3 vs. 86.8%, P = 0.03), and TT haplotype (57.2 vs. 83.6%, P = 0.003) compared to others (Kaplan-Meier estimates), and the results remained the same in univariate analysis. In multivariate analysis, older patients, and patients with PD1 and PD1.5 allele T, PD1 CT or TT plus PD1.5 CT or TT combined genotypes, and TT haplotype had 4.24, 2.90, 1.96, 3.81 and 2.90 more chances of evolving to death than others, respectively (**Table 2**).

Discussion

We investigated herein for the first time whether SNVs involved in PD1 pathway alter risk, clinicopathological aspects and outcome of patients with NMIBC.

We found that individuals with PD1.5 CC genotype were under increased risk of developing NMIBC. Similar associations were found in Hodgkin lymphoma [8] and gastrointestinal, lung, and breast cancers [15]. In fact, this association was not surprising since low expressions of *PDCD1* in leukocytes and PD1 in $TCD4^+$ lymphocytes were previously seen in carriers of PD1.5 allele C [9]. PD1 is produced only after activation and differentiation of naïve lymphocytes in effectors T cells [16], so it's possible that PD1.5 allele C is associated with a low number of circulating effector T lymphocytes and a decreased ability of the immune system for identifying and destroying abnormal cells, leading to NMIBC development consequently.

Table 1. PD1 and PD1.5 genotypes, alleles, and haplotypes in 160 non-muscle-invasive bladder cancer patients and 250 controls

Genotype/allele/haplotype	Patients N (%)	Controls N (%)	*p* value	OR[a] (95% CI)
PD1 (c.627+252C>T)				
CC	135 (84.4)	213 (85.2)	0.87	1.35 (0.03-51.07)
CT	23 (14.4)	33 (13.2)	0.97	1.08 (0.02-59.49)
TT	02 (1.2)	04 (1.6)		Reference
CC	135 (84.4)	213 (85.2)	0.91	1.06 (0.37-2.99)
CT or TT	25 (15.6)	37 (14.8)		Reference
CC or CT	158 (98.8)	246 (98.4)	0.88	1.31 (0.03-50.76)
TT	02 (1.2)	04 (1.6)		Reference
Allele C	0.92	0.92	0.94	1.03 (0.39-2.70)
Allele T	0.08	0.08		Reference
PD1.5 (c.804C>T)				
CC	66 (41.3)	85 (34.0)	0.61	1.35 (0.40-4.52)
CT	74 (46.2)	127 (50.8)	0.30	1.85 (0.56-6.07)
TT	20 (12.5)	38 (15.2)		Reference
CC	66 (41.3)	85 (34.0)	**0.04**[b]	2.30 (1.01-5.26)
CT or TT	94 (58.7)	165 (66.0)		Reference
CC or CT	140 (87.5)	212 (84.8)	0.66	1.27 (0.43-3.75)
TT	20 (12.5)	38 (15.2)		Reference
Allele C	0.64	0.59	0.24	1.38 (0.80-2.39)
Allele T	0.36	0.41		Reference
PD1 + PD1.5				
CC + CC	66 (72.5)	83 (70.3)	0.34	1.76 (0.54-5.68)
CT or TT + CT or TT	25 (27.5)	35 (29.7)		Reference
CC or CT + CC or CT	140 (98.6)	212 (98.1)	0.87	1.37 (0.03-60.47)
TT + TT	02 (1.4)	04 (1.9)		Reference
Haplotype				
CC	0.64	0.59	0.23	1.39 (0.80-2.39)
Others	0.36	0.41		Reference
CT	0.27	0.33	0.22	1.42 (0.80-2.53)
Others	0.73	0.67		Reference
TT	0.08	0.08	0.96	1.02 (0.38-2.69)
Others	0.92	0.92		Reference

Abbreviations: [a]OR, odds ratio adjusted by age, gender, and smoking habit by multivariate analysis; CI, confidence interval. *P* value < 0.05 is presented in bold letters; [b]*p* bootstrap = 0.04.

Similar frequencies of PD1 and PD1.5 genotypes and haplotypes were seen in patients stratified by clinicopathological aspects in this study. There are no other studies on the roles of PD1 and PD1.5 SNVs in clinicopathological aspects of NIMIB patients and thus the data suggest that they are not modified by the inherited abnormalities in regulation of T lymphocyte activity.

We also noted that patients with PD1 allele T, PD1.5 allele T, PD1 CT or TT plus PD1.5 CT or TT combined genotypes, and TT haplotype had up to 4.0-times greater chances of evolving to death from any cause than others, but analysis of the SNVs in NMIBC specific survival was not possible in study due to the small number of patients evolving to death during follow up (n = 5). PD1 did not alter OS in myeloid leukemia [17], renal carcinoma [18], and melanoma [9]. OS of esophageal carcinoma was not altered by PD1.5 [19], but melanoma patients with PD1.5 CC genotype had more chances of evolving to death by tumor effects [9]. Differences between

Table 2. Clinicopathological aspects and PD1 and PD1.5 genotypes of *PDCD1* gene in relapse-free survival and overall survival of 127 non-muscle-invasive bladder cancer patients

Characteristics	Relapse-free survival					Overall survival				
	Univariate analysis			Multivariate analysis		Univariate analysis			Multivariate analysis	
	N event/ N total	HR (95% CI)	*p* value	HR (95% CI)	*p* value	N event/ N total	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Median age (years)										
≤ 65	23/60	Reference	0.69	NA		04/60	Reference	**0.005**	Reference	**0.01**
> 65	23/67	1.12 (0.62-2.00)				17/67	5.02 (1.63-15.45)		4.24 (1.39-12.88)	
Gender										
Male	34/97	1.02 (0.52-1.97)	0.95	NA		20/97	7.75 (1.03-57.95)	**0.04**	5.56 (0.73-42.34)	0.09
Female	12/30	Reference				01/30	Reference		Reference	
Skin color										
White	42/119	1.38 (0.49-3.87)	0.53	NA		20/119	1.66 (0.22-12.39)	0.62	NA	
Non-white	04/08	Reference				01/08	Reference			
Smoking habit										
Yes	37/103	1.02 (0.49-2.13)	0.94	NA		17/103	1.05 (0.35-3.12)	0.92	NA	
No	09/24	Reference				04/24	Reference			
Tumor grade										
Low-grade	19/43	Reference	0.29	NA		02/43	Reference	**0.02**	Reference	0.13
High-grade	27/82	1.37 (0.76-2.46)				19/82	5.58 (1.29-23.99)		3.12 (0.71-13.74)	
PD1 (c.627+252C>T)										
CC	37/107	Reference	0.12	Reference	0.12	16/107	Reference	0.08	Reference	0.12
CT or TT	09/20	1.77 (0.85-3.70)		1.77 (0.85-3.70)		05/20	2.48 (0.89-6.89)		2.21 (0.79-6.17)	
Allele C	82/232	Reference	0.10	Reference	0.10	35/232	Reference	**0.005**	Reference	**0.01**
Allele T	10/22	1.73 (0.89-3.35)		1.73 (0.89-3.35)		07/22	3.22 (1.41-7.33)		2.90 (1.27-6.61)	
PD1.5 (c.804C>T)										
CC	20/52	Reference	0.88	NA		06/52	Reference	0.10	Reference	0.09
CT or TT	26/75	1.04 (0.58-1.87)				15/75	2.18 (0.84-5.66)		2.27 (0.87-5.95)	
Allele C	61/163	Reference	0.71	NA		22/163	Reference	0.05	Reference	**0.03**
Allele T	31/91	1.08 (0.70-1.67)				20/91	1.82 (1.00-3.35)		1.96 (1.06-3.61)	
PD1 plus PD1.5										
CC + CC	20/52	Reference	0.31	NA		06/52	Reference	**0.04**	Reference	**0.03**
CT or TT + CT or TT	09/20	1.50 (0.68-3.32)				05/20	3.47 (1.04-11.58)		3.81 (1.14-12.77)	
Haplotype										
CC	61/163	0.92 (0.59-1.42)	0.71	NA		22/163	0.54 (0.29-1.00)	**0.04**	0.52 (0.28-0.96)	**0.03**
Others	31/91	Reference				20/91	Reference		Reference	
CT	21/69	1.37 (0.84-2.24)	0.19	NA		13/69	1.19 (0.62-2.29)	0.59	NA	
Others	71/185	Reference				29/185	Reference			
TT	10/22	1.73 (0.89-3.35)	0.10	1.73 (0.89-3.35)	0.10	07/22	3.22 (1.41-7.33)	**0.005**	2.90 (1.27-6.61)	**0.01**
Others	82/232	Reference		Reference		35/232	Reference		Reference	

Abbreviations: HR, hazard ratio; CI, confidence interval; NA, characteristic not inserted in multivariate analysis. Genotype combination in recessive model was not presented due to a biological limitation (only two patients with PD1 TT genotype and five patients with PD1.5 TT genotype had events). *P* values < 0.05 are presented in bold letters.

studies might be explained by the heterogeneity of tumors and microenvironments. To explain our data, we initially postulated that PD1.5 allele T can increase PD1 protein in activated T lymphocytes [7] and PD1 allele T may induce structural modification on its receptors, leading to lymphocyte apoptosis, immunologic escape, and tumor expansion, as described [20]. However, as PD1 and PD1.5 SNVs did not alter RFS and NMIBC specific survival was not obtained, we cannot attribute the greater chance of evolving to death in patients with specific alleles, genotype, and haplotype to NMIBC recurrence or systemic effects determined by inherited deficiencies in T lymphocytic activity. The complexity of immune mechanisms activated by BCG in the innate and adaptative responses of tumor microenvironment may also have modified our patients' survival [20, 21].

Conclusion and future perspectives

We present for the first time, preliminary evidence that PD1.5 SNV alter risk of NMIBC development.

We are aware that a relatively small number of patients and controls were enrolled in analysis of NMIBC risk in the current study, and evaluation of NMIBC specific survival was not possible due to the small number of patients evolving to death by the tumor effects. Further larger epidemiological studies should be conducted to confirm the association of PD1.5 SNV with risk of NMIBC and to establish the roles of PD1 and PD1.5 SNVs in prognosis of patients with NMIBC. Functional studies should also be conducted to elucidate the roles of SNVs in T lymphocyte activity in development and progression of NMIBC. Moreover, we consider that associations PD1 and PD1.5 SNVs with the most aggressive form of bladder cancer, the muscle invasive bladder cancer, are of great interest to the area of knowledge and will be conducted in a future study by our group.

Disclosure of conflict of interest

None.

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Table S1. Clinical and tumor aspects of 160 patients with non-muscle-invasive bladder cancer and clinical aspects of 250 controls

Variable	Patients Median (range), N (%)	Controls Median (range), N (%)	*p* value
Age (years)	65 (39-86)	47 (19-63)	**< 0.01**
Gender			
Male	124 (77.5)	134 (53.6)	**< 0.01**
Female	36 (22.5)	116 (46.4)	
Skin color			
White	147 (91.9)	229 (91.6)	0.92
Non-white	13 (8.1)	21 (8.4)	
Tobacco consumption			
Smokers	133 (83.1)	26 (10.4)	**< 0.01**
Nonsmokers	27 (16.9)	223 (89.6)	
Tumor grade[a]			
Low-grade	51 (33.3)	NA	NA
High-grade	102 (66.7)	NA	NA
Treatment[b]			
TUR	127 (100.0)	NA	NA
Intravesical BCG	55 (43.3)	NA	NA
Follow-up time (months)[b]	60 (4.0-119.0)	NA	NA

Abbreviations: TUR, transurethral resection; BCG, bacillus Calmette-Guérin; NA, not applicable. [a]Tumor grade was identified using criteria of the World Health Organization Classification of Tumors of the Urinary System and Male Genital System (WHO 2004). In some cases, it was not possible to obtain information. [b]The number of patients were not the same included in the study, because there are patients who received both therapies. *P* values < 0.05 are presented in bold letters.

Table S2. PD1 and PD1.5 genotypes in 160 non-muscle-invasive bladder cancer patients stratified by clinical aspects

Genotypes	Age in years N (%)		Gender N (%)		Skin color N (%)		Smoking habit N (%)		Tumor grade[a] N (%)	
	≤ 65	> 65	Male	Female	White	Non-white	Yes	No	Low-grade	High-grade
PD1 (c.627+252C>T)										
CC	67 (83.8)	68 (85.0)	103 (83.1)	32 (88.9)	126 (85.7)	09 (69.2)	113 (85.0)	22 (81.5)	89 (83.2)	44 (86.3)
CT + TT	13 (16.2)	12 (15.0)	21 (16.9)	04 (11.1)	21 (14.3)	04 (30.8)	20 (15.0)	05 (18.5)	18 (16.8)	07 (13.7)
p value	0.82		0.60		0.12		0.77		0.81	
CC + CT	79 (98.7)	79 (98.7)	122 (98.4)	36 (100.0)	145 (98.6)	13 (100.0)	132 (99.2)	26 (96.3)	106 (99.1)	50 (98.0)
TT	01 (1.3)	01 (1.3)	02 (1.6)	0 (0.0)	02 (1.4)	0 (0.0)	01 (0.8)	01 (3.7)	01 (0.9)	01 (2.0)
p value	1.00		1.00		1.00		0.31		0.54	
PD1.5 (c.804C>T)										
CC	33 (41.2)	33 (41.2)	49 (39.5)	17 (47.2)	59 (40.1)	07 (53.8)	55 (41.4)	11 (40.7)	42 (39.3)	23 (45.1)
CT + TT	47 (58.8)	47 (58.8)	75 (60.5)	19 (52.8)	88 (59.9)	06 (46.2)	78 (58.6)	16 (59.3)	65 (60.7)	28 (54.9)
p value	1.00		0.40		0.38		0.95		0.48	
CC + CT	68 (85.0)	72 (90.0)	107 (86.3)	33 (91.7)	130 (88.4)	10 (76.9)	119 (89.5)	21 (77.8)	94 (87.9)	44 (86.3)
TT	12 (15.0)	08 (10.0)	17 (13.7)	03 (8.3)	17 (11.6)	03 (23.1)	14 (10.5)	06 (22.2)	13 (12.1)	07 (13.7)
p value	0.47		0.56		0.21		0.11		0.80	
PD1 + PD1.5										
CC + CC	33 (71.7)	33 (73.3)	49 (70.0)	17 (81.0)	59 (73.8)	07 (63.6)	55 (73.3)	11 (68.8)	42 (70.0)	23 (76.7)
CT + TT + CT + TT	13 (28.3)	12 (26.7)	21 (30.0)	04 (19.0)	21 (26.2)	04 (36.4)	20 (26.7)	05 (31.2)	18 (30.0)	07 (23.3)
p value	0.86		0.41		0.48		0.76		0.62	
CC + CT + CC + CT	68 (98.6)	72 (98.6)	107 (98.2)	33 (100.0)	130 (98.5)	10 (100.0)	119 (99.2)	21 (95.5)	94 (98.9)	44 (97.8)
TT + TT	01 (1.4)	01 (1.4)	02 (1.8)	0 (0.0)	02 (1.5)	0 (0.0)	01 (0.8)	01 (4.5)	01 (1.1)	01 (2.2)
p value	1.00		1.00		1.00		0.28		0.54	
Haplotypes										
CC	101 (63.1)	105 (65.6)	156 (62.9)	50 (69.4)	189 (64.3)	17 (65.4)	174 (65.4)	32 (59.3)	136 (63.6)	67 (65.7)
Others	59 (36.9)	55 (34.4)	92 (37.1)	22 (30.6)	105 (35.7)	09 (34.6)	92 (34.6)	22 (40.7)	78 (36.4)	35 (34.3)
p value	0.64		0.30		1.00		0.38		0.71	
CT	45 (28.1)	42 (26.2%)	69 (27.8%)	18 (25.0)	82 (27.9)	05 (19.2)	71 (26.7)	16 (29.6)	59 (27.6)	27 (26.5)
Others	115 (71.9)	118 (73.8%)	179 (72.2%)	54 (75.0)	212 (72.1)	21 (80.8)	195 (73.3)	38 (70.4)	155 (72.4)	75 (73.5)
p value	0.70		0.63		0.49		0.65		0.83	
TT	14 (8.8)	13 (8.1)	23 (9.3)	04 (5.6)	23 (7.8)	04 (15.4)	21 (7.9)	06 (11.1)	19 (8.9)	08 (7.8)
Others	146 (91.2)	147 (91.9)	225 (90.7)	68 (94.4)	271 (92.2)	22 (84.6)	245 (92.1)	48 (88.9)	195 (91.1)	94 (92.2)
p value	0.84		0.47		0.25		0.42		0.83	

[a]Tumor grade was identified using the criteria of the World Health Organization Classification of Tumors of the Urinary System and Male Genital System criteria (WHO 2004).