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HLA-G genetic variants and hepatocellular carcinoma: a meta-analysis

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ABSTRACT. Human leukocyte antigen (HLA)-G is a key tolerogenic molecule mainly expressed in the placenta and is crucial for implantation of the embryo and immunological tolerance of the fetus during pregnancy. However, under pathological conditions, such as cancer or viral infections, HLA-G can be expressed in other tissues. The gene coding for HLA-G (*HLA-G*, chromosome 6p21.3) presents numerous polymorphisms, some of them influencing its expression. One of the most studied, is the 14 bp ins/del (rs371194629) situated at the 3'-UTR of the gene. The insertion is thought to stabilize *HLA-G* mRNA. Different studies have analyzed the role of rs371194629 in hepatic injury, with either hepatotropic virus infection (i.e., HBV or HCV) or hepatocellular carcinoma (also induced by viral infection). Results from these studies are heterogeneous, differing with ethnicity and population age, and the role of rs371194629 is unclear. For these reasons, we decided to perform a meta-analysis of these results,

concluding that the 14-bp ins/del polymorphism does not significantly contribute to hepatic injury.

Key words: MHC class I; HLA-G; Liver neoplasms; Genetic polymorphism; Association studies

INTRODUCTION

HLA-G is a non-classical human leukocyte antigen (HLA) class Ib gene, located at chromosome 6 at position 6p21.3 (Geraghty et al., 1987). The protein possesses immunomodulatory functions that promote tolerance to the semi-allogenic fetus during pregnancy. HLA-G inhibits proliferation and the cytolytic function of cytotoxic T lymphocytes and natural killer cells (Rouas-Freiss et al., 1997; Riteau et al., 1999; Bahri et al., 2006; Caumartin et al., 2007). Furthermore, it can induce apoptosis via Fas/FasL (Fons et al., 2006) and differentiation of CD4+ regulatory cells (LeMaoult et al., 2007).

Under physiological conditions, HLA-G is expressed only in few cell types: trophoblasts (Kovats et al., 1990), cornea cells (Le Discorde et al., 2003), and erythroid and endothelial cells (Menier et al., 2004). Under pathological conditions, tumors (Urosevic and Dummer, 2003) and tissues affected by autoimmune diseases (Rosado et al., 2008) and viral infections (Lozano et al., 2002) may express HLA-G.

HLA-G mRNA levels are finely regulated by its complex promoter and terminal regions. Therefore, sequence variations in both areas could modify gene expression. For example, the single nucleotide polymorphism (SNP) rs1233334 (-725C/G/T) is located at the end of an interferon-sensitive response element motif and the -725 G allele creates a CpG site, which when methylated can inhibit transcription. Moreover, at the 3'-UTR, a 14-bp ins/del has been widely reported as causing mRNA instability (rs371194629) (Rousseau et al., 2003). The presence of a G allele at rs1063320 (3142C>G) is known to increase the site affinity for microRNA, favoring mRNA degradation (Tan et al., 2007). Being upstream of an AU-rich motif involved in mRNA degradation, the A allele at rs9380142 (3187A>G) decreases HLA-G expression (Yie et al., 2008). Specifically, the 14-bp ins/del has been the subject of numerous studies, first in spontaneous abortion investigations (Yan et al., 2006; Xue et al., 2007) then in autoimmune disease (Lee et al., 2015), viral infections (da Silva et al., 2014) and cancer (Li et al., 2015). Since HLA-G function is quite pleiotropic, the role of the 14-bp ins/del varies and depends on the pathology under scrutiny.

Viruses have been the subject of evolutionary pressure to avoid host immune system responses. One of the mechanisms that have been developed is increasing expression of HLA-G, either directly or indirectly by inflammatory milieu caused by viral infection. HLA-G expression has been analyzed in different chronic viral infections: human immunodeficiency virus, human papillomavirus, human cytomegalovirus, and hepatitis B/C virus (HBV/HCV) (Catamo et al., 2014). Specifically, the latter two viruses are involved in chronic liver injury that could be followed by malignant transformation (hepatocellular carcinoma). Increased expression of HLA-G has been associated either with HBV infection, via increased viral load (Souto et al., 2011), or HCV infection (Weng et al., 2011). Moreover, in early stages of HCV-associated liver infection, both soluble and membrane bound HLA-G protein production are increased (de Oliveira Crispim et al., 2012; Amiot et al., 2014). It is also possible that the presence of HLA-G expression in the context of HBV and HCV infections could favor virus

spread, but in early phases of chronic injury, it could have a protective effect.

Given the different studies on the role of HLA-G and *HLA-G* genetic variations in chronic hepatitis and HBV/HCV infection, we decided to perform a meta-analysis on the role of a 14-bp ins/del polymorphism (the most studied *HLA-G* genetic variations as reported in the literature) in hepatitis and hepatocellular carcinoma.

MATERIAL AND METHODS

Literature search strategy and study selection

We searched hepatitis and hepatocellular carcinoma (HCC) literature, paying special attention to genetic association studies analyzing the *HLA-G* 3'-UTR 14-bp indel polymorphism. Full texts were searched through the PubMed database (www.ncbi.nlm.nih.gov/pubmed) using these key words: "hla-g polymorphisms hepatitis" or "hla-g polymorphism hcc". After full-text retrieval, we incorporated in the analysis all the studies attending to the following inclusion criteria: be an observational genetic association study that includes patients with HCC or chronic hepatitis due to viral infections. We excluded reviews and studies that analyzed genetic variations in more than one non-classical HLA (to avoid confounding effects).

Data about each study are summarized in Table 1, including country of origin of the sampled individuals and ethnicity, study sample size, male/female ratio in cases, mean age, type of infection (HBV or HCV), and concurrent diseases prevailing in the sampled individuals.

Table 1. Summary of characteristics of *HLAG* 3'-UTR 14-bp ins/del polymorphism (rs371194629) genetic association studies with hepatocellular carcinoma (HCC).

Study	Country	Sample size		Male/Female ratio in cases	Mean age (SD[a])	Type of infection	Concurrent disease
		Cases	Controls				
Cordero et al., 2009	Brazil	21	72	1/1	27.3	HCV	Sickle-cell disease[b]
Jiang et al., 2011	China	318	599	3/1	52.3	HBV	HCC
Kim et al., 2013	Korea	180	181	NR[c]	NR	HBV (chronic)	HCC[d]
Teixeira et al., 2013	Brazil	109[e]	202	4/1	55.8	HBV or HCV	HCC
da Silva et al., 2014	Brazil and Italy	43[f]	185[f]				
		60[g]	105[g]	1.5/1	43.8	HCV	HIV
		30[h]	144[h]				
Laaribi et al., 2015	Tunisia	263	246	1/1	36.75	HBV	NR

[a]Standard deviation (SD). [b]Both cases and controls present sickle-cell disease. [c]Not reported (NR) by the authors. [d]HCC present only on cases. [e]HCC present in both HBV-infected and HCV-infected patients. [f]Brazilian individuals with European-descendant ethnicity. [g]Brazilian individuals with African-descendant ethnicity. [h]Italian individuals. The listed studies were included in a random-effect model meta-analysis.

Statistical analysis

The adherence of allelic frequencies to the Hardy-Weinberg equilibrium for the case and control groups was assessed by χ^2 tests. Odds ratios (OR) were calculated for each genetic association study included in the analysis through 2 x 2-contingency tables, which distributed 3'-UTR 14-bp indel allelic counts in cases (individuals with hepatitis or HCC) versus controls (individuals with no hepatitis or HCC).

A meta-analysis was performed with all included data to obtain a pooled OR representing the overall measure of association of *HLA-G* indel alleles with hepatitis or HCC. The meta-analysis was performed by using the R software version 3.1.1.

Heterogeneity between studies was quantified by the I^2 measure, which is a transformation of the τ^2 statistic and estimated by the DerSimonian and Laird method (DerSimonian and Kacker, 2007). The Cochran's Q-test with n-1 degrees of freedom (in which n is the number of studies included) was used to assess if heterogeneity was significantly different from zero. For this test only, significance level was set at 0.10 ($\alpha = 0.10$), as recommended by the literature, since this test is prone to yield false-negative conclusions (Higgins et al., 2003). For all remaining analysis, a significance level of 0.05 ($\alpha = 0.05$) was assumed. If heterogeneity was to be deemed significantly different from zero, a random-effect model was assumed (OR pooling by inverse variance weighting). A fixed-effect model was chosen if no heterogeneity was detected (OR pooling by Mantel-Haenszel method) (Mantel and Haenszel, 1959). Ninety-five percent confidence intervals (95%CI) were calculated for each included OR and the final pooled OR. The *t*-test was used to calculate the associated P value for the pooled OR. Presence of publication bias, commonly defined as disproportional preference for positive association results over no association reports (Rothstein et al., 2006), was assessed using the Egger test.

HLA-G 14-bp indel polymorphism across populations

As a side analysis, we evaluated *HLA-G* 14-bp indel polymorphism frequency distribution across selected populations from the 1000 Genomes database (1000 Genomes Project Consortium et al., 2012) to assess if different genetic backgrounds could influence the results of genetic association studies performed in diverse populations.

We downloaded *HLA-G* 14-bp indel polymorphism (rs371194629) genotypes from eight populations deposited in the 1000 Genomes database. These populations came from similar geographic backgrounds to the samples included in the meta-analysis. Two came from East Asia (Han Chinese in Bejing, China - CHB and Japanese in Tokyo, Japan - JPT), one from European descendants [Utah (USA) residents (CEPH) with Northwestern European Ancestry - CEU], one from Africa (Yoruba in Ibadan, Nigeria - YRI), and four admixed populations from Latin America (Colombians from Medellin, Colombia - CLM; Mexican ancestry from Los Angeles, USA - MXL; Peruvians from Lima, Peru - PEL; and Puerto Ricans from Puerto Rico - PUR). Then, we calculated allele frequencies in the control samples and the 1000 Genomes reference populations through direct genotype counting and compared the populations' diversity through pairwise Wright's fixation index (F_{ST}) computed with a locally developed algorithm. We assumed F_{ST} values lower than 0.05 to represent low genetic divergence; values between 0.05 and 0.15 indicated moderate divergence; values between 0.15 and 0.25 indicated large divergence; and values greater than 0.25 meant very large divergence (Wright, 1951).

RESULTS

The literature search produced six genetic association studies evaluating *HLA-G* 3'-UTR 14-bp indel polymorphism association with hepatitis. Cordero et al. (2009) recruited HCV-infected individuals with sickle-cell disease from Brazil; Jiang et al. (2011) sampled HBV-infected subjects with HCC from China; Kim et al. (2013) sampled Korean individuals with chronic HBV infection and HCC; Teixeira et al. (2013) sampled both HBV- and HCV-infected Brazilian patients; da Silva et al. (2014) sampled HIV-HCV co-infected Brazilian and Italian subjects; and Laaribi et al. (2015) recruited HBV-infected Tunisian subjects. Since the

study of da Silva et al. (2014) sampled three distinct ethnic groups, we considered them as three independent sampling events, so they were stratified and included in the meta-analysis separately.

The meta-analysis included a total of 1114 cases and 1644 controls. No deviations from Hardy-Weinberg equilibrium were observed for *HLA-G* 3'-UTR 14-bp indel allelic frequencies in both cases and controls. Table 1 summarizes the study characteristics as reported by the authors.

The heterogeneity estimated by the DerSimonian and Laird method was significantly different from zero, as suggested by the Cochran's Q-test result (I^2 = 72.4%; Q = 25.3 with degrees of freedom = 7 and P = 0.0007). Thus, a random-effect model was assumed. The individual ORs from all genetic association studies included in the meta-analysis were pooled, resulting in a summary OR. The *t*-test suggested that the 14-bp insertion allele might not influence susceptibility to hepatitis or hepatocellular cancer occurrences (pooled OR = 1.05; 95%CI = 0.76-1.43; t = 0.34 and P = 0.75). The Egger test showed no evidence for publication bias (test statistic = 0.89, P = 0.37). The meta-analysis results, including OR and 95%CIs from each study, are summarized in Figure 1. The sensitivity analysis revealed no bias by removing any study from analysis or from sampling stratification, as detailed above.

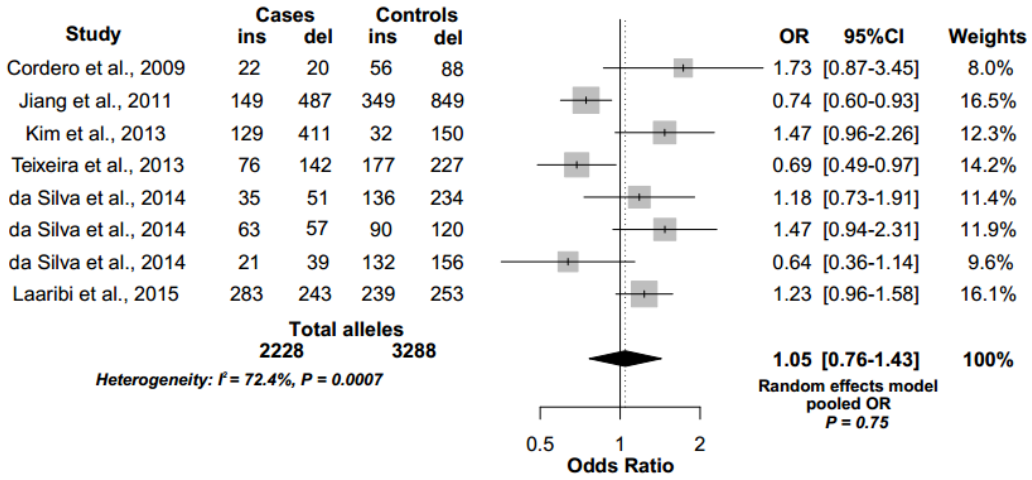

Figure 1. Forest plot of individual odds ratios (OR), individual 95% confidence intervals (95%CI), and pooled ORs from a random-effect model meta-analysis involving association studies examining the 14-bp ins/del polymorphism (rs371194629) in the 3'-UTR of HLA-G and its relation to hepatocellular carcinoma.

The F_{ST} analysis indicates an overall genetic uniformity across worldwide populations, with the possible exception of Asian-origin samples. The Asian populations had the lowest minor allele frequencies (MAF) of the 14-bp ins allele. The Chinese sample (Jiang et al., 2011) had an MAF = 0.29, whereas the Korean (Kim et al., 2013) had an MAF = 0.18. The 1000 Genomes JPT reference sample had an MAF = 0.25. In contrast, all the remaining non-Asian samples had MAF > 0.30, with a mean frequency of 0.40 (whereas the Asian samples had a mean of 0.24) and a range between 0.33 in CLM and YRI to 0.49 in the Tunisian sample (Laaribi et al., 2015) (Table 2).

Table 2. Pairwise comparison of *HLA-G* 3'-UTR ins/del polymorphism (rs371194629) 14-bp ins allele in the studies selected for meta-analysis (control samples only) and in reference populations from 1000 Genomes database.

MAF (14-bp ins allele)		Cordeiro et al., 2009	Jiang et al., 2011	Kim et al., 2013	Teixeira et al., 2013	da Silva et al., 2014 (1)	da Silva et al., 2014 (2)	da Silva et al., 2014 (3)	Laaribi et al., 2015	CEU	CHB	JPT	YRI	CLM	MXL	PEL	PUR
0.39	Cordeiro et al., 2009																
0.29	Jiang et al., 2011	0.0043															
0.18	Kim et al., 2013	***0.0568***	0.0076														
0.44	Teixeira et al., 2013	0.0019	0.0184	***0.0642***													
0.37	da Silva et al., 2014 (1)	0.0004	0.0049	0.0384	0.0052												
0.43	da Silva et al., 2014 (2)	0.0016	0.0111	***0.0741***	0.0001	0.0036											
0.46	da Silva et al., 2014 (3)	0.0044	0.0199	***0.0834***	0.0004	0.0084	0.0009										
0.49	Laaribi et al., 2015	0.0066	0.0344	***0.0788***	0.0023	0.0139	0.0028	0.0007									
0.35	CEU	0.0013	0.0022	0.0401	0.0065	0.0002	0.0059	0.0109	0.0145								
0.38	CHB	0.0001	0.0045	0.0504	0.0033	0.0001	0.0026	0.0063	0.0096	0.0007							
0.25	JPT	0.0219	0.0011	0.0081	0.0339	0.0145	0.0355	0.0453	0.0478	0.0127	0.0192						
0.33	YRI	0.0032	0.0011	0.0319	0.0104	0.0012	0.0096	0.0159	0.0200	0.0005	0.0022	0.0084					
0.33	CLM	0.0037	0.0008	0.0313	0.0106	0.0014	0.0103	0.0164	0.0197	0.0006	0.0026	0.0077	0.00001				
0.44	MXL	0.0024	0.0088	***0.0817***	0.000001	0.0039	0.0001	0.0004	0.0015	0.0071	0.0034	0.0380	0.0109	0.0119			
0.44	PEL	0.0028	0.0114	0.0831	0.00001	0.0049	0.0002	0.0003	0.0015	0.0080	0.0040	0.0405	0.0122	0.0131	0.00001		
0.36	PUR	0.0011	0.0025	0.0407	0.0063	0.0001	0.0056	0.0105	0.0143	0.00001	0.0006	0.0132	0.0006	0.0007	0.0066	0.0076	

CEU = Utah Residents (CEPH) with Northern Western European Ancestry; CHB = Han Chinese in Bejing, China; JPT = Japanese in Tokyo, Japan; YRI = Yoruba in Ibadan, Nigeria; CLM = Colombians from Medellin, Colombia; MXL = Mexican Ancestry from Los Angeles, USA; PEL = Peruvians from Lima, Peru; PUR = Puerto Ricans from Puerto Rico. F_{ST} values lower than 0.05, low genetic divergence; values between 0.05 and 0.15, moderate divergence; values between 0.15 and 0.25, large divergence; and values greater than 0.25, very large divergence. Bold italic values represent F_{ST} values >0.05.

Therefore, the F_{ST} indexes revealed a small but significant ($0.05 > F_{ST} > 0.09$) presence of genetic diversity when comparing the Korean (Kim et al., 2013) sample to some of the other non-Asian admixed samples, namely the reference MXL sample and Brazilian samples (Cordero et al., 2009; Teixeira et al., 2013; da Silva et al., 2014). In contrast, all non-Asian samples and the Chinese sample (Jiang et al., 2011) had very low pairwise F_{ST} indexes ($F_{ST} < 0.05$) when compared (Table 2).

These results may point to true non-classical major histocompatibility complex differences in some Asian populations in relation to other populations (namely lower frequencies of the 14-bp ins allele), but since we are analyzing a single *HLA-G* locus, we cannot speculate any further. However, the observed overall diversities were very small.

DISCUSSION

The meta-analysis data presented here argue against a possible role of rs371194629 (14-bp ins/del) in chronic hepatic injury or HCC. Indeed, the association studies examined in this analysis did not provide a very strong correlation between the 14-bp ins/del and hepatitis or HCC. Moreover, in some cases, the 14-bp ins allele appeared to be protective, while in other cases, this allele was associated with risk. Our analysis demonstrates that the 14-bp ins/del polymorphism and whichever allele is present do not provoke any significant overall effect. It is possible that the functional effect of the rs371194629 SNP depends on ethnicity; however, our analysis suggests that there is little variation in rs371194629 locus distribution among non-Asian populations. This reinforces our conclusions that the allele has little influence over hepatitis or HCC, since the meta-analysis would not be biased by ethnicity in this case.

Regarding Asian populations, a study published in 2014 found a significant association between the presence of the ins/ins genotype and breast cancer susceptibility in this demographic (Ge et al., 2014). HLA-G pathological expression is well correlated to HCC. As an example, Cai et al. (2009) found that HLA-G expression was negatively associated with patient survival and time between relapses, suggesting that HLA-G could worsen the HCC outcome. This study was subsequently confirmed and extended by two studies in the following years (Lin et al., 2010; Wang et al., 2011), confirming once more that increased HLA-G levels appear to be detrimental towards patient survival. Thus, the role of HLA-G in HCC appears to be important albeit unrelated to the 14-bp ins/del allele, and much more research has to be done to better elucidate how HLA-G could influence survival in cancer. The 14-bp ins allele generates a more stable HLA-G mRNA (Rousseau et al., 2003) it could be hypothesized that this polymorphism has a role in HCC. However, our analysis shows that this is not the case; other mechanisms are probably taking place, such as promoter strength through epigenetic modulation or mRNA stability regulated by miRNA (Catamo et al., 2014).

In conclusion, our meta-analysis demonstrates that the 14-bp ins/del probably has no role in chronic hepatitis or HCC, and stimulates other research towards different *HLA-G* polymorphisms or even other genetic factors, which could lead to the discovery of markers with more impact and better diagnostic potential. Further association analysis and review are needed to pinpoint such genetic variants, potentially revealing data that could be useful for diagnostic procedures.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Amiot L, Vu N, Rauch M, L’Helgoualc’h A, et al. (2014). Expression of HLA-G by mast cells is associated with hepatitis C virus-induced liver fibrosis. *J. Hepatol.* 60: 245-252. http://dx.doi.org/10.1016/j.jhep.2013.09.006

Bahri R, Hirsch F, Josse A, Rouas-Freiss N, et al. (2006). Soluble HLA-G inhibits cell cycle progression in human alloreactive T lymphocytes. *J. Immunol.* 176: 1331-1339. http://dx.doi.org/10.4049/jimmunol.176.3.1331

Cai MY, Xu YF, Qiu SJ, Ju MJ, et al. (2009). Human leukocyte antigen-G protein expression is an unfavorable prognostic predictor of hepatocellular carcinoma following curative resection. *Clin. Cancer Res.* 15: 4686-4693. http://dx.doi.org/10.1158/1078-0432.CCR-09-0463

Catamo E, Zupin L, Crovella S, Celsi F, et al. (2014). Non-classical MHC-I human leukocyte antigen (HLA-G) in hepatotropic viral infections and in hepatocellular carcinoma. *Hum. Immunol.* 75: 1225-1231. http://dx.doi.org/10.1016/j.humimm.2014.09.019

Caumartin J, Favier B, Daouya M, Guillard C, et al. (2007). Trogocytosis-based generation of suppressive NK cells. *EMBO J.* 26: 1423-1433. http://dx.doi.org/10.1038/sj.emboj.7601570

Cordero EA, Veit TD, da Silva MA, Jacques SM, et al. (2009). HLA-G polymorphism influences the susceptibility to HCV infection in sickle cell disease patients. *Tissue Antigens* 74: 308-313. http://dx.doi.org/10.1111/j.1399-0039.2009.01331.x

da Silva GK, Vianna P, Veit TD, Crovella S, et al. (2014). Influence of HLA-G polymorphisms in human immunodeficiency virus infection and hepatitis C virus co-infection in Brazilian and Italian individuals. *Infect. Genet. Evol.* 21: 418-423. http://dx.doi.org/10.1016/j.meegid.2013.12.013

de Oliveira Crispim JC, Silva TG, Souto FJ, Souza FF, et al. (2012). Upregulation of soluble and membrane-bound human leukocyte antigen G expression is primarily observed in the milder histopathological stages of chronic hepatitis C virus infection. *Hum. Immunol.* 73: 258-262. http://dx.doi.org/10.1016/j.humimm.2011.12.004

DerSimonian R and Kacker R (2007). Random-effects model for meta-analysis of clinical trials: an update. *Contemp. Clin. Trials* 28: 105-114. http://dx.doi.org/10.1016/j.cct.2006.04.004

Fons P, Chabot S, Cartwright JE, Lenfant F, et al. (2006). Soluble HLA-G1 inhibits angiogenesis through an apoptotic pathway and by direct binding to CD160 receptor expressed by endothelial cells. *Blood* 108: 2608-2615. http://dx.doi.org/10.1182/blood-2005-12-019919

Ge YZ, Ge Q, Li MH, Shi GM, et al. (2014). Association between human leukocyte antigen-G 14-bp insertion/deletion polymorphism and cancer risk: a meta-analysis and systematic review. *Hum. Immunol.* 75: 827-832. http://dx.doi.org/10.1016/j.humimm.2014.06.004

1000 Genomes Project Consortium, Abecasis GR, Auton A, Brooks LD, et al. (2012). An integrated map of genetic variation from 1,092 human genomes. *Nature* 491: 56-65.

Geraghty DE, Koller BH and Orr HT (1987). A human major histocompatibility complex class I gene that encodes a protein with a shortened cytoplasmic segment. *Proc. Natl. Acad. Sci. USA* 84: 9145-9149. http://dx.doi.org/10.1073/pnas.84.24.9145

Higgins JP, Thompson SG, Deeks JJ and Altman DG (2003). Measuring inconsistency in meta-analyses. *BMJ* 327: 557-560.

Jiang Y, Chen S, Jia S, Zhu Z, et al. (2011). Association of HLA-G 3¢ UTR 14-bp insertion/deletion polymorphism with hepatocellular carcinoma susceptibility in a Chinese population. *DNA Cell Biol.* 30: 1027-1032. http://dx.doi.org/10.1089/dna.2011.1238

Kim SK, Chung JH, Jeon JW, Park JJ, et al. (2013). Association between HLA-G 14-bp insertion/deletion polymorphism and hepatocellular carcinoma in Korean patients with chronic hepatitis B viral infection. *Hepatogastroenterology* 60: 796-798.

Kovats S, Main EK, Librach C, Stubblebine M, et al. (1990). A class I antigen, HLA-G, expressed in human trophoblasts. *Science* 248: 220-223. http://dx.doi.org/10.1126/science.2326636

Laaribi AB, Zidi I, Hannachi N, Ben Yahia H, et al. (2015). Association of an HLA-G 14-bp Insertion/Deletion polymorphism with high HBV replication in chronic hepatitis. *J. Viral Hepat.* 22: 835-841. http://dx.doi.org/10.1111/jvh.12395

Le Discorde M, Moreau P, Sabatier P, Legeais JM, et al. (2003). Expression of HLA-G in human cornea, an immune-privileged tissue. *Hum. Immunol.* 64: 1039-1044. http://dx.doi.org/10.1016/j.humimm.2003.08.346

Lee YH, Bae SC and Song GG (2015). Meta-analysis of associations between functional HLA-G polymorphisms and susceptibility to systemic lupus erythematosus and rheumatoid arthritis. *Rheumatol. Int.* 35: 953-961. http://dx.doi.org/10.1007/s00296-014-3155-3

LeMaoult J, Caumartin J, Daouya M, Favier B, et al. (2007). Immune regulation by pretenders: cell-to-cell transfers of HLA-G make effector T cells act as regulatory cells. *Blood* 109: 2040-2048. http://dx.doi.org/10.1182/blood-2006-05-024547

Li T, Huang H, Liao D, Ling H, et al. (2015). Genetic polymorphism in HLA-G 3'UTR 14-bp ins/del and risk of cancer: a meta-analysis of case-control study. *Mol. Genet. Genomics* 290: 1235-1245. http://dx.doi.org/10.1007/s00438-014-0985-3

Lin A, Chen HX, Zhu CC, Zhang X, et al. (2010). Aberrant human leucocyte antigen-G expression and its clinical relevance in hepatocellular carcinoma. *J. Cell. Mol. Med.* 14: 2162-2171. http://dx.doi.org/10.1111/j.1582-4934.2009.00917.x

Lozano JM, González R, Kindelán JM, Rouas-Freiss N, et al. (2002). Monocytes and T lymphocytes in HIV-1-positive patients express HLA-G molecule. *AIDS* 16: 347-351. http://dx.doi.org/10.1097/00002030-200202150-00005

Mantel N and Haenszel W (1959). Statistical aspects of the analysis of data from retrospective studies of disease. *J. Natl. Cancer Inst.* 22: 719-748.

Menier C, Rabreau M, Challier JC, Le Discorde M, et al. (2004). Erythroblasts secrete the nonclassical HLA-G molecule from primitive to definitive hematopoiesis. *Blood* 104: 3153-3160. http://dx.doi.org/10.1182/blood-2004-03-0809

Riteau B, Menier C, Khalil-Daher I, Sedlik C, et al. (1999). HLA-G inhibits the allogeneic proliferative response. *J. Reprod. Immunol.* 43: 203-211. http://dx.doi.org/10.1016/S0165-0378(99)00034-0

Rosado S, Perez-Chacon G, Mellor-Pita S, Sanchez-Vegazo I, et al. (2008). Expression of human leukocyte antigen-G in systemic lupus erythematosus. *Hum. Immunol.* 69: 9-15. http://dx.doi.org/10.1016/j.humimm.2007.11.001

Rothstein HR, Sutton AJ and Borenstein M (2006). Publication bias in meta-analysis: Prevention, assessment and adjustments. 1st edn. John Wiley & Sons, Ltd., Chichester, UK.

Rouas-Freiss N, Gonçalves RM, Menier C, Dausset J, et al. (1997). Direct evidence to support the role of HLA-G in protecting the fetus from maternal uterine natural killer cytolysis. *Proc. Natl. Acad. Sci. USA* 94: 11520-11525. http://dx.doi.org/10.1073/pnas.94.21.11520

Rousseau P, Le Discorde M, Mouillot G, Marcou C, et al. (2003). The 14 bp deletion-insertion polymorphism in the 3¢ UT region of the HLA-G gene influences HLA-G mRNA stability. *Hum. Immunol.* 64: 1005-1010. http://dx.doi.org/10.1016/j.humimm.2003.08.347

Souto FJ, Crispim JC, Ferreira SC, da Silva AS, et al. (2011). Liver HLA-G expression is associated with multiple clinical and histopathological forms of chronic hepatitis B virus infection. *J. Viral Hepat.* 18: 102-105. http://dx.doi.org/10.1111/j.1365-2893.2010.01286.x

Tan Z, Randall G, Fan J, Camoretti-Mercado B, et al. (2007). Allele-specific targeting of microRNAs to HLA-G and risk of asthma. *Am. J. Hum. Genet.* 81: 829-834. http://dx.doi.org/10.1086/521200

Teixeira AC, Mendes-Junior CT, Souza FF, Marano LA, et al. (2013). The 14bp-deletion allele in the HLA-G gene confers susceptibility to the development of hepatocellular carcinoma in the Brazilian population. *Tissue Antigens* 81: 408-413. http://dx.doi.org/10.1111/tan.12097

Urosevic M and Dummer R (2003). HLA-G and IL-10 expression in human cancer--different stories with the same message. *Semin. Cancer Biol.* 13: 337-342. http://dx.doi.org/10.1016/S1044-579X(03)00024-5

Wang Y, Ye Z, Meng XQ and Zheng SS (2011). Expression of HLA-G in patients with hepatocellular carcinoma. *HBPD INT* 10: 158-163. http://dx.doi.org/10.1016/S1499-3872(11)60025-8

Weng PJ, Fu YM, Ding SX, Xu DP, et al. (2011). Elevation of plasma soluble human leukocyte antigen-G in patients with chronic hepatitis C virus infection. *Hum. Immunol.* 72: 406-411. http://dx.doi.org/10.1016/j.humimm.2011.02.008

Wright S (1951). The genetical structure of populations. *Ann. Eugen.* 15: 323-354. http://dx.doi.org/10.1111/j.1469-1809.1949.tb02451.x

Xue S, Yang J, Yao F, Xu L, et al. (2007). Recurrent spontaneous abortions patients have more -14 bp/+14 bp heterozygotes in the 3'UT region of the HLA-G gene in a Chinese Han population. *Tissue Antigens* 69 (Suppl 1): 153-155. http://dx.doi.org/10.1111/j.1399-0039.2006.763_7.x

Yan WH, Lin A, Chen XJ, Dai MZ, et al. (2006). Association of the maternal 14-bp insertion polymorphism in the HLA-G gene in women with recurrent spontaneous abortions. *Tissue Antigens* 68: 521-523. http://dx.doi.org/10.1111/j.1399-0039.2006.00723.x

Yie SM, Li LH, Xiao R and Librach CL (2008). A single base-pair mutation in the 3'-untranslated region of HLA-G mRNA is associated with pre-eclampsia. *Mol. Hum. Reprod.* 14: 649-653. http://dx.doi.org/10.1093/molehr/gan059