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ORIGINAL ARTICLE

Association of PD-1 and PDL-1 gene polymorphisms with colorectal cancer risk and prognosis

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Abstract

Background Programmed Cell Death-1 (PD-1) together with Programmed Death Ligand 1 (PDL-1) have crucial roles in anti-tumor immune response, cancer susceptibility and prognosis. Since PD-1 and PDL-1 have been considered as important genetic risk factors in cancer development and their functions can be affected by polymorphic sites, we investigated the effects of PD-1 rs2227981, rs2227982, rs36084323 and PDL-1 rs2282055, rs822336 gene polymorphisms on colorectal cancer (CRC) risk and prognosis in Turkish subjects.

Methods and results Our study group consisted of 5-FU or Capacitabine prescribed CRC diagnosed patients and healthy controls. Genotype analyses of PD1 and PDL-1 polymorphisms were performed with Agena MassARRAY platform. rs36084323 CT genotype frequency was found to be higher in controls compared to cases ($p < 0.001$). rs36084323 CT genotype was highly associated with reduced CRC risk compared to CC genotype (OR 0.068, 95% CI 0.022–0.211, $p < 0.001$). In adjusted analysis, rs2282055 GG genotype was found to be associated with reduced CRC risk (OR 0.271, 95% CI 0.078–0.940, $p = 0.040$). rs2282055 TT genotype was found to be related to longer progression-free (Bonferroni corrected Log rank $p = 0.013$) and overall survival (Bonferroni corrected Log rank $p = 0.009$) to that of GG genotypes. Patients with rs822336 GC+CC genotypes showed longer overall survival times compared to GG (Log rank $p = 0.044$).

Conclusions According to our results, PD-1 rs822336 G > C polymorphism might be useful in predicting CRC prognosis. PDL-1 rs2282055 T > G polymorphism might be useful in predicting both CRC risk and prognosis. Further studies should be conducted in larger and different populations to clear the roles of PD-1 and PDL-1 polymorphisms in CRC risk and prognosis.

Keywords Colorectal cancer · Immune check point · PD-1 · PDL-1 · rs36084323 · rs822336 · rs2282055

Introduction

Despite of advances in treatment regimens, colorectal cancer (CRC) is still among the leading causes of death and disease worldwide. CRC is the third most common and diagnosed cancer type, and it is the fourth most common cause of cancer related death [1, 2]. CRC is also the 3rd common cancer among women and men [3]. In both 2018 and 2019, aggressive CRC was the 4th leading cause of death in Turkish population with the mortality rate 7.6% and 7.4%, respectively [4]. In the light of available data, the prevalence of CRC is expected to increase by 60% worldwide until 2030 [5].

Under normal physiological conditions, certain tumor cells in the initial stage can be recognized by immune system elements as abnormal cells, infiltrated and finally eliminated. However, tumor cells are able to develop different mechanisms such as suppressing the function of immune

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cells in order to escape from immune elements [6, 7]. Escaping from the immune system plays very important role in cancer development [8]. T cells are thought to play key role for tumor cells to escape of from the host immune system by CD28/B7 family members, Programmed Cell Death-1, (PD-1) and its ligands Programmed Death Ligand 1/2, (PDL-1/2) which involve T cell activation [9, 10].

PD-1, which is encoded by PDCD1 (Programmed cell death protein 1 gene), localized on 2q37.3, belongs to the Ig superfamily, is a trans-membrane protein of about 50 ~ 55 kDa consisting 288 amino acids and functions as an immunosuppressive receptor [11–13]. As a negative regulator of the immune system, PD-1 is expressed in a variety of activated immune cells such as $TCD4^+$, $TCD8^+$, B, natural killer, and $TCD4^-$ $CD8^-$ cells, activated monocytes, dendritic cells and macrophages [14, 15].

Programmed death ligand 1 (PDL-1), also known as CD274 or B7-H1, plays an important role in maintaining peripheral and central immune tolerance by binding to the PD-1 receptor [16]. PDL-1, encoded by the PDCDL1 gene, is located on chromosome 9 (p24.1) and is a 33 kDa glycoprotein consisting of 290 amino acids with Ig and IgC-like domains [17]. PDL-1 is constitutively expressed on the surface of professional antigen presenting cells (APCs) such as macrophages, B cells, specialized DCs, and non-professional APCs that presenting antigen to cytotoxic $CD8^+$ T cells [11]. PDL-1 is also commonly expressed on malignant tumor cells to target PD-1 receptors and activates the PD-1/PDL-1 immune checkpoints that cause inhibition of cytotoxic T cell response and evade host immune surveillance [18].

The interaction between PD-1 and PDLs is crucial for proper peripheral tolerance and autoimmunity [19]. PD-1/PDL-1 pathway provides protective immune responses, maintaining T cell homeostasis, and sustaining self-tolerance by regulating the balance between stimulating and inhibitory signals in normal physiological condition [20]. However, cancer cells may have the ability to escape from surveillance of the immune system by limiting normal anti-tumor immune responses through the PD-1/PDL-1 pathway [21].

The pivotal roles of PD-1 and PDL-1 in anti-tumor immune responses and immune surveillance make them considered as important genetic risk factors in cancer susceptibility and prognosis. Since both PD-1 and PDL-1 have polymorphic sites, recent studies focused on the effects of PD-1 and PDL-1 gene polymorphisms on development and prognosis of various tumors including colon cancer [22–29]. Studies have also shown that differences in expression levels of both PD-1 and PDL-1 are associated with tumor prognosis and risk [11, 30]. However, the effects of PD-1 and PDL-1 variations on gene expression levels and cancer development, especially colorectal cancer, have not been fully elucidated.

Since, there is a strong link between inflammation and CRC [31], we believe it is crucial to understand the connection between PD-1/PDL-1 polymorphisms and CRC characteristics. Thus, in the current study, we genotyped PD-1 rs2227981, rs2227982, rs36084323, PDL-1 rs2282055 and rs822336 polymorphisms in CRC patients and healthy controls and investigated the relationship between the PD-1/PDL-1 polymorphisms with CRC risk and prognosis.

Materials and methods

Study population

This study was approved by Marmara University Local Ethical Committee with the protocol number 09.2018.174. The study subjects consisted a total of 103 sporadic CRC patients and 86 controls. Cases were selected randomly from histologically confirmed colorectal adenocarcinoma patients without the history of former inflammatory bowel disease or any of the known hereditary cancers. Controls, who visited the hospital during sampling period, were selected randomly from volunteers meeting the criteria of being without history of CRC and other malignancy or autoimmune disorders. Both case and control subjects signed a well written consent complying with the ethical criteria in the Helsinki Declaration. 5 ml EDTA blood samples were recruited from each patient at Group Florence Nightingale Sisli Hospital, Medical Oncology Department and control subjects were recruited in the same hospital at other departments. All of our patients were treated with 5-fluorouracil (5-FU) or capacitabine based mono or combined therapies during research period and blood samples were recruited just before the treatment. Following the sampling, the blood regimens were transferred under suitable cold conditions to Marmara University Molecular Metabolism Research Laboratory for genomic DNA isolation on the same day.

Together with the blood samples demographic and clinical data of the subjects have been recorded. For all subjects the data about gender, smoking status (current, former, never) and age were recorded as self-reported data during in-person interview; whereas weight, length, etc. data were collected by measuring. Body–mass index (BMI) was calculated for each patients by dividing their weight (kg) to square of height (m^2). Together with these data, primary tumor site [right (cecum, ascending colon, hepatic flexure, and transverse colon) or left (splenic flexure, descending colon, sigmoid colon, and rectum)], therapy regimens, metastasis status, number of metastatic sites (organs), K-RAS mutation status were recorded for patients. Overall survival of patients was defined as the date from the beginning of the treatment until death from any cause and survivors were censored at the date of last contact. Progression-free survival times were

calculated considering the date of treatment starts to first observation of disease progression, death from any cause or last contact.

Genotyping

Genomic DNA was isolated from peripheral blood using Kurabo, Quick Gene DNA Whole Blood Kit. DNA samples with sufficient quantity and quality were aliquoted and stored at − 20 °C for short and − 80 °C for long term in case of possibility where the analysis must be repeated. Genotyping was performed with Agena MassARRAY platform. The platform combines iPLEX and Mass ARRAY technology (Agena Bioscience, San Diego, CA, USA) which is based on MALDI-TOF Mass Spectrometry assay. To design forward, reverse and single base extension primers Agena Bioscience online tool, Assay Design Suite software version 2.0 was utilized. PCR reaction was performed with 10 ng/µl DNA samples, dNTPs, forward and reverse primers, reaction buffers, and DNA polymerase (Agena iPLEX Gold Genotyping Kit). After PCR reaction, to neutralize the uncombined deoxynucleotides, PCR mixture was treated with shrimp alkaline phosphatase (SAP) (Agena). After SAP treatment a second PCR, iPLEX extension, was performed with mass modified ddNTPs and extension primer. PCR conditions were performed according to the protocol reported by Gabriel et al. [32]. After the final PCR, for desalting the iPLEX Extension reaction, PCR products were treated with resin and then transferred to 384 well Spectro-CHIP using Mass ARRAY Nanodispenser. Spectro-CHIPs were transferred to MALDI-TOF mass spectrometry analyzer and the data analyzed by Typer Analyzer v 4.0 software. Assay plate included both positive and negative controls. Electropherogram examples for genotype calls were given in Fig. 1.

Statistical analysis

Statistical analysis was performed using IBM SPSS statistics version 26. Data are expressed as numbers and percentage for discrete variables as mean±standard error (SE) (min-max) for continuous variables. $\chi 2$ tests were performed to analyze the accordance of genotype distributions with Hardy–Weinberg equilibrium. $\chi 2$ or Fisher's Exact test was used to determine the genotype frequencies between cases and controls. Binary logistic regression analysis was used to estimate odds ratio (OR) and 95% confidence intervals (CI). Binary logistic regression analysis was performed as both unadjusted and adjusted in sex, age and smoking. Kolmogorov Smirnov and Shapiro Wilk test was used to determine whether the distributions of continuous measured variables were normal. Log-Rank test was used for comparisons of survival times and the Bonferroni correction was performed to adjust multiple comparison values with significant results. p values below 0.05 were considered significant.

Results

Demographic data of the case and control groups and clinical data of the cases were given in Table 1. Participation rates for cases and controls were slightly different from each other (68.21% and 65.15%) and did not differ notably in cases by gender, but in controls men were slightly higher. The number of both female and male individuals was found to be higher in the case group compared to the control group. BMI values were higher in the control group. Smoking status during or before the study was higher in cases compared to controls.

Genotype frequencies of PD-1 rs2227982, PD-1 rs36084323, PDL-1 rs2282055 and PDL-1 rs822336

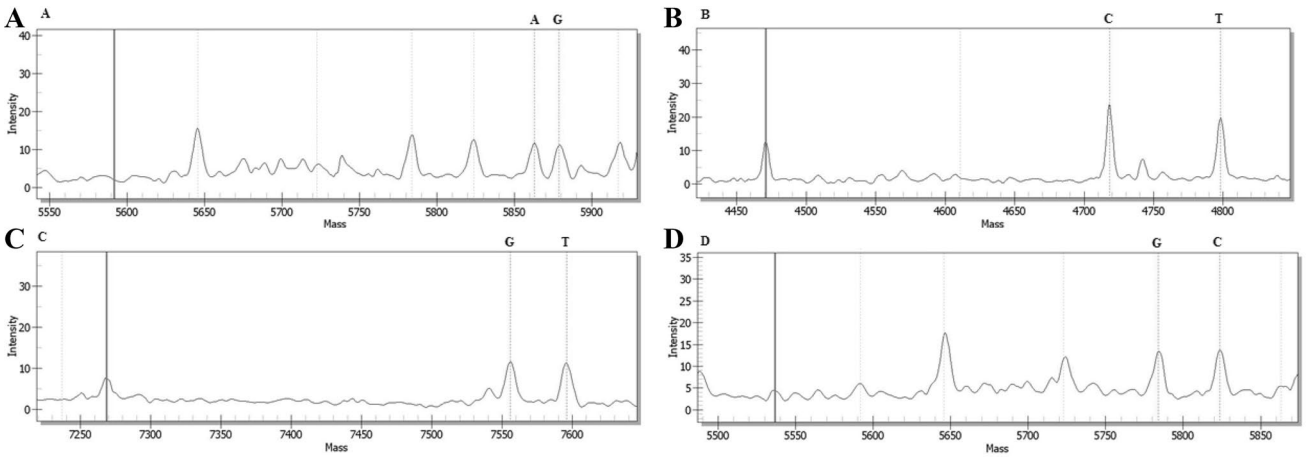

Fig. 1 Example electropherograms for genotype calls. **A** PD-1 rs2227982 heterozygous (GA) genotype. **B** PD-1 rs36084323 heterozygous (CT) genotype. **C** PDL-1 rs2282055 heterozygous (TG) genotype. **D** PDL-1 rs822336 heterozygous (GC) genotype

Table 1 Clinopathological characteristics of the whole study group

	Case	Control
Sex, n (%)		
Female	45 (54.9)	37 (45.1)
Male	58 (54.2)	49 (45.8)
Age	59.67 ± 1.151 (28–84)	52.67 ± 1.9 (19–85)
Weight	71.81 ± 1.5 (40–115)	75.85 ± 1.7 (44–120)
BMI	25.80 ± 0.5 (15–41)	27.26 ± 0.5 (18–47)
Smoking, n (%)		
No	44 (53.7)	38 (46.3)
Yes	11 (27.5)	29 (72.5)
Former	48 (76.2)	15 (23.8)
PFS	12.69 ± 0.98 (3–49)	
OS	22.82 ± 1.72 (2–91)	
Right column	22 (21.4)	
Left column	81 (78.6)	
Metastasis, n (%)	84 (81.6)	
KRAS mutant, n (%)	34 (33.0)	
Death	36 (37.9)	

BMI Body Mass Index (kg/m^2), *PFS* Progression Free Survival, *OS* Overall Survival

Categorical variables were expressed as n (%). Age, weight, BMI, PFS, OS values were expressed as mean± standard error (Min–Max)

polymorphisms were given in Table 2. We did not find any PD-1 rs2227981 heterozygous or mutant in our study group. While genotype distributions of rs2227982, rs2282055 and rs822336 polymorphisms conformed to Hardy Weinberg equilibrium in cases and controls, rs36084323 polymorphism was deviated in controls.

rs36084323 homozygous wild (CC) and heterozygous (CT) genotypes were detected as 89.6% and 10.4% in cases, 47.7% and 52.3% in controls, respectively ($p < 0.001$). rs36084323 CT genotype carries were found to be higher in controls compared to cases. Synchronously, the frequency of rs36084323 CC genotype was higher in cases. No rs36084323 homozygous mutant (TT) was detected in our study group. rs2227982, rs2282055 and rs822336 genotype frequencies were not significantly differ between cases and controls.

Adjusted and unadjusted odds ratios that predict CRC risk for rs2227982, rs36084323, rs2282055 and rs822336 gene polymorphisms were given in Table 3. rs36084323 CT genotype was highly associated with reduced risk of colorectal cancer compared to CC genotype (OR 0.068, 95% CI 0.022–0.211, $p < 0.001$).

In adjusted analysis, rs2282055 GG genotype was found to be associated with reduced risk of CRC (OR 0.271, 95% CI 0.078–0.940, $p = 0.040$). However, this association decreased without adjusting sex, age and smoking (OR

Table 2 The distributions of rs2227982, rs36084323, rs2282055 and rs822336 genotype and minor allele frequencies between cases and controls

Genotypes	Cases	MAF	$\chi2$	p^a	Controls	MAF	$\chi2$	p^a	p^b
rs2227982	n: 82 (%)				n: 77 (%)				
GG	76 (92.7)	0.04	0.118	0.73	73 (94.8)	0.03	0.055	0.81	0.747
GA	6 (7.3)				4 (5.2)				
rs36084323	n:77 (%)				n: 44 (%)				
CC	69 (89.6)	0.05	0.231	0.63	21 (47.7)	0.26	5.509	0.02	**< 0.001**
CT	8 (10.4)				23 (52.3)				
rs2282055	n:79 (%)				n:76 (%)				
TT	44 (55.7)	0.25	0.001	0.97	32 (42.1)	0.36	0.042	0.84	0.153
TG	30 (38.0)				34 (44.7)				
GG	5 (6.3)				10 (13.2)				
TT + TG/GG	74 (93.7)				66 (86.8)				0.181
TT/TG + GG	35 (44.3)				44 (57.9)				0.109
rs822336	n: 80 (%)				n: 76 (%)				
GG	21 (26.3)	0.48	0.222	0.64	25 (32.9)	0.43	0.002	0.96	0.653
GC	42 (52.5)				37 (48.7)				
CC	17 (21.3)				14 (18.4)				
GG + GC/CC	63 (78.8)				62 (81.6)				0.692
GG/GC + CC	59 (73.8)				51 (67.1)				0.385

MAF Minor allele frequency

p^a and $\chi2$ belong to Hardy–Weinberg equilibrium

p^b shows $\chi2$ or Fisher's exact test results of the comparison of genotypes between cases and control groups

Categorical variables were expressed as n (%)

$p < 0.05$ was considered statistically significant and presented by bold numbers

Table 3 Odds ratios of rs2227982, rs36084323, rs2282055 and rs822336 polymorphisms in colorectal cancer risk

	Unadjusted OR (95% CI)	p	Adjusted OR* (95% CI)	p
PD-1 rs2227982				
GG	References		References	
GA	1.441 (0.391–5.315)	0.583	1.484 (0.335–6.568)	0.603
PD-1 rs36084323				
CC	References		References	
CT	0.106 (0.041–0.271)	**< 0.001**	0.068 (0.022–0.211)	**< 0.001**
PDL-1 rs2282055				
TT	References		References	
TG	0.642 (0.328–1.254)	0.194	0.613 (0.298–1.260)	0.183
GG	0.364 (0.113–1.167)	0.089	0.271 (0.078–0.940)	**0.040**
TT + TG/GG	0.446 (0.145–1.372)	0.159	0.341 (0.103–1.125)	0.077
TT/TG + GG	1.729 (0.915–3.265)	0.092	1.891 (0.953–3.750)	0.068
PDL-1 rs822336				
GG	References		References	
GC	1.351 (0.652–2.802)	0.418	1.707 (0.764–3.815)	0.192
CC	1.446 (0.579–3.609)	0.430	1.578 (0.584–4.263)	0.368
GG + GC/CC	1.195 (0.543–2.632)	0.658	1.136 (0.481–2.685)	0.771
GG/GC + CC	1.377 (0.690–2.748)	0.364	1.668 (0.784–3.550)	0.184

OR odds ratio, *CI* confidence interval

*Odds ratios were adjusted for age, gender and smoking

$p < 0.05$ was considered statistically significant and presented by bold numbers

0.364, 95% CI 0.113–1.167, p = 0.089). rs2282055 TG (OR 0.613, 95% CI 0.298–1.260, p = 0.183), TT + TG (OR 0.341, 95% CI 0.103–1.125, p = 0.077) and TG + GG (OR 1.891, 95% CI 0.953–3.750, p = 0.068) genotypes did not show similar results for CRC risk.

The best logistic regression model we applied to investigate the relationship between the genotypes of PD-1 and PDL-1 polymorphisms, patient characteristics and the colorectal cancer risk was given in Table 4. rs822336 GC + CC (OR 4.791, 95% CI 1.436–15.983, p = 0.011) genotypes were found to be associated increased CRC risk. rs36084323 CT was associated with reduced colorectal cancer risk (OR 0.048, 95% CI 0.012–0.188, p < 0.001). Addition of other genotypes did not show significant improvement in the fit of the model. According to the model, CRC risk increases with increasing age (OR 1.084, 95% CI 1.040–1.131, p < 0.001) and decreases with BMI (OR 0.883, 95% CI 0.799–0.975, p = 0.014).

The relationships between progression-free survival and overall survival times of CRC patients in our study group with rs2227982, rs36084323, rs2282055 and rs822336 genotypes were given in Table 5.

In multiple comparison rs2282055 T > G polymorphisms was found to be correlated CRC patients progression free survival times (Log rank p = 0.046). Only patients with rs2282055 TT genotypes showed statistically significant longer progression survival times than those with GG genotypes (Log rank p = 0.013; significant threshold was corrected by Bonferroni method, α = 0.017). There was no significant difference in progression-free survival times between neither rs2282055 TT and TG (Bonferroni corrected log rank p = 0.745) nor TG and GG (Bonferroni

Table 4 Binary logistic regression model for genotypes and patient characteristics predicting colorectal cancer

	B	S.E.	Wald	df	OR (95% CI)	p
rs36084323 CT	− 3.041	0.699	18.916	1	0.048 (0.012–0.188)	**< 0.001**
rs822336 GC+CC	1.567	0.615	6.497	1	4.791 (1.436–15.983)	**0.011**
Age	0.081	0.021	14.342	1	1.084 (1.040–1.131)	**< 0.001**
BMI	− 0.125	0.051	6.038	1	0.883 (0.799–0.975)	**0.014**
Constant	− 0.759	1.541	0.243	1	0.468	0.622

B regression coefficient, *SE* standard error, *df* degrees of freedom, *OR* odds ratio, *CI* confidence interval

Significant p values are indicated in bold

$p < 0.05$ was considered statistically significant

Table 5 Association of rs2227982, rs36084323, rs2282055 and rs822336 polymorphisms with progression free and overall survival times in colorectal cancer patients

	Progression free survival (Month)	p	p*	Overall survival (Month)	p	p*
rs2227982						
GG	26.38 ± 2.85 (20.792–31.967)	0.368		52.98 ± 6.20 (40.83–65.13)	0.328	
GA	27.20 ± 4.29 (18.785–35.615)			48.50 ± 6.85 (35.08–61.92)		
rs36084323						
CC	22.47 ± 2.82 (16.94–28.009	0.095		42.26 ± 6.91 (28.72–55.81)	0.074	
CT	28.57 ± 3.17 (22.35–34.79)			52.13 ± 5.50 (41.35–62.90)		
rs2282055						
TT	22.88 ± 2.34 (18.29–27.46)	**0.046**	0.745[a]	41.18 ± 3.62 (34.10–48.27)	**0.037**	0.936[a]
TG	27.55 ± 4.57 (18.60–36.51)		0.036[b]	57.87 ± 9.61 (39.04–76.70)		0.051[b]
GG	8.00 ± 1.24 (5.56–10.44)		**0.013**[c]	10.50 ± 4.07 (2.52–18.48)		**0.009**[c]
TT + TG vs. GG	27.98 ± 2.81 (22.47–33.50)	**0.014**		56.34 ± 6.22 (44.15–68.52)	**0.010**	
TG + GG vs. TT	24.83 ± 4.26 (16.47–33.18)	0.381		53.24 ± 8.99 (35.62–70.85)	0.630	
rs822336						
GG	22.25 ± 4.67 (13.09–31.41)	0.400		30.40 ± 5.53 (19.56–41.24)	0.111	
GC	26.93 ± 4.01 (19.06–34.79)			58.67 ± 8.84 (41.35–75.99)		
CC	23.13 ± 3.38 (16.50–29.75)			40.44 ± 5.60 (29.45–51.42)		
GG + GC vs. CC	25.59 ± 3.16 (19.39–31.79)	0.709		53.14 ± 7.13 (39.18–67.11)	0.954	
GC + CC vs. GG	27.30 ± 3.12 (21.18–33.42)	0.177		57.03 ± 6.90 (43.51–70.55)	**0.044**	

Survival times were represented as estimate of mean ± standard error (95% CI)

p: Uncorrected, log rank (α = 0.05). Significant values were presented by bold numbers

p*: Bonferroni corrected, log rank (α = 0.017). Significant values were presented by bold numbers

[a]Bonferroni corrected p values for TT vs. TG comparisons

[b]Bonferroni corrected p values for TG vs. GGcomparisons

[c]Bonferroni corrected p values for TT vs. GG comparisons

corrected log rank p = 0.036) genotypes. While this relationship continued to exist in the recessive model (TT + TG vs. GG, Log rank p = 0.014), it disappeared in the dominant model (TG + GG vs. TT, Log rank p = 0.381) (Table 5).

Similar to progression-free survival times, in multiple comparison, statistically significant differences were detected for rs2282055 genotypes and overall survival times (Log rank p = 0.037). When we continued with Bonferroni correction, only the difference between TT and GG genotypes remained to be statistically significant. rs2282055 TT genotypes resulted with longer overall survival times than GG genotypes (Bonferroni corrected log rank p = 0.009). There was no clear difference in comparisons between TT vs. TG (Bonferroni corrected log rank p = 0.936) and TG vs. GG (Bonferroni corrected log rank p = 0.051) genotypes. This correlation continued to exist in the recessive model (TT + TG vs. GG, Log rank p = 0.010) and disappeared in the dominant model with higher number of variant alleles (TG + GG vs. TT, Log rank p = 0.630) (Table 5).

We did not find any significant relationships between rs2227982 and rs822336 genotypes for both progression free and overall survival times except for rs822336 genotypes in multiple comparisons. However, when we compared rs822336 genotypes in dominant model (GC + CC vs. GG), a significant difference was detected between GG and GC + CC genotypes and overall survival times (Log rank p = 0.044). Although non-significant, there was a slightly considerable difference between rs36084323 homozygous wild (CC) and heterozygous (CT) genotypes in both progression-free and overall survival times of the patients in our study group (Log rank p = 0.095 and Log rank p = 0.074, respectively) (Table 5).

No association was found between primary tumor location (right and left column) neither with PD-1 nor PDL-1 gene polymorphisms.

Discussion

In this study, we examined the effects of PD-1 rs2227981, rs2227982, rs36084323 and PDL-1 rs2282055, rs822336 gene polymorphisms on CRC risk and prognosis in Turkish subjects. To the best of our knowledge, this study is the first polymorphism study to comprehensively investigate the relationship between the genetic variants in the PD-1 and

PDL-1 genes with the risk and prognosis of CRC in Turkish subjects.

There are conflicting results in the literature from different populations regarding rs36084323 polymorphism and the risk of various cancer types. rs36084323 polymorphism was not found to be associated with several types of cancer [24, 25]. However, studies exist reporting the relationship between rs36084323 C (G) allele and cancer risk. In a meta-analysis based on data from ten different studies, investigated different types of cancer including breast cancer, non-small cell lung cancer, esophageal squamous cell carcinoma, epithelial ovarian cancer, esophagogastric junction adenocarcinoma, cutaneous melanoma and CRC, it was reported that the rs36084323 polymorphism was associated with reduced cancer risk in Asian populations. The researchers also reported that the reason for heterogeneous data in the literature on the relationship between rs36084323 and cancer is significantly related to ethnicity ($p = 0.029$), but not with cancer type ($p = 0.792$), sample number ($p = 0.585$) and control source ($p = 0.207$) [33]. rs36084323 CC (GG) ($p = 0.020$) genotype was reported to be lower in breast cancer patients than controls in Chinese population [22]. Shamsdin et al. investigated the relationship between PD-1 rs36084323 polymorphism and colon cancer risk in Iranian population. Similar to our results, researchers determined the frequency of rs36084323 CC genotype and C allele were significantly higher in colon cancer patients ($p < 0.001$ and $p < 0.001$); and TT (AA) and CT (GA) genotypes were significantly higher in control subjects ($p < 0.001$ and $p = 0.012$) [34]. In our study, rs36084323 CT genotype frequency was found to be higher in controls whereas CC genotype frequency was higher in cases. Similar to Shamsdin et al. our results indicate that PD-1 rs36084323 C allele is associated with CRC risk.

In a study focused on subacute sclerosing panencephalitis, the frequency of PD-1 rs36084323 C allele and promoter activity of C allele were found to be higher in patients compared to healthy controls, and PD1 relative expression levels were also reported to be significantly higher in patients compared to controls [35]. According to their results PD-1 rs36084323 C allele causes higher promoter activity, leading to high gene expression and increasing susceptibility to disease [35]. With a similar motif, it may be assumed that PD-1 rs36084323 C allele can affect CRC development through gene transcription and activation. High PD-1 gene expression resulting from C allele may cause immune system cells to have more PD-1 receptors as high number of target to PDL-1 molecules released from cancer cells, and consequently a decreased tumor immune response. However, further studies are required to fully elucidate the relationship of colorectal cancer with the PD-1 rs36084323 C allele.

Along with the studies reporting no significant relationship between rs36084323 polymorphism and survival times in different cancers, there are also studies reporting that patients with rs36084323 CC genotype had significantly lower survival times than those with heterozygous (CT) or homozygous mutant (TT) genotypes, and that rs36084323 homozygous wild (CC) genotype is associated with poor prognosis [26, 36]. In our study group, no significant association was detected between rs36084323 CC or CT genotypes and progression-free survival times of CRC patients. However, although not statistically significant, patients with CC genotype showed in near-limit value and considerable shorter overall survival compared to CT genotype ($p = 0.074$).

In our study group, PDL-1 rs2282055 homozygous mutant genotype (GG) was found to be associated with reduced CRC risk comparing homozygous wild genotype (TT) ($p = 0.040$). However, this relationship decreases the importance when analyzed without adjusting for gender, age and smoking ($p = 0.089$). Since the best of our knowledge, there was no other study investigating the relationship between PDL-1 rs2282055 polymorphism and the risk of colorectal cancer in the literature, our study reports the first results on this subject. In a study focused on non-small cell lung cancer patients under nivolumab treatment, shorter progression-free survival was reported in patients with the rs2282055 TT genotype compared to those with the GG and TG genotype ($p = 0.0163$) [27]. In our study, CRC patients with rs2282055 TG genotype had the highest progression-free and overall survival times compared to other genotypes ($p = 0.046$ and $p = 0.037$, respectively). Patients with rs2282055 TT genotypes showed statistically significant longer progression-free and overall survival times than those with GG genotypes (Bonferroni corrected Log rank $p = 0.013$ and Bonferroni corrected Log rank $p = 0.009$, respectively). The difference of the results between two studies may be arising from the presence of PD-1 ligand Nivolumab, or different interactions in different populations or different types of cancer.

In our basic case–control comparison analyzes, we found no significant difference between PDL-1 rs822336 genotypes and CRC risk. Similar to our results Zhao et al. did not find a statistically significant relationship between non-small cell lung cancer risk and rs822336 polymorphism [37]. However, in our logistic regression model, we found that the rs822336 GC + CC genotype had an effect on the increased risk of CRC in its presence in other parameters ($p = 0.011$).

We found no statistically significant difference between PDL-1 rs822336 polymorphism and progression-free survival in all genetic models tested. While there was no significant difference between PDL-1 rs822336 polymorphism and overall survival of the patients in co-dominant and recessive models; a statistically significant difference was detected in dominant genetic model. The overall survival times of patients with rs822336 GG genotype was found

to be quite low compared to patients with GC + CC (Log rank p = 0.044) in our subjects. There are only few studies which investigated the relationship between CRC patients survival time and PDL-1 rs822336 G > C polymorphism and have been reported results contradictory to our study. Huijian et al. did not report any association between PDL-1 rs822336 G > C polymorphism and disease-free and overall survival times of CRC patients, and PDL-1 gene expression [29]. Similar results have come from another study with R0 resected colorectal cancer patients under capecitabine treatment and reported no statistically significant association among PDL-1 rs822336 G > C polymorphism, clinical survival and PDL-1 gene expression [28]. We suggest different results might be attributed to different populations depending on the number or heterogeneity of the patients involved.

On the other hand, our results from dominant model are similar to the findings of Zhao and et al. The researchers found disease-free and overall survival times of non-small cell lung cancer patients with rs822336 GG genotype were lower compared to GC + CC genotypes (p = 0.010 and p = 0.008) [38]. The researchers also reported that PDL-1 mRNA expression was significantly higher in peripheral blood mononuclear cells of patients with the GG genotype compared to the GC + CC genotype (p < 0.001) [38]. Although there are studies reporting conflicting results between PDL-1 rs822336 polymorphism and PDL-1 gene expression [23, 36, 39] and lower overall survival times resulting from higher PDL-1 levels caused by the GG genotype might lead to more PD-1/PDL-1 ligation, allow cancer cells to escape from the immune system more easily and resulting with more aggressive cancer phenotype. However, since high PDL-1 expression on tumor infiltrating cells was determined as independent factor for prolonged overall survival in colorectal cancer [30], the effects of PDL-1 rs822336 polymorphism on CRC prognosis should be investigated in detail.

The distributions of genotype frequencies for specific polymorphisms vary between studies and especially populations. rs2227982 GG, GA and AA genotype frequencies were found as 93.7, 6.3 and 0.0%, respectively, in our study group. Different studies from Chinese population rs2227982 genotype frequencies were reported as 20.7–66.2% for GG; 28.0–53.0% for GA, and 5.8–27.2% for AA genotypes [22, 36, 40]. Studies in Japanese population, rs2227982 genotype frequencies were reported as 29.3–47.4% for GG, 28.7–51.0% for GA and 19.7–26.0% for AA genotype [24, 26, 27]. In other studies reporting from Asian populations, rs2227982 GG, GA and AA genotype frequencies were reported as 24.6%, 50.7% and 24.6% in Korean [23], 95.6%, 4.0% and 0.4% in Iranian [41] population. Apart from Asian populations, rs2227982 GG, GA and AA genotype frequencies were reported respectively as 96.9%, 3.1% and 0.0% in Polish [42] population. In Brazilian population GG was reported as 90.4% and GA + AA combined frequency was reported as 9.6% [43]. Similar with our results rs2227982 GG, GA and AA genotype frequencies were reported as 89.9%, 9.2% and 0.9% in another study conducted in Turkish population [44]. According to results from different populations, rs2227982 AA genotype seems more frequent in Asian populations.

rs36084323 GG, GA and AA genotype frequencies were reported respectively as 21.6–26.6%, 48.0–54.7% and 22.8–25.4% in Chinese [22, 36, 40], 21.2–25.6%, 47.8–50.8% and 25.0–28.1% in Japanese [23, 24, 26], 42.3–95.2%, 4.8–57.7% and 0.0–28.9% in Iranian [34, 41, 45], 97.0%, 3.0% and 0.0% in Polish [25], 95.0%, 4.4% and 0.6% in Spanish population [46]. It was also reported as 90.5%, 9.5% and 0.0% in another Turkish population based study [47]. In the present study rs36084323 GG, GA and AA genotype frequencies were found as 74.4%, 25.6% and 0.0%, respectively. Comparing to Asian populations, the distribution of PD-1 rs36084323 genotypes in Turkish population were found to be similar to European populations.

rs2282055 TT, TG and GG frequencies were reported as 20.0%, 40.0% and 40.0% in Japanese [27], 49.0%, 46.0% and 3.0% in French [48], 78.5%, 15.2% and 6.3% in Egyptian Populations [49]. In our study group rs2282055 TT, TG and GG genotype frequencies were found as 49.0%, 41.3% and 9.7%, respectively.

As far as we know, there are very limited numbers of studies focusing on PDL-1 rs822336 polymorphism. Results were reported mostly from Asian populations. rs822336 GG, GC and CC genotype frequencies were reported respectively as 55.7%, 34.2%, 10.1% in Korean [23]; 47.8%, 44.9%, 7.2% [36] and 47.8%, 47.3%, 4.8% in Chinese [50]; and 23.4%, 51.1%, 25.5% in Polish [39] population. In the present study with Turkish subjects, rs822336 GG, GC and CC genotype frequencies were detected as 29.5%, 50.6% and 19.9%, respectively.

In summary, our results suggest several PD-1 and PDL-1 polymorphisms are associated with risk and prognosis of CRC in Turkish subjects. We believe that the identification of CRC susceptibility and prognosis associated polymorphisms, and elucidating their functions will lead us to prepare personalized CRC susceptibility and prognosis tests to apply in clinical practice in near future.

Conclusions

Our results indicate that PD-1 rs36084323 CT genotype is a genetic risk factor affecting the development of CRC. PD-1 rs822336 G > C polymorphism might be useful in predicting CRC prognosis. PDL-1 rs2282055 T > G polymorphism was found to be effective in both CRC risk and prognosis. Although our study has limitations such as the low

number of patients involved, our results indicate that PD-1 and PDL-1 polymorphisms have respectable role in CRC risk and prognosis and, encourage the investigations on the effects of genetic polymorphisms on CRC risk and clinical outcomes. Further validation studies should be conducted to verify our results in larger and different populations to understand the effects of causal polymorphisms and their exact functional mechanisms.

Author Contributions MC contributed to the experimental molecular genetic analysis, study design and literature. EN, UIK, NDS and CC as the clinicians selected and provided the blood samples and clinical data of subjects to participate in this study. AK contributed to the interpretation and evaluation of the results. BS contributed to the whole study management, development of the final protocol of the experiments, interpretation of the results and organization of the manuscript. All authors were involved in preparing the manuscript.

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Declarations

Conflict of interest No conflict of interest was declared by the authors.

Ethical approval The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by Marmara University Local Ethical Committee with the protocol number 09.2018.174.

Informed consent Written informed consents were obtained from all patients prior to recruitment and sample collection. The study was performed in accordance with the Declaration of Helsinki.

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