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Certain haplotypes of the 3′-UTR region of the HLA-G gene are linked to breast cancer

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ABSTRACT

Background: Human leukocyte antigen G belongs to the family of non-classical HLA class I genes, its expression considered an important immune escape mechanism of cancer cells. The polymorphisms in the 3′-untranslated region (UTR) region of HLA-G influence the magnitude of the protein by modulating HLA-G mRNA stability. We hypothesised links between any of eight (UTR) single nucleotide polymorphisms (SNPs) and their haplotype of the HLA-G gene with breast cancer.

Materials and Methods: Peripheral blood DNA from 100 patients affected by breast cancer and 100 controls was PCR sequenced for genotyping of 25 HLA-G 3′-UTR regions, including rs371194629 (+2960), rs1707 (+3003), rs1710 (+3010), rs17179101 (+3027), rs1063320 (+3142), rs9380142 (+3187), rs1610696 (+3196), and rs1233331 (+3227).

Results: The 14-bp deletion ($p = 0.01$), and the +3010 ($p = 0.021$), +3142 ($p = 0.006$) and +3187 ($p = 0.046$) variants were significantly more prevalent in patients than in controls. In combining these data, two haplotypes of all eight SNPs and deletion/insertion (UTR-1 and UTR-4) are associated with breast cancer.

Conclusion: Certain variants in the 3-UTR, and their combination as a haplotype, of the HLA-G gene are linked to breast cancer.

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HLA-G; 3'-UTR; haplotype; breast cancer

Introduction

The International Agency of Research on Cancer estimates of new breast cancer cases place this cancer in the first rank for women, followed by lung cancer and colorectal cancer, and the second cause of death worldwide [1]. Genetic and epigenetic alterations play a role in the development of cancer, although it can also be influenced by environmental factors, physicochemical factors and viruses [2].

HLA-G is a non-classical HLA class I molecule, whose expression was initially observed in extravillous cytotrophoblasts and that plays a role in the maintenance of foetal-maternal immune tolerance [3,4]. In addition to extravillous cytotrophoblasts, HLA-G expression is restricted to few healthy adult tissues, including the cornea, the thymic medulla and the pancreatic islets [5,6]. However, HLA-G expression is increased in various pathological conditions such as cancers, viral infection, organ transplantation, and autoimmune and inflammatory diseases [7].

Much *in vitro* and *in vivo* evidence shows that HLA-G could directly interact with its receptors expressed on almost all types of immune cells or by the pathway of 'trogocytosis', revealing a broad immune inhibiting function on both innate and adaptive immune responses [8]. Various studies have demonstrated that aberrantly expressed HLA-G in various types of cancers, including those of the breast, is related to advanced tumour grade, more aggressive behaviour and worse outcome [9]. However, the role of changes to the 3-untranslated region (UTR) of HLA-G and breast cancer are unexplored. We hypothesised links between variants of the 3-UTR and breast cancer. The existence of a haplotype associated with breast cancer susceptibility in the population was assessed, and the value of *linkage disequilibrium* (LD) was determined. Study of these haplotypes and polymorphisms could highlight their possible role in breast cancer susceptibility.

Methods and materials

This case-control study included 100 female patients affected by breast cancer and 100 female controls with no history of cancer. Full written consent was obtained, the study protocol followed the ethical guidelines of the 1975 Declaration of Helsinki, and was approved by the Ethics Committee of the University of Tabriz.

Genomic DNA was isolated from EDTA-treated peripheral blood samples using magnetic nanoparticles from a commercial extraction kit (www.ziaviz.com). The target sequence containing the polymorphic region was amplified by forward primer: 5′-

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GTGCTATGAGGTTTCTTTGACTTCAATG-3′ and reverse primer: 5′-GTCTTCCATTTATTTTGTCTCT-3′. PCR amplification was performed using PCR Master Mix (Cinaclone-Iran) with a volume of 50 µl. An initial denaturation at 95 °C for 2 min; 35 cycles of denaturation at 95 °C for 30 s, annealing at 57 °C for 30 s, and extension at 72 °C for 40s; and a final extension at 72 °C for 2 min. Corbett CGI-96 Palm-Cycler Thermal cycler was used. Sequencing results of PCR products were analysed by Chromas software (v.2.4; Technelysium Pty Ltd., Australia), and eventually all the SNPs in the 3′-UTR region of the HLA-G were genotyped in all study subjects. After SNP genotyping, allele and genotype frequencies were calculated using SPSS software. The frequency of polymorphisms and the combination of these markers as haplotypes were evaluated by Haploview v.4.2 software based on p-values < 0.05 as significant. Also, calculating the linkage disequilibrium for finding D′ between each marker was done.

Results

The mean [SD] age of patients and controls were 47.9 [2.3] and 51.5 [2.4] years, respectively [p > 0.05]. Thirty-eight per cent of patients had a family history of breast cancer. Tumour type was ductal in 64% and lobular in 36%. Metastasis was present in 59% of the patients.

Twenty-five SNPs were analysed; the allele and genotype frequencies of eight SNPs are summarised in Table 1. According to the genotyping results, the rs371194629 deletion allele was significantly more

Table 1. Alleles and genotypes distribution of the 3′-UTR region of HLA-G polymorphisms in breast cancer patients compared to controls. D indicates 14bp deletion allele and I indicate 14bp Insertion allele.

HLA-G	Patients, n = 100	Controls, n = 100	OR (95%CI)	P-value
rs371194629(+2960)				
D	148 (74%)	123 (61.5%)	1.78 (1.16–2.72)	0.01
I	52 (26%)	77 (38.5%)		
DD	56 (56%)	40 (40%)	1.90 (1.08–3.34)	0.034
DI	36 (36%)	43 (43%)	0.75 (0.42–1.31)	0.385
II	8 (8%)	17 (17%)	0.43 (0.15–1.04)	0.087
rs1707(+3003)				
T	156 (78%)	158 (79%)		
C	44 (22%)	42 (21%)	1.06 (0.66–1.71)	0.903
TT	67 (67%)	63 (63%)	1.192 (0.67–2.13)	0.657
TC	22 (22%)	32 (32%)	0.59 (0.32–1.13)	0.152
CC	11 (11%)	5 (5%)	2.35 (0.79–7.03)	0.193
rs1710(+3010)				
G	108 (54%)	84 (42%)	1.62 (1.09–2.41)	0.021
C	92 (46%)	116 (58%)		
GG	32 (32%)	21 (21%)	1.77 (0.94–3.35)	0.109
GC	44 (44%)	42 (42%)	1.08 (0.62–1.9)	0.886
CC	24 (24%)	37 (37%)	0.53 (0.29–0.99)	0.065
rs17179101(+3027)				
C	183 (91.5%)	176 (88%)	1.46 (0.76–2.83)	0.323
A	17 (8.5%)	24 (12%)		
CC	85 (85%)	78 (78%)	1.59 (0.77–3.29)	0.275
CA	13 (13%)	20 (20%)	0.59 (0.28–1.28)	0.253
AA	2 (2%)	2 (2%)	1 (0.13–7.24)	0.614
rs1063320(+3142)				
G	99 (49.5%)	127 (63.5%)		
C	101 (50.5%)	73 (36.5%)	1.78 (1.19–2.65)	0.006
GG	30 (30%)	42 (42%)	0.59 (0.33–1.06)	0.105
GC	39 (39%)	43 (43%)	0.85 (0.48–1.49)	0.666
CC	31 (31%)	15 (15%)	2.55 (1.27–5.09)	0.012
rs9380142(+3187)				
A	150 (75%)	167 (83.5%)		
G	50 (25%)	33 (16.5%)	1.69 (1.04–2.78)	0.046
AA	57 (57%)	69 (69%)	0.59 (0.33–1.06)	0.107
AG	36 (36%)	29 (29%)	1.377 (0.76–2.49)	0.365
GG	7 (7%)	2 (2%)	3.69 (0.74–18.21)	0.172
rs1610696(+3196)				
C	147 (73.5%)	130 (65%)	1.49 (0.97–2.29)	0.083
G	53 (26.5%)	70 (35%)		
CC	54 (54%)	47 (47%)	1.32 (0.76–2.31)	0.396
CG	39 (39%)	36 (36%)	1.14 (0.64–2.02)	0.77
GG	7 (7%)	17 (17%)	0.37 (0.15–0.93)	0.05
rs1233331(+3227)				
G	193 (96.5%)	190 (95%)	1.45 (0.54–3.89)	0.63
A	7 (3.5%)	10 (5%)		
GG	93 (93%)	90 (90%)	1.48 (0.54–4.05)	0.612
GA	7 (7%)	10 (10%)	0.68 (0.25–1.86)	0.612
AA	0	0		

D = deletion, I = insertion. Other letters are nucleotides.

prevalent in patients than in controls. Furthermore, deletion/deletion genotype was significantly common in patients; therefore, the deletion allele can be regarded as a risk factor of breast cancer in this population. Moreover, the significant difference of +3010 G allele in patients and controls, +3142 C allele and +3187 G allele were significant associations with the breast cancer patient group. The other SNPs – rs551200694, rs941671211, rs567747015, rs1001428984, rs1036044443, rs1020986754, rs146339774, rs17179108, rs569057854, rs180827037, rs554784083, rs760052251, rs138249160, rs984061571, rs554076817, rs187320344, and rs531180553 – had little minor allele frequency and showed no significant association with breast cancer, either as a risk or a protective factor. Twenty-five SNPs were included in the 3′-UTR region of the HLA-G as a haplotype block and constructed by Haploview v.4.2 software; then a linkage disequilibrium plot was generated for 25 SNPs (Figure 1) and for nine selected SNPs (Figure 2). The highest D′ value was related to rs371194629, rs17179108 and rs1063320, which demonstrated significant linkage disequilibrium between these pairwise (Figure 2). The results summarised in Table 2 show that UTR-2 was the most frequent haplotype in the assembly of two groups of patient and control samples, and the second most frequent haplotype was UTR-1. The UTR-1 and UTR-4 haplotypes were significantly associated with breast cancer.

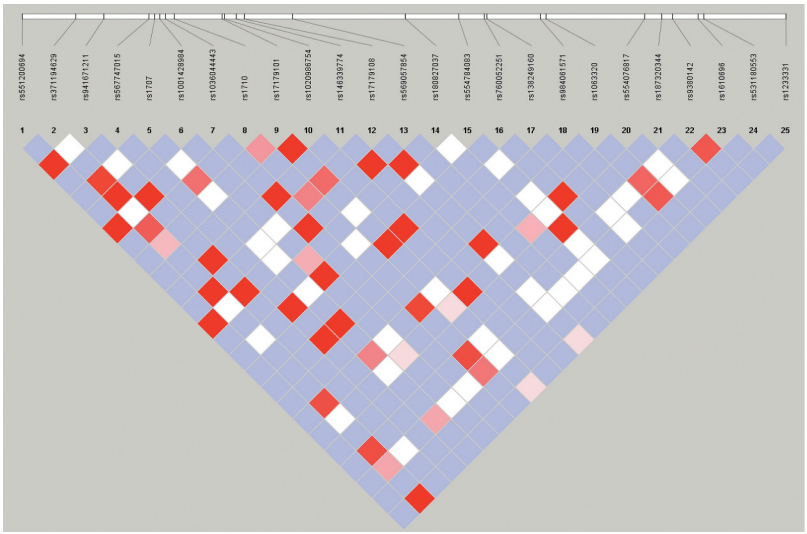

Figure 1. Linkage disequilibrium between all pairwise polymorphisms in 3′-UTR region of HLA-G gene. Red colour represents a significant D′ value and white colour demonstrates a low D′ value.

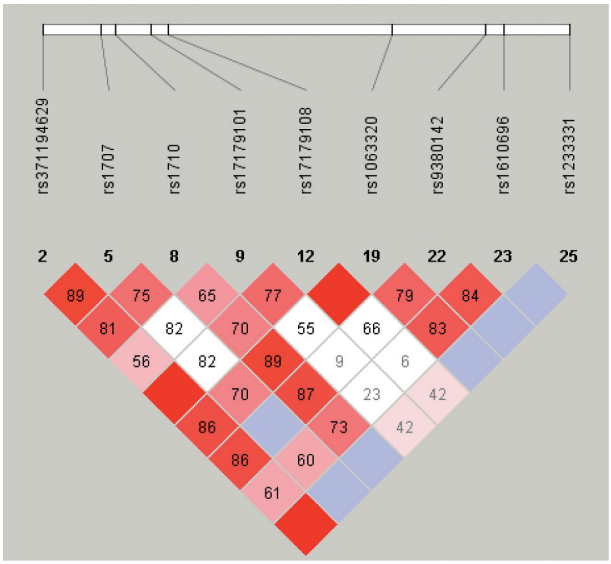

Figure 2. Linkage disequilibrium between selected nine pairwise polymorphisms in 3′-UTR region of HLA-G gene. Red colour represents a significant D′ value and white colour demonstrates a low D′ value.

Table 2. The HLA-G 3′-UTR haplotype frequency in breast cancer and healthy control samples.

dbSNP	rs371194629	rs1707	rs1710	rs17179101	rs17179108	rs1063320	rs9380142	rs1610696	rs1233331	Breast cancer 2 *n* = 200	Healthy sample 2 n = 200	p-Value
HLA-G position	2960	3003	3010	3027	3035	3142	3187	3196	3227			
UTR-2	Ins	T	C	C	C	G	A	G	G	0.163	0.203	0.309
UTR-1	Del	T	G	C	C	C	G	C	G	0.215	0.115	0.007
UTR-4	Del	C	G	C	C	C	A	C	G	0.182	0.112	0.048
UTR-3	Del	T	C	C	C	G	A	C	G	0.114	0.124	0.773
UTR-6	Del	T	G	C	C	C	A	C	G	0.068	0.068	0.989
UTR-10	Del	T	C	C	C	G	A	G	G	0.061	0.044	0.431
UTR-7	Ins	T	C	A	T	G	A	C	G	0.037	0.055	0.407
Others										0.160	0.279	

Discussion

Studies of polymorphism located in the 3′UTR region of HLA-G and its relationship with cancer for each polymorphism may be contradictory in different populations [9,10]. This discrepancy may be due to differences in the genetic context of different populations, as well as to the effect of continuous polymorphisms; therefore, more haplotype studies seem necessary. The 14bp In-Del has been a widely studied polymorphism in HLA-G. The association of the deleted variant with various cancers has been reported, although in some studies no association has been found [10]. Previous reports indicate that the 14bp deletion/insertion polymorphism influences HLA-G mRNA stability and isoform splicing patterns [11]. High expression of the HLA-G gene allows the cancer cell to protect itself from the immune system [12] and, as a result, the high frequency of 14-bp deletion alleles may be of pathological importance in breast cancer patients.

The presence of 14bp can act as a cryptic branch-point sequence for HLA-G mRNA splicing that causes a 92bp elimination of mRNA. Short-length mRNA, although more stable, has been reported to have low levels of the HLA-G protein. As a result, the insertion allele decreases protein expression and the deletion allele increases HLA-G protein expression [11]. An important point to consider is that short mRNA contains a region of 14-bp and +3003 and +3010 polymorphisms loci, and because multiple mRNAs bind in this region, it may explain why short mRNA is more stable [13]. There was a significant relationship between the G allele in +3010 polymorphism and breast cancer, which may be due to the direct effect of this polymorphism and/or due to the high linkage of this +3010 G allele with 14-bp deletion.

In haplotypes 2 and 4, there are G nucleotides at the +3010 site, which may merge to become one despite the mismatch and the reduced binding of some miRNAs, such as mir-513a [13]. Lack of strong binding is associated with decreased RNA degradation and increased protein expression, so the high frequency of this allele in the breast cancer group is to be expected, as observed in this study. This theory is reinforced by the reports of both HLA-G increase and decrease in miR-513 in some cancers, including breast cancer.

The C allele at +3142 showed a significant association with breast cancer. This association was also observed in a Tunisian homozygous CC status, which was also observed in a Tunisian population [14]. The results showed a significant relationship between allele A at +3187 and allele C at +3196 with breast cancer which has also been reported in ovarian cancer [15].

The order of frequency of haplotypes in the study population is almost consistent with global frequency. The study of haplotype distribution shows that the UTR-1 and UTR-4 haplotypes are more frequent in breast cancer patients and are risk factor haplotypes. However, the association of the UTR-1 haplotype as a risk factor for breast cancer is very strong ($P < 0.01$). Both UTR-1 and UTR-4 haplotypes have 14bp deletion and G nucleotide in +3010 loci with high LD (Figure 2). Reports show high expression of HLA-G genes by these haplotypes and their alleles, although both are common haplotypes in global populations [16]. It seems that the high ability of these haplotypes to be HLA-G expressed in the embryonic stage has been useful for immune tolerance in pregnancy and for natural selection, but this ability can be a risk factor for breast cancer and cancer cell immune evasion in adulthood.

We recognise certain limitations in our study, notably the relatively small sample size. Additional studies in different populations with larger sample sizes are necessary and useful. Identifying those polymorphisms and haplotypes that affect various cancers, including breast cancer, in different populations can enhance our understanding of the molecular nature of this disease.

This work represents an advance in biomedical science because it shows that 14-bp Del, +3010 G, +3142 C and +3187 G alleles and UTR-1 and UTR-4 haplotypes are associated with breast cancer.

Summary table

What is known about this subject?

- HLA-G expression and its role in various cancers including breast cancer have been reported.
- 3′-UTR polymorphisms and haplotypes of HLA-G play an important role in gene expression.

What this paper adds

- 14-bp Del, +3010 G, +3142 C and +3187 G alleles show significant association with breast cancer.
  UTR-1 and UTR-4 haplotypes of HLA-G gene show significant association with breast cancer.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin. 2019;69:7–34.
[2] Papaccio F, Paino F, Regad T, et al. Concise review: cancer cells, cancer stem cells, and mesenchymal stem cells: influence in cancer development. Stem Cells Transl Med. 2017;6:2115–2125.
[3] Wedenoja S, Yoshihara M, Teder H, et al. Fetal HLA-G mediated immune tolerance and interferon response in preeclampsia [published online ahead of print, 2020 Jul 11]. EBioMedicine. 2020;59:102872.
[4] Ferreira LMR, Meer TB, Tilburgs T, et al. HLA-G: at the interface of maternal-fetal tolerance. Trends Immunol. 2017;38:272–286.
[5] Cirulli V, Zalatan J, McMaster M, et al. The class I HLA repertoire of pancreatic islets comprises the nonclassical class Ib antigen HLA-G. Diabetes.2006;55:1214–1222.
[6] Le Discorde M, Moreau P, Sabatier P, et al. Expression of HLA-G in human cornea, an immune-privileged tissue. Hum Immunol. 2003;64:1039–1044.
[7] González A, Rebmann V, LeMaoult J, et al. The immunosuppressive molecule HLA-G and its clinical implications. Crit Rev Clin Lab Sci2012;49:63–84.
[8] Carosella ED, Gregori S, LeMaoult J. The tolerogenic interplay(s) among HLA-G, myeloid APCs, and regulatory cells. Blood. 2011;118:6499–6505.
[9] Lin A, Yan WH. Human Leukocyte Antigen-G (HLA-G) expression in cancers: roles in immune evasion, metastasis and target for therapy. Mol Med. 2015;21:782–791.
[10] Jiang Y, Lu J, Wu YE, et al. Genetic variation in the *HLA-G* 3′UTR 14-bp insertion/deletion and the associated cancer risk: evidence from 25 case-control studies. Biosci Rep. 2019 Published 2019 May 10;39:BSR20181991.
[11] Rousseau P, Le Discorde M, Mouillot G, et al. The 14 bp deletion-insertion polymorphism in the 3′ UT region of the HLA-G gene influences HLA-G mRNA stability. Hum Immunol. 2003;64:1005–1010.
[12] Krijgsman D, Roelands J, Hendrickx W, et al. HLA-G: a new immune checkpoint in cancer? Int J Mol Sci. 2020 Published 2020 Jun 25;21:4528.
[13] Castelli EC, Moreau P. Oya e Chiromatzo A, et al. In silico analysis of microRNAS targeting the HLA-G 3′ untranslated region alleles and haplotypes. Hum Immunol. 2009;70:1020–1025.
[14] Ouni N, Chaaben AB, Kablouti G, et al. The impact of *HLA-G* 3′UTR polymorphisms in breast cancer in a Tunisian population. Immunol Invest. 2019;48:521–532.
[15] Schwich E, Rebmann V, Michita RT, et al. HLA-G 3′ untranslated region variants +3187G/G, +3196G/G and +3035T define diametrical clinical status and disease outcome in epithelial ovarian cancer. Sci Rep. 2019;9:5407.
[16] Castelli EC, Ramalho J, Porto IO, et al. Insights into HLA-G genetics provided by worldwide haplotype diversity. Front Immunol. 2014;5:476.