Biomarkers, 2012; 17(1): 9–15

ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.3109/1354750X.2011.633242

informa
healthcare

RESEARCH ARTICLE

Relationship between HLA-G gene polymorphism and the susceptibility of esophageal cancer in Kazakh and Han nationality in Xinjiang

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Abstract

Objective: To explore the association between polymorphism of the human leukocyte antigen G (HLA-G) and susceptibility of esophageal carcinoma (EC) in Kazakh and Han nationality in Xinjiang.

Methods: The 14 bp deletion/insertion (rs16375) and 0105N (rs41557518) of HLA-G genotyping were determined by PCR and PCR-RFLP, respectively in 239 patients and 467 controls.

Results: There was a 2.69-fold (P_c = 0.04, 95% CI: 1.30–5.55) increased risk of developing EC in individuals with the −14 bp/−14 bp genotype (rs16375) compared with those carrying +14 bp/+14 bp genotype in Kazakh after Bonferroni correction, there was no association of 0105N (rs41557518) both in Kazak and Han population. And there was a 2.82-fold (P_c = 0.04, 95% CI: 1.32–6.04) increased risk of developing EC in individuals with −14 bp/−14 bp and C/C genotypes compared with those who had +14 bp/+14 bp and C/C genotypes in Kazakh.

Conclusions: The study demonstrates that EC is associated with polymorphism of HLA-G14 bp in Chinese Kazak population. The 14 bp deletion/insertion of HLA-G gene may play a role in EC susceptibility of Kazakh.

Keywords: Human 8-hydroxyguanine glycosylase, genetic polymorphism, esophageal cancer, Kazakh

Introduction

The incidence of esophageal cancer varies considerably with geographic location and also, to some extent, among ethnic groups within a common area. Some of the highest rates occur in northern China and northern Iran, where incidence exceeds 100 in 100,000 individuals. The nonclassical human leukocyte antigen G (HLA-G) class I locus can be distinguished from classical class I loci in terms of cellular and tissue expression patterns, peptide-binding properties and functions (Pyo et al. 2006). It is predominantly expressed at the maternal–fetal interface, particularly in the extravillous cytotrophoblast, and has primarily been associated with maternal–fetal tolerance. HLA-G is involved in the inhibition of maternal cytotoxic T lymphocyte (CTL) and natural killer (NK) cytolytic functions (Hviid 2006), prevents proliferation of CD4+ T cells and tolerizes dendritic cells (DCs) (Ristich et al. 2005). HLA-G plays an important role in the modulation of the maternal immune system during pregnancy, enabling fetuses to survive unharmed in a genetically foreign environment during successful pregnancies (Castelli et al. 2008). In addition, HLA-G expression is observed at low levels, in a variety of normal human adult tissues (Hviid 2006). HLA-G expression has been identified in several tumors, including renal cell carcinoma, esophageal squamous cell carcinoma, gastric carcinoma, ovarian carcinoma, breast cancer, glioblastoma, and cutaneous lymphoma, endometrial adenocarcinoma (Dunker et al. 2008, Yie et al. 2007, Yie et al. 2007, Menier et al. 2009, Chen et al. 2010, Barrier et al. 2006). The expression of HLA-G is not specific for just the malignant cells in tumors, but can be found in the immune cells that infiltrate the tumor site such as antigen presenting cells and T cells and favors cancer progression (Rouas-Freiss et al.

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(Received 11 May 2011; revised 10 October 2011; accepted 15 October 2011)

2003). The aberrant expression of HLA-G by tumor cells has been suggested to be part of the strategies they use to escape from the host's immuno-surveillance.

HLA-G expression has been extensively evaluated in several disorders of distinct etiologies; however, HLA-G gene polymorphism has not been studied to the same extent. Since the magnitude of HLA-G expression is regulated by the promoter and 3′ untranslated regions (3′ UTR) many studies have focused on these regions, particular on the 3′ UTR polymorphic sites (Lajoie et al. 2010, Matte et al. 2004, Martinetti et al. 2006, La Nasa et al.2007, Rizzo et al. 2008). In our study, two polymorphic sites of HLA-G 14 bp deletion/insertion (rs16375) and 0105N (rs41557518) were chosen due to their functional properties to explore whether HLA-G gene polymorphisms play an important role in risk of the esophageal carcinoma (EC) incidence in Kazak population.

Material and methods

Patients and controls

Participants were collected to the study from the six hospitals (Xinjiang Autonomous Region People's Hospital, the First Affiliated Hospital of Xinjiang Medical University, the Second Affiliated Hospital of Xinjiang Medical University, the Third Affiliated Hospital of Xinjiang Medical University, the Friendship Hospital of Ili State, the Xinhua Hospital of Ili State) from 2005–2007. There were 239 patients (132 Kazakans, 107 Han) with primary esophageal cancer which were diagnosed by endoscopy, X-ray and pathology, and the patients were unrelated. And there were 467 healthy controls (254 Kazakans, 213 Han) of non-blood relationship, the same nationality, gender, age difference of ±5 years old, over the same period of hospitalization in non-tumor, non-autoimmune diseases. Informed consent was obtained from all participants, and then EDTA blood (5 mL) was achieved from EC patients and healthy controls, preserving in –20°C.

Genotyping assays

Genome DNA was extracted from 1 mL EDTA anti-coagulated peripheral blood sample using the improved Miller salting-out procedure (Miller et al. 1988)

HLA-G exon 8 was amplified by polymerase chain reaction (PCR) using the forward primer 5′-GTGATGGGCTGTTTAAAGTGTCACC-3′ and reverse primer 5′-GGAAGGAATGCAGTTCAGCATGA-3′ for the HLA-G 14 bp insertion/deletion polymorphism analysis (rs16375); PCR amplification was performed with 50 ng genomic DNA by the following conditions: 10 × PCR Buffer 4 μL, 10 mM dNTP 1μL, 25 mM $Mgcl_2$ 4 μL, 10 pM of each primer 1 μL, 2.5 U Taq DNA polymerase (Sangon, BBI) in a final volume of 20 μL. The PCR amplification was completed with initial denaturation at 95°C for 4 min, 35 cycles at 95°C for 30 s, 59°C for 30 s and 72°C for 30 s and final extension at 72°C for 7 min (Mycycler, Bio-Rad, America). PCR products were of either 224 or 210 bp, respectively, depending on the insertion/deletion of the 14 bp in exon 8. Genotyping for 14 bp insertion/deletion polymorphism (rs16375) was performed by electrophoresis. PCR products were run on 4% agarose gel, the number of 14 bp insertion/deletion alleles was calculated.

HLA-G exon 3 was amplified by PCR using the forward primer 5'-CCAGTGGATGATTGGCTGCG-3' and reverse primer 5'-CCGTTCTCCAGGTATCTGTG-3' for the HLA-G*0105N polymorphism analysis (rs41557518); PCR amplification were performed with 50 ng genomic DNA by the following conditions: 10 × PCR Buffer 4 μL, 10 mM dNTP 1 μL, 25 mM $MgCl_2$ 2 μL, 10 pM of each primer 1 μL, 2.5 U Taq DNA polymerase (Sangon, BBI) in a final volume of 20 μL. The PCR amplification was completed with initial denaturation at 95°C for 4 min, 35 cycles at 95°C for 30 s, 59°C for 30 s and 72°C for 30 s and final extension at 72°C for 10 min (Mycycler, Bio-Rad, USA). Genotyping for HLA-G*0105N polymorphism was carried out by restriction fragment length polymorphism with the enzyme PpumI (New England Biolabs, USA) according to the instruction. The normal sequence shows 104 and 136 bp bands. The deletion sequence eliminated a cutting site, resulting in a 240 bp band PpumI-digested PCR products were run on 2% agarose gel, so the heterozygote for HLA-G*0105N was digested into three bands with the 240, 104 and 136 bp, respectively.

The homozygous genotype of the two polymorphic sites were confirmed with automatic sequencing (ABI 3130; Applied Bio-systems, CA) and the results were compared with the HLA-G gene sequence in GeneBank Blast.

Statistical analysis

Descriptive statistics were used to calculate frequencies and percentages of discrete variables. The test for goodness of fit between observed and estimated 14 bp insertion/deletion and 0105C/del genotype frequencies according to the Hardy–Weinberg equilibrium was determined by the chi-squared test. Allele frequencies and genotype frequencies of two sites in cases and controls were tested by chi-squared test. The odds ratio (OR) and 95% confidence interval (95% CI) were calculated by logistic regression. Multiple-testing was performed by Bonferroni correction, adjusted P_c values <0.05 were considered significant. Statistical analysis was conducted by SPSS 13.0 software (SPSS, inc., Chicago, IL).

Results

Characteristics of cases and controls

The characteristics of 239 cases and 467 controls are presented in Table 1. There were no statistically significant differences between the cases and controls in gender, age, education level, and marital status.

PCR amplification and genotyping

In this study, there are three kinds of PCR products for the HLA-G14 bp del/ins, in case of a deletion, one band and the fragment length was 210 bp (del–), the genotype

was the homozygous –14 bp/–14 bp, in case of an insertion, one band, it was 224 bp (del+), the genotype was the homozygous +14 bp/+14 bp, in case of two bands, it was the heterozygote –14 bp/+14 bp (Figure 1). There are two genotypes for the HLA-G*0105N, no homozygote was observed. The heterozygote genotype (C/–) has three bands with the 240, 104 and 136 bp, the genotype (–/–) has two bands with 104 and 136 bp (Figure 3).

The association between HLA-G polymorphisms and esophageal cancer

HLA-G gene on the risks of EC in Kazakh's and Han's cases and controls are presented in Tables 2 and 3. Genotype frequencies of these genetic polymorphisms were calculated from the control group by a Hardy–Weinberg equilibrium (χ^2-test, $P > 0.05$). In the Kazakans, the frequency of the cases with HLA-G 14 bp del (–14 bp/–14 bp, rs16375)

Table 1. General demographic characteristics of cases and controls.

	Kazakan (n=386)				Han (n=320)			
Characteristics	Case n (%)	Control n (%)	χ^2	P	Case n (%)	Control n (%)	χ^2	P
Sex								
Male	80 (60.61)	157 (61.80)	0.05	0.82	76 (71.00)	159 (74.60)	0.48	0.49
Female	52 (39.39)	97 (38.20)			31 (29.00)	54 (25.40)		
Age (years)								
≤45	13 (9.80)	25 (9.80)	4.39	0.22	4 (3.70)	16 (7.50)	3.67	0.30
46~	34 (25.80)	61 (24.00)			11 (10.30)	28 (13.10)		
56~	59 (44.70)	94 (37.00)			40 (37.40)	62 (29.10)		
≥66	26 (19.70)	74 (29.10)			52 (48.60)	107 (50.20)		
Education level								
Yes	20 (15.20)	36 (14.20)	0.07	0.80	13 (12.10)	29 (13.60)	0.13	0.71
No	112 (84.80)	218 (85.80)			94 (87.90)	184 (86.40)		
Marital status								
Married	119 (91.70)	234 (92.10)	3.98	0.26	100 (94.30)	197 (92.50)	0.44	0.80
Widowed	9 (6.80)	20 (7.90)			5 (4.70)	14(6.60)		
Divorce	1 (0.80)	0 (0.00)			1 (0.90)	2(0.90)		
Unmarried	1 (0.80)	0 (0.00)			0 (0.00)	0 (0.00)		

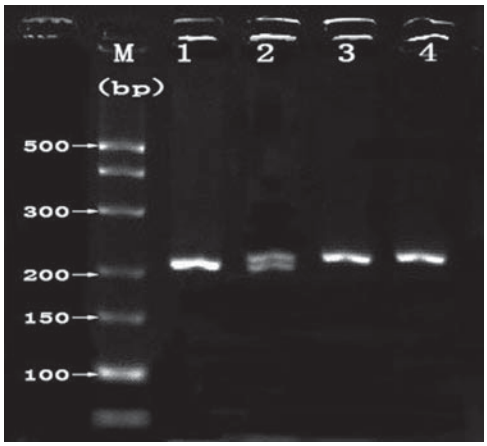

Figure 1. The PCR amplification products of the gene HLA-G 14 bp del (rs16375) (M: Low MW DNA Marker-A; Lane 1: the length is 210 bp; Lane 2: the lengths are 210 bp and 224 bp; Lanes 3 and 4: the length is 224 bp).

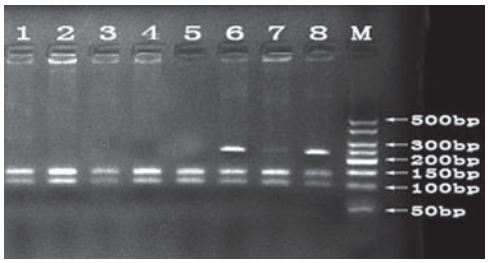

Figure 3. The PCR amplification products of the gene HLA-G*0105N (rs41557518) (M: 50 bp Ladder DNA Marker; Lanes 6 and 8: the lengths are 240 bp, 136 bp and 104 bp; the other lengths are 136 bp and 104 bp).

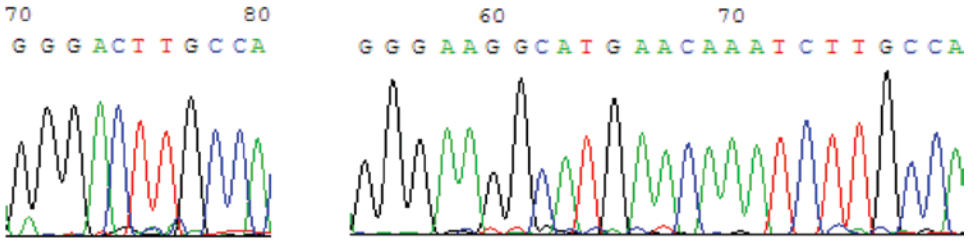

Figure 2. The sequencing of the PCR products of the gene HLA-G 14 bp del (rs16375) (the left is genotype of –14 bp/–14 bp, the right is genotype of +14 bp/+14 bp, arrow mark as a variant fragment).

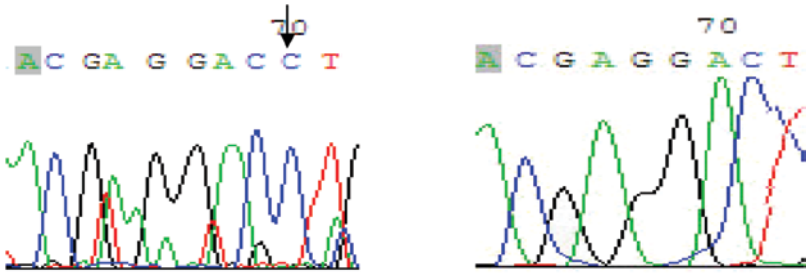

Figure 4. The sequencing of the PCR products of the gene HLA-G*0105N (rs41557518) (the left is genotype of C/C, the right is genotype of C/–, arrow mark as the mutant fragment).

Table 2. Association between HLA-G polymorphism and esophageal cancer in Kazakh.

	Case (n = 132)		Control (n = 254)						
	N	%	N_o	%	χ^2	P	OR	95% CI	P_c
HLA-G14 bp del (rs16375)									
Genotype									
+14 bp/+14 bp	12	9.09	46	18.33	–	–	1.00	–	–
−14 bp/+14 bp	66	50.00	128	51.00	3.71	0.05	1.98	0.98–3.99	0.20
−14 bp/−14 bp	54	40.91	77	30.67	7.46	0.01	2.69	1.30–5.55	0.04
Allele									
+14 bp	90	34.10	220	43.80	–	–	1.00	–	–
−14 bp	174	65.90	282	56.20	6.80	0.01	1.51	1.11–2.06	0.04
HLA-G*0105N (rs41557518)									
Genotype									
C/– + –/–	11	8.33	30	11.95	–	–	1.00	–	–
C/C	121	91.67	221	88.05	1.19	0.28	0.67	0.32–1.38	1.12
Allele									
–	11	4.17	30	5.98	–	–	1.00	–	–
C	253	95.83	472	94.02	1.13	0.29	1.46	0.72–2.97	1.16

Table 3. Association between HLA-G polymorphism and esophageal cancer in Han.

	Case (n = 107)		Control (n = 213)						
	N_o	%	N_o	%	χ^2	P	OR	95% CI	P_c
HLA-G14 bp del									
Genotype									
+14 bp/+14 bp	18	16.82	24	11.37	–	–	1.00	–	–
−14 bp/+14 bp	57	53.27	109	51.66	1.06	0.30	0.70	0.35–1.39	0.12
−14 bp/−14 bp	32	29.91	78	36.97	2.61	0.11	0.55	0.26–1.14	0.44
Allele									
+14 bp	93	43.46	157	37.20	–	–	1.00	–	–
−14 bp	121	56.54	265	62.80	2.33	0.13	1.30	0.93–1.81	0.52
HLA-G*0105N									
Genotype									
C/– + –/–	25	23.36	30	14.22	–	–	1.00	–	–
C/C	82	76.64	181	85.78	4.15	0.04	1.84	1.02–3.32	0.16
Allele									
–	25	11.68	30	7.11	–	–	1.00		–
C	189	88.32	392	92.89	3.76	0.05	0.58	0.33–1.01	0.20

genotype was higher than that of controls (40.91 vs. 30.67%), and also there was a significant difference by Bonferroni correction (P_c = 0.04), the individual with −14 bp/−14 bp genotype had a 2.69-fold (95% CI = 1.30–5.55) increased risk of EC compared with those carrying genotype (+14 bp/+14 bp). No significant difference was found in the HLA-G*0105N (rs41557518) genotype distribution between the cases and controls (P_c = 1.12).

In the Han's, no significant difference was found in the HLA-G14 bp del (rs16375) genotype distribution between the cases and controls (P > 0.05) whether the Bonferroni correction was performed or not. And the frequency of

Table 4. Interaction of two polymorphic sites of HLA-G gene and susceptibility to EC in Kazakh.

0105N (rs41557518)	14 bp del (rs16375)	Case	Control	χ^2	P	OR	95% CI	P_c
C/C	+14 bp/+14 bp	11	41	–	–	1.00	–	–
C/C	+14 bp/–14 bp	60	114	3.30	0.07	1.96	0.94–4.09	0.28
C/C	–14 bp/–14 bp	50	66	7.48	0.01	2.82	1.32–6.04	0.04
C/–	+14 bp/+14 bp	1	5	–	–	1.00	–	–
C/–	+14 bp/–14 bp	6	14	0.42	0.52	2.14	0.20–22.48	2.08
C/–	–14 bp/–14 bp	4	11	0.24	0.63	1.82	0.16–20.71	2.52

Table 5. Interaction of two polymorphic sites of HLA-G gene and susceptibility to EC in Han.

0105N (rs41557518)	14 bp del (rs16375)	Case	Control	χ^2	P	OR	95% CI	P_c
C/C	+14 bp/+14 bp	14	20	–	–	1.00	–	–
C/C	+14 bp/–14 bp	41	88	1.06	0.30	0.67	0.31–1.45	1.20
C/C	–14 bp/–14 bp	27	72	2.29	0.13	0.54	0.24–1.21	0.52
C/–	+14 bp/+14 bp	4	4	–	–	1.00	–	–
C/–	+14 bp/–14 bp	16	21	0.12	0.73	0.76	0.17–3.52	2.92
C/–	–14 bp/–14 bp	5	6	0.04	0.85	0.83	0.13–5.17	3.40

the cases with two copies of HLA-G*0105N (rs41557518) variation genotype (C/C) was higher than that of controls (76.64 vs. 85.78%), but there was no statistical difference between them after Bonferroni test ($P_c = 0.16$).

Interaction of two polymorphic sites of HLA-G gene and genetic susceptibility to EC

The interactions of two sites of HLA-G gene and susceptibility to EC are presented in Tables 4 and 5. Table 4 showed that there was a 2.82-fold ($P_c = 0.04$, 95% CI: 1.32–6.04) increased risk of EC in subjects with the HLA-G14 bp del genotype (–14 bp/–14 bp) and the 0105N genotype (C/C) compared with those carrying HLA-G14 bp del genotype (+14 bp/+14 bp) and the 0105N genotype (C/C) in the Kazakh. And there was no significant difference between the interaction of HLA-G genetic polymorphisms and susceptibility to EC in Han nationality.

Discussion

Cancer cells display tumor-associated antigens coded by mutated or normal deregulated genes that, once presented by classical MHC class I molecules, may be recognized by the host immune system, being usually eliminated. Even in the presence of a competent immune system, neoplastic cells can grow and progress to very aggressive malignant lesions, evolving by tumor immunoediting, a process that comprises three major mechanisms. First, most immunogenic tumor cells are eliminated by cytotoxic T and NK cells. Second, tumor cells with reduced immunogenicity are selected. Third, variants that no longer respond to the host immune system are maintained (Carosella et al.2007, Dunn et al.2006, Dunn et al.2004). HLA-G is supposed to play a pivotal role in cancer immunoediting by decreasing the elimination of tumor cells, inhibiting the cytotoxic function of T and NK cells, and by trogocytosis, i.e. the intercell transference of viable HLA-G molecules, rendering competent cytotoxic cells unresponsive to tumor antigens (LeMaoult et al. 2007, Caumartin et al. 2007).

Most studies have evaluated HLA-G expression in tumor cells, tumor cell lines or even soluble HLA-G levels, and few genetic studies have been performed. Underlying these tumor-associated studies is the concept that some HLA-G polymorphisms may influence HLA-G expression (promoter and 3′ UTR polymorphisms), the pattern of alternative splicing of the primary transcript and mRNA turnover (coding region, including introns, and 3′ UTR polymorphisms).

The 3′ UTR of HLA-G has an important role on the regulation of gene expression. HLA-G alleles presenting the 14-bp sequence (5′-ATTTGTTCATGCCT-3′) have been associated with low HLA-G mRNA production for most membrane-bound and soluble isoforms in trophoblast samples (Castelli et al. 2008), which may affect HLA-G function (Lefebvre et al. 2002). HLA-G mRNA transcripts presenting the 14-base insertion can be further processed by the removal of the first 92 bases of exon 8, resulting in HLA-G transcripts that have been reported to be more stable than the complete mRNA forms (Veit & Chies 2008). In physiological conditions, monocyte secretes a small amount of sHLA-G in human's peripheral blood. Low levels of sHLA-G can not play a role, and the high levels of serum sHLA-G suggest that human being had a lower immune surveillance level, which is not conducive to NK cells and T cells to clear mutant cells but in favor of the development of the tumors. An experiment by ELISA method was found that the level of serum sHLA-G in esophageal cancer patients was higher than control (Contini et al. 2003). A 14 bp insertion/deletion polymorphism is related with the soluble HLA-G molecule expression level. Generally, HLA-G +14 bp is considered as a low secretor allele gene, HLA-G –14 bp is considered as a high secretor allele gene. However heterozygote –14 bp/+14 bp genotype, the level of serum sHLA-G is lower (Tripathi et al. 2004, O'Brien et al. 2001). In our study, we found that there was a 2.69-fold ($P_c = 0.04$,

95% CI: 1.30–5.55) increased risk of developing EC in individuals with the –14 bp/–14 bp genotype (rs16375) compared with those carrying +14 bp/+14 bp genotype in Kazakh after Bonferroni correction, but not in Han. The results demonstrate that the polymorphism of HLA-G 14 bp deletion/insertion (rs16375) may be related to the higher level of serum sHLA-G, so as to increase the risk of the esophageal cancer in Kazakh.

The HLA-G*0105N null allele is characterized by a single base pair deletion in exon 3 with the deletion of a single cytosine at the first base of codon 130 or the last base of codon 129, which results in a gap in the open reading frame, causing a premature stop at either codon 189 (TGA), which blocks translation of HLA-G1 and -G5, or codon 297 (TAG), blocking the translation of HLA-G4 (Le Discorde et al. 2005). It was confirmed that HLA-G*0105N does not encode the complete HLA-G1 and HLA-G4 membrane-bound isoforms, or HLA-G5 soluble isoform of HLA-G, but does encode both membrane-bound and soluble functional HLA-G proteins, such as HLA-G2, HLA-G3, HLA-G6, and HLA-G7 proteins (Lin et al. 2009). Thus HLA-G isoforms due to genetic polymorphic site of HLA-G*0105N may not affect on its function. In our study, we found that comparing with the C/– genotype, individuals with C/C genotype had a 1.84-fold (95% CI: 1.02–3.32) increased risk of developing EC in Han nationality, but there was no association after Bonferroni test. When we explored the relationship between interactions of two sites and susceptibility to EC, it indicated that there was a 2.82-fold (95% CI: 1.32–6.04) increased risk of EC in subject with the HLA-G14 bp del genotype (–14 bp/–14 bp) and the 0105N genotype (C/C) compared with those carrying HLA-G14 bp del genotype (+14 bp/+14 bp) and the 0105N genotype (C/C) in the Kazakh, but not in the Han population. According to the frequency distribution of genetic polymorphisms exist the variance in different ethnic groups, we got the different results in Kazakh and Han population. In addition, the mechanism of the HLA-G molecular still exist controversy, these suggestive results need to be verified in further study.

Declaration of interest

This work was supported by National Natural Science Foundation of China (Grant No. 30460118).

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