Int J Clin Exp Med 2015;8(5):8086-8093
www.ijcem.com /ISSN:1940-5901/IJCEM0007567

Original Article

Programmed death-1 (*PD-1*) polymorphism is associated with gastric cardia adenocarcinoma

Weifeng Tang[1*], Yu Chen[2*], Shuchen Chen[1], Bin Sun[3], Haiyong Gu[3], Mingqiang Kang[1]

[1]Department of Thoracic Surgery, The Union Clinical Medical College of Fujian Medical University, Fuzhou, Fujian Province, China; [2]Department of Medical Oncology, Fujian Provincial Cancer Hospital, Fuzhou, Fujian Province, China; [3]Department of Cardiothoracic Surgery, Affiliated People's Hospital of Jiangsu University, Zhenjiang, Jiangsu Province, China. [*]Equal contributors.

Received March 4, 2015; Accepted April 30, 2015; Epub May 15, 2015; Published May 30, 2015

Abstract: Polymorphisms in gene encoding programmed death-1 (PD-1) were suggested to be associated with the risk of multiple cancers. To the best of our knowledge, no investigation has been carried out for gastric cardia adenocarcinoma (GCA) and *PD-1* polymorphisms. Thus, we studied *PD-1* rs10204525 A>G, rs7421861 T>C and rs2227982 C>T single nucleotide polymorphisms (SNPs) in 330 GCA cases and 608 cancer-free controls. Genotypes of *PD-1* SNPs were determined by ligation detection reaction (LDR) assays. The distributions of the allele and genotype were not statistically significant in two groups for the three *PD-1* SNPs. However, in stratified analyses by various characteristics (eg., age, sex, smoking condition and alcohol consumption status), we found a significantly increased risk of GCA associated with the *PD-1* rs2227982 C>T polymorphism was evident among ever drinking cases (TT vs. CC: adjusted OR = 2.53, 95% CI = 1.11-5.79, P = 0.028; TT+CT vs. CC: adjusted OR = 2.04, 95% CI = 1.01-4.13, P = 0.047). For the other SNPs, in stratified analyses, no correlation between the SNPs and susceptibility of GCA was observed. In conclusion, our results highlight that rs2227982 C>T polymorphism in *PD-1* gene may contribute to the risk of GCA.

Keywords: Polymorphism, programmed death-1, immunoglobulin superfamily, gastric cardia adenocarcinoma, susceptibility

Introduction

Gastric cardia adenocarcinoma (GCA) is one of the most common upper gastrointestinal malignancies with the leading causes of cancer relative death worldwide [1, 2]. Previous studies have identified that several risk factors, such as genetic abnormalities, alcohol consumption, tobacco use, inflammation, and diet, were involved in the development of GCA [3, 4]. It is now confirmed that GCA is a complex disease which is the results of genetic susceptibility and environmental triggers interaction.

The programmed death-1 (PD-1) (cluster of differentiation 279, CD 279), a type of the immunoglobulin superfamily, is commonly expressed on activated CD4+/CD8+ T cells, B lymphocytes and natural killer cells, as well as myeloid cells, and behaves as a down-regulator of activation of T cells [5]. There are accumulating evidences that PD-1 pathway plays an important role in peripheral tolerance [6]. Two ligands of PD-1, programmed death-1 ligand 1 (PD-L1) and PD-L2, engagement with PD-1, suppress proliferation and activation of T cells, then may result in tumor escape from immune surveillance [7, 8]. The expression of PD-1 on tumor infiltrating lymphocytes (TILs) and PD-Ls on multiple tumor cells suggests the presence of an immunosuppressive milieu for tumor incidence and/or progression [9-11].

Since the PD-1 acts as a crucial inhibitor in antitumor responses, it is considered as a cogent candidate for genetic risk of individuals to a number of malignancies. Recent studies have highlighted that PD-1 polymorphisms are associated with susceptibility to several types of cancer, such as gastric cancer [12], colon can-

Table 1. Distribution of selected demographic variables and risk factors in GCA cases and controls

Variable	Cases (n = 330)		Controls (n = 608)		P^a
	n	%	n	%	
Age (years) mean ± SD	65.06 (± 8.37)		64.19 (± 6.66)		0.103
Age (years)					0.746
< 60	89	26.97	170	27.96	
≥ 60	241	73.03	438	72.04	
Sex					0.965
Male	223	67.58	410	67.43	
Female	107	32.42	198	32.57	
Tobacco use					**0.006**
Never	209	63.33	438	72.04	
Ever	121	36.67	170	27.96	
Alcohol use					0.072
Never	233	70.61	462	75.99	
Ever	97	29.39	146	24.01	

[a]Two-sided x^2 test and student t test; Bold values are statistically significant ($P < 0.05$).

cer [13], breast cancer [14, 15], esophageal cancer [16] and hepatocellular carcinoma [17]. However, the association between PD-1 polymorphisms and GCA was unclear. Accordingly, we explored, in this case-control study, the *PD-1* polymorphisms to determine whether there was a correlation between *PD-1* single nucleotide polymorphisms (SNPs) and susceptibility of GCA in a Chinese Han population.

Materials and methods

Study subjects

Three hundred and thirty unrelated patients with newly diagnosed GCA and 608 hospital-based controls were recruited in this study. All GCA patients were pathologically confirmed in the Affiliated People's Hospital and Affiliated Hospital of Jiangsu University (Zhenjiang city, Jiangsu Province, China) during October 2008 and June 2013. The exclusion criteria were GCA cases with a history of other malignancy or recurrent tumors. The controls were selected from participators who admitted to these two hospitals for cure of trauma during the same time and frequency-matched with cases by age (± 5-years) and sex. This study was approved by Ethics Committee of Jiangsu University. All participators signed the informed consent and provided the information of demographic data and risk factors (e.g., smoking and drinking condition).

Genotyping for PD-1 rs10204525 A>G, rs7421861 T>C and rs2227982 C>T polymorphisms

Every participator donated 2 ml EDTA anti-coagulated venous blood. Genomic DNA was extracted from lymphocytes using the DNA Extraction Kit (Qiagen, Berlin, Germany) following the instructions. Genotypes of *PD-1* rs10204525 A>G, rs7421861 T>C and rs2227982 C>T polymorphisms were determined by ligation detection reaction (LDR) assays as described previously [16, 18, 19]. Genotyping value was more than 95% (95.31%-99.47%). To ensure quality control, 110 randomly selected samples of all participators were genotyped again by LDR, and the reproducibility was 100%.

Statistical analysis

All statistical calculations were performed using SAS software package (version 9.0; SAS Institute, Cary, NC). The differences in demographic data among two groups were evaluated using Chi-square test (x^2). Genotype frequencies between different groups were compared using the x^2 test, and odds ratios (ORs) with corresponding 95% confidence intervals (CIs) were computed using conditional logistic regression assay. A $P < 0.05$ (two-sided) was considered the criterion of statistical significance.

Results

Demographic characteristics of study population

Table 1 gives baseline characteristics in two groups. Of the 330 GCA cases and 608 controls, the mean age was 65.06 ± 8.37 years and 64.19 ± 6.66 years, respectively. There were no significant difference in the mean age, gender and alcohol consumption distributions among GCA cases and controls (P = 0.746, P = 0.965 and P = 0.076, respectively), suggesting that the variables frequency matching was adequate. However, smoking rate between GCA

Table 2. Primary information for *PD-1* rs10204525, rs7421861 and rs2227982 polymorphisms

Genotyped SNPs	rs10204525 A>G	rs7421861 T>C	rs2227982 C>T
Chromosome	2	2	2
Function	UTR-3	intron	Missense
Chr Pos (Genome Build 36.3)	242440994	242444023	242442106
Regulome DB Score[a]	5	5	5
TFBS[b]	-	-	-
Splicing (ESE or ESS)	-	-	Y
miRNA (miRanda)	Y	-	-
MAF[c] for Chinese in database	0.302	0.165	0.488
MAF in our controls (n = 608)	0.280	0.177	0.488
P value for HWE[d] test in our controls	0.120	0.368	0.448
Genotyping method[e]	LDR	LDR	LDR
% Genotyping value	95.31%	98.29%	99.47%

[a]http://www.regulomedb.org/; [b]TFBS: Transcription Factor Binding Site (http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm); [c]MAF: minor allele frequency; [d]HWE: Hardy-Weinberg equilibrium; [e]LDR: ligation detection reaction.

patients and controls was significantly different (P = 0.006). Total cancer cases were sporadic GCA cases. The distribution of *PD-1* rs10204525 A>G, rs7421861 T>C and rs2227982 C>T genotypes were in accordance with Hardy-Weinberg equilibrium (HWE) in the GCA cases and controls, and missing data were < 5%. The relatively primary information for *PD-1* SNPs is summarized in **Table 2** as described previously [16].

PD-1 rs10204525 A>G, rs7421861 T>C and rs2227982 C>T polymorphisms and the risk of GCA

The frequencies of genotype and allele of the three *PD-1* SNPs in GCA cases and controls are listed in **Table 3**. When comparing the genotype frequencies distribution in GCA cases with those of controls, no significant increased risk for developing GCA was found for *PD-1* rs10204525 A>G, rs7421861 T>C and rs2227982 C>T polymorphisms. However, following stratified analyses of various characteristics (eg., age, sex, smoking condition and alcohol-consumption status), a significantly increased risk of GCA associated with the *PD-1* rs2227982 C>T polymorphism was evident among ever drinking patients (TT vs. CC: adjusted OR = 2.53, 95% CI = 1.11-5.79, P = 0.028; TT+CT vs. CC: adjusted OR = 2.04, 95% CI = 1.01-4.13, P = 0.047) (**Table 4**), but no significant association was found between *PD-1* rs10204525 or rs7421861 T>C SNPs and the risk of GCA (data not shown).

Discussion

GCA is a relatively common malignancy with high morbidity and poor prognosis in China. The etiology of GCA is very complicated and until now, has not been clarified utterly. There may be a multifactorial interaction between a number of risk factors and genetic abnormalities in a combined manner rather than individually. Of late, several studies have kept a watchful eye on the important role of the immune system in GCA (20-24). Here, we conducted a case-control study to explore the potential correlation of *PD-1* SNPs with the risk of GCA for the first time. The results suggested that *PD-1* rs2227982 C>T polymorphism might conduce to the risk of GCA among drinking patients and then backed up the hypothesis that genetic abnormalities, influencing the activation and effector functions of T cells, might modify the risk of multiple malignancies.

PD-1 polymorphisms might contribute to cancer risk and autoimmune diseases pathogenesis. Some of the studies focused on the association of *PD1* polymorphisms with autoimmune diseases [6, 25-27]. However, there are only several investigations in the field of malignancy [12-17, 28-31] and according to our knowledge, the correlation of PD-1 polymorphisms with GCA was unclear. In view of such findings, we selected *PD-1* polymorphisms to investigate their roles in GCA.

In *PD-1* rs2227982 C>T polymorphism, we confirmed that the TT and TT+CT genotypes were relevant to increased risk of GCA among ever drinking cases. It has been reported that *PD-1* rs2227982 C>T polymorphisms are not correlated with breast cancer [14] and esophageal cancer [16]. However, in this study, we found that T allele of this variant was associated with

Table 3. Logistic regression analyses of associations between *PD-1* rs10204525, rs7421861 and rs2227982 polymorphisms and risk of GCA

Genotype	Cases (n = 330)		Controls (n = 608)		Crude OR (95% CI)	*P*	Adjusted OR[a] (95% CI)	*P*
	n	%	n	%				
PD-1 rs10204525 A>G								
AA	169	53.99	309	53.18	1.00		1.00	
AG	123	39.30	219	37.69	1.03 (0.77-1.37)	0.857	1.02 (0.76-1.37)	0.901
GG	21	6.71	53	9.12	0.72 (0.42-1.24)	0.241	0.71 (0.41-1.21)	0.208
GG vs. AG vs. AA								0.451
AG+GG	144	46.01	272	46.82	0.97 (0.74-1.28)	0.817	0.96 (0.72-1.27)	0.757
AA+AG	292	93.29	528	90.88	1.00		1.00	
GG	21	6.71	53	9.12	0.72 (0.42-1.21)	0.214	0.70 (0.41-1.19)	0.186
PD-1 rs7421861 T>C								
TT	226	69.75	408	68.23	1.00		1.00	
TC	91	28.09	168	28.09	0.98 (0.72-1.32)	0.885	0.98 (0.72-1.33)	0.906
CC	7	2.16	22	3.68	0.58 (0.24-1.37)	0.210	0.59 (0.25-1.42)	0.242
CC vs. TC vs. TT								0.447
TC+CC	98	30.25	190	31.77	0.93 (0.70-1.25)	0.633	0.94 (0.70-1.26)	0.675
TT+TC	317	97.84	576	96.32	1.00		1.00	
CC	7	2.16	22	3.68	0.58 (0.24-1.37)	0.213	0.60 (0.25-1.42)	0.244
PD-1 rs2227982 C>T								
CC	75	22.73	163	27.03	1.00		1.00	
CT	168	50.91	292	48.42	1.25 (0.90-1.74)	0.188	1.27 (0.91-1.78)	0.160
TT	87	26.36	148	24.54	1.28 (0.87-1.87)	0.207	1.30 (0.88-1.90)	0.186
TT vs. CT vs. CC								0.351
CT+TT	255	77.27	440	72.97	1.26 (0.92-1.72)	0.150	1.28 (0.93-1.76)	0.127
CC+CT	243	73.64	455	75.46	1.00		1.00	
TT	87	26.36	148	24.54	1.10 (0.81-1.50)	0.541	1.10 (0.81-1.50)	0.534

[a]Adjusted for age, sex, smoking and drinking status.

the increased risk of GCA, which might be interpreted that *PD-1* gene plays different role in different cancer even the same SNP. *PD-1* rs2227982 C>T polymorphism is a missense mutation, encoding a valine → alanine substitution at amino acid residue of PD-1 during protein synthesis, thereby probably changes the activity of PD-1. *PD-1* rs2227982 C>T may accordingly have an impact on the risk of GCA through altering the structures and functions of PD-1, and this study demonstrated that *PD-1* rs2227982 TT genotype and T allele could affect gastric cardia carcinogenesis. In this study, minor allele frequency (MAF) of *PD-1* rs2227982 C>T G was 0.488 among 608 cancer-free controls, which is in agreement with the data for Chinese in database (http://www.ncbi.nlm.nih.gov/SNP) (0.488). The power of our analysis ($\alpha = 0.05$) was evaluated using a web-based Power and Sample Size program (PS, version 3.0, 2009, http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSample Size). The power was 0.938 in TT vs. CC genetic model with adjusted OR 2.53 and 0.596 in TT+CT vs. CC genetic model with adjusted OR 2.04 for *PD-1* rs2227982 C>T in ever drinking subgroup.

In the present investigation, the SNPs *PD-1* rs10204525 A>G and rs7421861 T>C were also included. *PD-1* rs10204525 is located on 3' untranslated region and may affect the inflammatory cytokines levels via modulating polyadenylation. And previous studies reported that mutation in *PD-1* rs10204525 might decreased the risk of esophageal squamous cell carcinoma [16]. Li et al. suggested that *PD-1* rs10204525 A>G polymorphism combined with chronic HBV infection might contribute

Table 4. Stratified analyses between *PD-1* rs2227982 C>T polymorphism and GCA risk by sex, age, smoking status and alcohol consumption

Variable	*PD-1* rs2227982 C>T (case/control)[a]				Adjusted OR[b] (95% CI); *P*; P_h[c]				
	CC	CT	TT	CT+TT	CC	CT	TT	CT+TT	TT vs. (CT+TT)
Sex									
Male	46/104	114/202	63/100	177/302	1.00	1.30 (0.86-1.98); P: 0.217; P_h: 0.901	1.46 (0.91-2.34); *P*: 0.119; P_h: 0.416	1.35 (0.91-2.01); *P*: 0.135; P_h: 0.672	1.22 (0.84-1.76); *P*: 0.305; P_h: 0.389
Female	29/59	54/90	24/48	78/138	1.00	1.16 (0.65-2.06); *P*: 0.618; P_h: 0.901	1.06 (0.54-2.07); *P*: 0.875; P_h: 0.416	1.12 (0.66-1.92); *P*: 0.675; P_h: 0.672	0.96 (0.55-1.70); *P*: 0.898; P_h: 0.389
Age									
< 60	14/40	46/89	29/40	75/129	1.00	1.39 (0.66-2.91); *P*: 0.384; P_h: 0.629	1.79 (0.80-4.00); *P*: 0.159; P_h: 0.154	1.52 (0.75-3.07); *P*: 0.241; P_h: 0.363	1.41 (0.78-2.57); *P*: 0.261; P_h: 0.157
≥ 60	61/123	122/203	58/108	180/311	1.00	1.21 (0.82-1.77); *P*: 0.337; P_h: 0.629	1.09 (0.70-1.71); *P*: 0.695; P_h: 0.154	1.17 (0.81-1.67); *P*: 0.400; P_h: 0.363	0.97 (0.67-1.40); *P*: 0.865; P_h: 0.157
Smoking status									
Never	51/116	108/207	50/111	158/318	1.00	1.21 (0.80-1.84); *P*: 0.375; P_h: 0.676	1.11 (0.69-1.80); *P*: 0.663; P_h: 0.121	1.18 (0.79-1.75); *P*: 0.423; P_h: 0.353	0.98 (0.66-1.45); *P*: 0.931; P_h: 0.106
Ever	24/47	60/85	37/37	97/122	1.00	1.42 (0.77-2.64); *P*: 0.263; P_h: 0.676	2.00 (0.99-4.04); *P*: 0.054; P_h: 0.121	1.59 (0.89-2.86); *P*: 0.119; P_h: 0.353	1.57 (0.90-2.75); *P*: 0.113; P_h: 0.106
Alcohol consumption									
Never	59/122	115/220	59/116	174/336	1.00	1.13 (0.76-1.68); *P*: 0.550; P_h: 0.161	1.11 (0.70-1.74); *P*: 0.661; P_h: 0.094	1.12 (0.77-1.62); *P*: 0.547; P_h: 0.099	1.02 (0.71-1.48); *P*: 0.905; P_h: 0.308
Ever	16/41	53/72	28/32	81/104	1.00	1.84 (0.88-3.85); *P*: 0.106; P_h: 0.161	**2.53 (1.11-5.79); *P*: 0.028**; P_h: 0.094	**2.04 (1.01-4.13); *P*: 0.047**; P_h: 0.099	1.64 (0.88-3.05); *P*: 0.119; P_h: 0.308

[a]The genotyping was successful in 330 (100.0%) GCA cases and 603 (99.2%) controls for *PD-1* rs2227982 C>T; [b]Adjusted for age, sex, smoking status and alcohol consumption (besides stratified factors accordingly) in a logistic regression model; [c]P_h for heterogeneity. Bold values are statistically significant ($P < 0.05$).

to the development of hepatocellular carcinoma [17]. In this regard, we explored the association between *PD-1* rs10204525 A>G and GCA, but we found no significant correlation. As it is known, rs7421861 is located on intron 1 which involves many splicing control components and regulatory elements, and some mutations, if there are, may lead to disruption of the splice site, translation suppression and even alteration of the mRNA secondary structure [32-34]. Recent reports showed that *PD-1* 7421861 T>C was not involved in the risk of breast cancer and esophageal carcinoma in a Chinese population [14, 16]. In this case-control study, we found no significant correlation of this SNP with GCA susceptibility, which was consistent with previous study. Thus, in the future, further large-scale studies with detailed individual information will be needed to clarify whether *PD-1* rs10204525 A>G and rs7421861 T>C do influence the activation of PD-1 protein.

Additionally, some limitations in this study should be addressed here. We are aware that the moderate sample size of our study is a relative limitation; however, in a field where no similar study is available, we suppose that any new knowledge on *PD-1* SNPs might help to validate these associations. Moreover, since the participants were enrolled from local hospitals, the selection bias cannot be avertible and have a limited power to fully represent the general population. Furthermore, we only studied the *PD-1* SNPs in Chinese Han patients with GCA, which lessens the possibility of confounding from ethnicity, so these SNPs should be explored in other ethnic groups. Finally, the SNPs we examined may not provide a comprehensive assessment of *PD-1* genetic mutation. In the future, well designed fine-mapping studies are required to confirm our present results.

Taken together, our study highlights the influence of the interaction between drinking and a functional polymorphism in *PD-1* to the risk of sporadic GCA in Chinese Han population, suggesting the prospect of investigation in individual malignancy prevention strategies to alcohol-associated GCA.

Acknowledgements

We appreciate all subjects who participated in this study. We wish to thank Dr. Yiqun Chen (Biowing Applied Biotechnology Company, Shanghai, China) for technical support. This study was supported in part by National Natural Science Foundation of China (81472332, 81370001, 81300037 and 81341006), Jiangsu Province Natural Science Foundation (BK2010333 and BK2011481), Fujian Province Natural Science Foundation (2013J01126, 2013J05116 and 2015J01435), Fujian Medical University professor fund (JS12008) and Fujian Province science and technology programmed fund (2012Y0030).

Disclosure of conflict of interest

None.

Address correspondence to: Mingqiang Kang, Department of Thoracic Surgery, The Union Clinical Medical College of Fujian Medical University, Fuzhou 350001, China. E-mail: Mingqiang_Kang@126.com

References

[1] Vial M, Grande L and Pera M. Epidemiology of adenocarcinoma of the esophagus, gastric cardia, and upper gastric third. Recent results in cancer research. Fortschritte der Krebsforschung. Progres Dans Les Recherches Sur Le Cancer 2010; 182: 1-17.

[2] Jemal A, Siegel R, Xu J and Ward E. Cancer statistics. CA 2010; 60: 277-300.

[3] Zhang L, Du C, Guo X. Interleukin-8-251A/T polymorphism and Helicobacter pylori infection influence risk for the development of gastric cardiac adenocarcinoma in a high-incidence area of China. Mol Biol Rep 2010; 37: 3983-3989.

[4] Chen MJ, Wu DC, Lin JM, Wu MT and Sung FC. Etiologic factors of gastric cardiac adenocarcinoma among men in Taiwan. World J Gastroenterol 2009; 15: 5472-5480.

[5] Sharpe AH, Wherry EJ, Ahmed R and Freeman GJ. The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. Nature Immunol 2007; 8: 239-245.

[6] Kristjansdottir H, Steinsson K, Gunnarsson I, Grondal G, Erlendsson K; Alarcon-Riquelme ME. Lower expression levels of the programmed death 1 receptor on CD4+CD25+ T cells and correlation with the PD-1. 3A genotype in patients with systemic lupus erythematosus. Arthritis Rheum 2010; 62: 1702-1711.

[7] Blank C, Gajewski TF and Mackensen A. Interaction of PD-L1 on tumor cells with PD-1 on tumor-specific T cells as a mechanism of immune evasion: implications for tumor immu-

notherapy. Cancer Immunol Immunother 2005; 54: 307-314.
[8] Wang W, Lau R, Yu D, Zhu W, Korman A and Weber J. PD1 blockade reverses the suppression of melanoma antigen-specific CTL by CD4+CD25 (Hi) regulatory T cells. Int Immunol 2009; 21: 1065-1077.
[9] Ghebeh H, Mohammed S, Al-Omair A. The B7-H1 (PD-L1) T lymphocyte-inhibitory molecule is expressed in breast cancer patients with infiltrating ductal carcinoma: correlation with important high-risk prognostic factors. Neoplasia 2006; 8: 190-198.
[10] Karim R, Jordanova ES, Piersma SJ. Tumor-expressed B7-H1 and B7-DC in relation to PD-1+ T-cell infiltration and survival of patients with cervical carcinoma. Clin Cancer Res 2009; 15: 6341-6347.
[11] Thompson RH, Dong H, Lohse CM. PD-1 is expressed by tumor-infiltrating immune cells and is associated with poor outcome for patients with renal cell carcinoma. Clin Cancer Res 2007; 13: 1757-1761.
[12] Savabkar S, Azimzadeh P, Chaleshi V, Nazemalhosseini Mojarad E and Aghdaei HA. Programmed death-1 gene polymorphism (PD-1.5 C/T) is associated with gastric cancer. Gastroenterol Hepatol Bed Bench 2013; 6: 178-182.
[13] Mojtahedi Z, Mohmedi M, Rahimifar S, Erfani N, Hosseini SV and Ghaderi A. Programmed death-1 gene polymorphism (PD-1.5 C/T) is associated with colon cancer. Gene 2012; 508: 229-232.
[14] Hua Z, Li D, Xiang G. PD-1 polymorphisms are associated with sporadic breast cancer in Chinese Han population of Northeast China. Breast Cancer Res Treat 2011; 129: 195-201.15.
[15] Haghshenas MR, Naeimi S, Talei A, Ghaderi A and Erfani N. Program death 1 (PD1) haplotyping in patients with breast carcinoma. Mol Biol Rep 2011; 38: 4205-4210.
[16] Qiu H, Zheng L, Tang W, Yin P, Cheng F and Wang L. Programmed death-1 (PD-1) polymorphisms in Chinese patients with esophageal cancer. Clinical Biochemistry 2014; 47: 612-617.
[17] Li Z, Li N, Zhu Q. Genetic variations of PD1 and TIM3 are differentially and interactively associated with the development of cirrhosis and HCC in patients with chronic HBV infection. Infect Genet Evol 2013; 14: 240-246.
[18] Tang W, Zhang S, Qiu H. Genetic variations in MTHFR and esophageal squamous cell carcinoma susceptibility in Chinese Han population. Med Oncol 2014; 31: 915.
[19] Tang W, Qiu H, Jiang H. Lack of association between cytotoxic T-lymphocyte antigen 4 (CTLA-4) -1722T/C (rs733618) polymorphism and cancer risk: from a case-control study to a meta-analysis. PLoS One 2014; 9: e94039.
[20] Guo W, Wang N, Wang YM. Interleukin-10 -1082 promoter polymorphism is not associated with susceptibility to esophageal squamous cell carcinoma and gastric cardiac adenocarcinoma in a population of high-incidence region of north China. World J Gastroenterol 2005; 11: 858-862.
[21] Sun T, Zhou Y, Yang M. Functional genetic variations in cytotoxic T-lymphocyte antigen 4 and susceptibility to multiple types of cancer. Cancer Res 2008; 68: 7025-7034.
[22] Yin J, Liu C, Wang X. Interleukin 15 receptor alpha rs2228059 A>C polymorphism decreased risk of gastric cardiac adenocarcinoma in a Chinese population. Tumour Biol 2014; 35: 6593-6600.
[23] Yin J, Wang X, Wei J, Wang L, Shi Y, Zheng L, Tang W, Ding G, Liu C, Liu R, Chen S, Xu Z, Gu H. Interleukin 12B rs3212227 T>G polymorphism was associated with an increased risk of gastric cardiac adenocarcinoma in a Chinese population. Dis Esophagus 2015; 28: 291-8.
[24] Wang X, Yin J, Zheng L. The variant interleukin 1f7 rs3811047 G>A was associated with a decreased risk of gastric cardiac adenocarcinoma in a Chinese Han population. Tumour Biol 2014; 35: 3509-3515.
[25] Tahoori MT, Pourfathollah AA, Akhlaghi M, Daneshmandi S, Nicknam MH and Soleimanifar N. Association of programmed cell death-1 (PDCD-1) gene polymorphisms with rheumatoid arthritis in Iranian patients. Clin. Exp. Rheumatol 2011; 29: 763-767.
[26] Momin S, Flores S, Angel BB, Codner DE, Carrasco PE and Perez-Bravo F. Interactions between programmed death 1 (PD-1) and cytotoxic T lymphocyte antigen 4 (CTLA-4) gene polymorphisms in type 1 diabetes. Diabetes Res Clin Pract 2009; 83: 289-294.
[27] Liu X, Hu LH, Li YR, Chen FH, Ning Y and Yao QF. Programmed cell death 1 gene polymorphisms is associated with ankylosing spondylitis in Chinese Han population. Rheumatol Int 2011; 31: 209-213.
[28] Bayram S, Akkiz H, Ulger Y, Bekar A, Akgollu E and Yildirim S. Lack of an association of programmed cell death-1 PD1.3 polymorphism with risk of hepatocellular carcinoma susceptibility in Turkish population: a case-control study. Gene 2012; 511: 308-313.
[29] Dehaghani AS, Kashef MA, Ghaemenia M. PDCD1, CTLA-4 and p53 gene polymorphism and susceptibility to gestational trophoblastic diseases. J Reprod Med 2009; 54: 25-31.

[30] Ivansson EL, Juko-Pecirep I and Gyllensten UB. Interaction of immunological genes on chromosome 2q33 and IFNG in susceptibility to cervical cancer. Gynecol Oncol 2010; 116: 544-548.
[31] Guzman VB, Yambartsev A, Goncalves-Primo A. New approach reveals CD28 and IFNG gene interaction in the susceptibility to cervical cancer. Hum Mol Genet 2008; 17: 1838-1844.
[32] Gazave E, Marques-Bonet T, Fernando O, Charlesworth B and Navarro A. Patterns and rates of intron divergence between humans and chimpanzees. Genome Biol 2007; 8: R21.
[33] Majewski J and Ott J. Distribution and characterization of regulatory elements in the human genome. Genome Res 2002; 12: 1827-1836.
[34] Baralle D and Baralle M. Splicing in action: assessing disease causing sequence changes. J Med Genet 2005; 42: 737-748.