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Research article

Meta-analysis of HLA-G 14bp insertion/deletion polymorphism and soluble HLA-G revealed an association with digestive cancers initiation and prognosis

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ABSTRACT

Background/Objective: Conflicting results on the association between HLA-G and digestive cancers were reported. We conducted a meta-analysis to further investigate the true relationship between HLA-G and digestive cancers (DC).

Methods: Following PRISMA guidelines, we performed a meta-analysis including 7 case-control studies on HLA-G 14-bp Insertion/deletion (I/D) polymorphism, and 15 studies on soluble HLA-G (sHLA-G). Odds ratios (OR) and their corresponding 95% confidence intervals (CI) for genetic polymorphisms were calculated. The pooled OR was calculated under three genetic models: allelic, recessive, and dominant models. Concerning sHLA-G meta-analysis, standardized mean differences (SMDs) were calculated.

Results: The HLA-G 14-bp I/D was not associated with the risk of DC. However, in the subset of HBV/HCV positive hepato-cellular cancer (HCC) patients, we reported a significant association of HLA-G 14-bp I/D with the disease initiation under allelic (D vs. I; OR = 1.698, 95% CI = 1.263–2.282, p = 0.000), dominant (DD + ID vs. II; OR = 2.321, 95% CI = 1.277–4.218, p = 0.006)and recessive (DD vs. DI + II; OR = 1.739, 95% CI = 1.173–2.577, p = 0.006) genetic models. Interestingly, HLA-G 14-bp I/D was not associated with the disease initiation in HBV/HCV negative HCC patients. However, the infection by HBV/HCV seems to be implicated in the HCC development when we compared HBV/HCV positive patients to HBV/HCV negative patients under allelic (D *vs.* I; OR = 1.429, 95% CI = 1.029–1.983, p = 0.033, and dominant (DD + ID *vs.*II; OR = 1.981, 95% CI = 1.002–3.916, p = 0.049) genetic models.

Overall analysis of DC showed significant increased sHLA-G in patients compared to healthy controls (SMD = 3.341, 95% CI = 2.415–4.267, p = 0.000). In Asian patients with gastric cancer, sHLA-G was significantly increased in grade 3 compared to low grades (SMD = 0.448, 95% CI = 0.109–0.787, p = 0.000). Further analysis showed that sHLA-G was significantly increased in positive DC vascular invasion (SMD = 0.743, 95% CI = 0.385–1.100, p = 0.000). Accordingly, sHLA-G was associated with a poor prognosis for DC.

Conclusion: The current meta-analysis supports the significant role of HLA-G in DC. The HLA-G 14-bp I/D polymorphism was associated with HCC patients with concomitant HBV/HCV viral infections. Increased sHLA-G indicated a poor prognosis for DC cancer patients.

1. Introduction

Digestive cancers (DC), composed of oesophageal, colorectal, pancreatic, stomach, and liver cancers are common malignancies and account for one-quarter of the global cancer incidence [1]. Fortunately, recent years showed considerable improvements in DC diagnosis and treatment, including relief of symptoms and prolonged survival [2]. However, the efficacy of surgery and chemotherapy remains unsatisfactory, particularly if tumours aren't detected and removed at an early stage. Therefore, novel biomarkers to improve cancer diagnosis and prognosis are crucial for reducing cancer burden and mortality. The expression of HLA-G, an immune tolerant and tumour promoting factor,

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has been extensively investigated, and its role as a novel immune checkpoint has been established [3, 4]. HLA-G has been described as a potent immune suppressive mediator observed in various malignancies and is strongly associated with tumour immune escape and metastasis [5]. Yie et al. reported that HLA-G protein was expressed in a majority of the primary site of gastric carcinomas and significantly correlated with tumour location, histological grade, depth of invasion, histological grade, host immune response, lymph nodal metastasis, and clinical stages of the disease [6]. A high frequency of tumour cell HLA-G expression and/or increased sHLA-G has been found in various body fluids in a variety of cancers [7]. Similarly to membrane HLA-G, peripheral sHLA-G was associated with advanced disease stage, tumour metastasis and/or poor prognosis [8, 9]. Both membrane-bound and sHLA-G have immune suppressive function [10]. The abnormal expression of HLA-G might be caused by genetic polymorphisms in HLA-G gene, as previously announced. Particularly, the HLA-G 14-bp insertion/deletion has been widely investigated in the context of cancer susceptibility and progression [11, 12, 13].

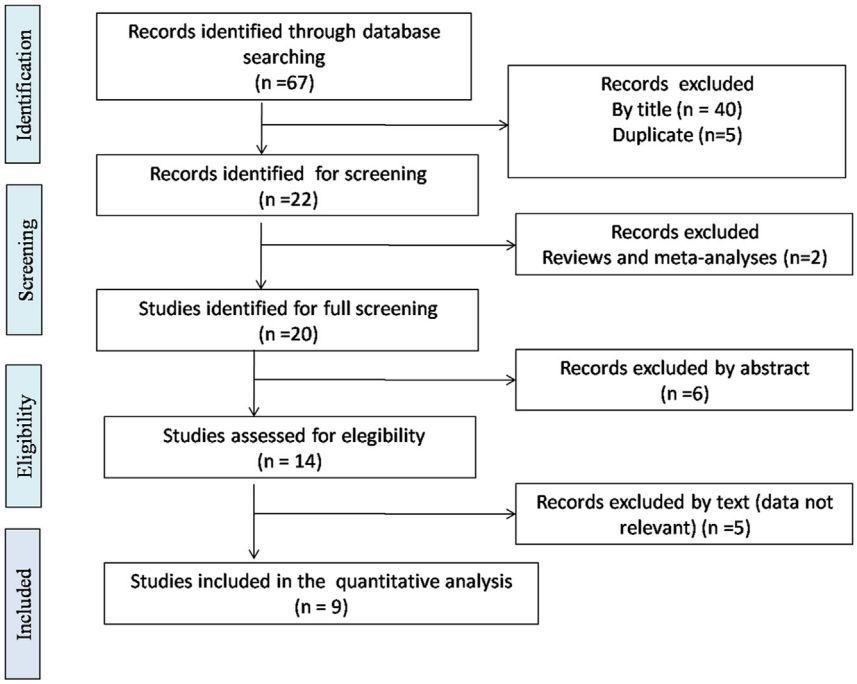

Figure 1. Flow diagram representing the selection process concerning HLA-G 14bp I/D polymorphism studies in digestive cancer.

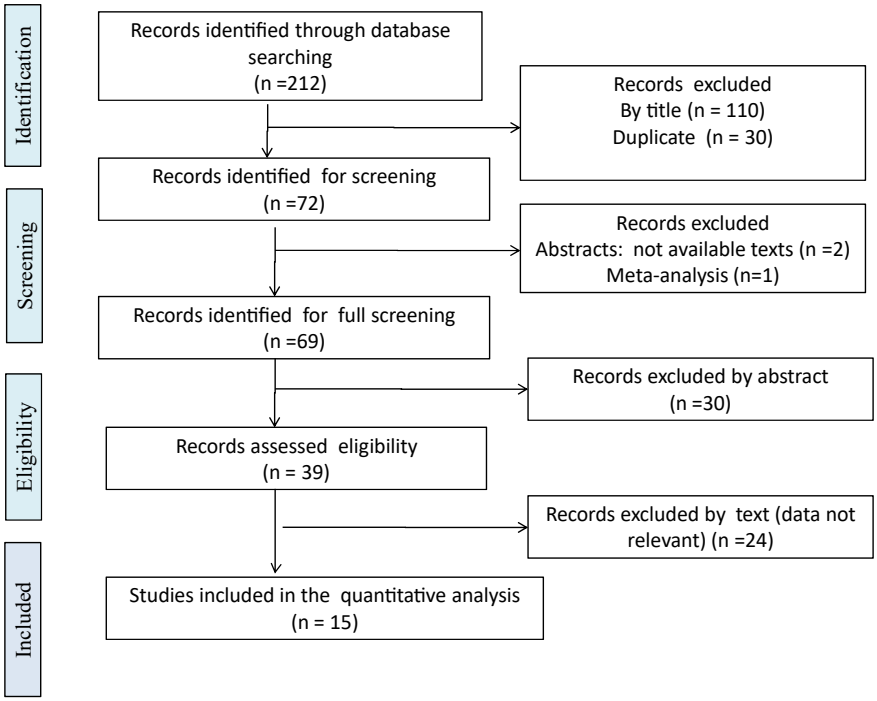

Figure 2. Flow diagram representing the selection process concerning sHLA-G dosage studies in digestive cancer.

We aimed through the current meta-analysis to assess the significance of HLA-G in DC by studying the association of HLA-G 14-bp and sHLA-G with DC susceptibility and progression.

2. Methods

2.1. Literature search and inclusion/exclusion criteria

MEDLINE, EMBASE, Web of Science, and Cochrane databases (up to May, 2021) were searched using the terms "HLA-G" "polymorphism", "sHLA-G," and "digestive cancer". The review process followed PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [14]. Eligible studies included: (1) published cases/controls cohort studies evaluating the association of the HLA-G polymorphism with DC; (2) Availability of mean or median and standard deviation (SD) or range data of sHLA-G levels in patients and controls. Studies were excluded if they were reviews or case reports or if they present duplicate or incomplete data. The literature searches were performed in triplicate by three independent reviewers (SD, KT and IZ). Reviewers extracted the data on the methods and results from the original studies. Discrepancies were resolved by consensus among the reviewers. Flow diagrams of research strategy, exclusion and exclusion criteria are presented in Figures 1 and 2.

Table 1. Characteristics of individual studies included in the meta-analysis of HLA-G 14bp I/D polymorphism in digestive cancer.

Study	Genotyping method	Country	Ethnicity	Cases		Controls	
				Sample	Number	Number	HWE p-value
Dhouioui 2022	PCR	Tunisia	Caucasian	CRC	233	241	0.186
Vaquero-Yuste 2021	PCR	Spain	Caucasian	GC	107	55	0.597
Abu Hassan 2019	PCR	Saudi-Arabia	Caucasian	CRC	105	119	0.519
El Bassiouny 2019	PCR	Egypt	Egyptian	HCC	40	20	0.371
Garziera 2016	PCR	Italy	Caucasian	CRC	308	294	**0.017**
Kim 2013	PCR	South Korea	Asian	HCC	270	91	0.556
Teixeira 2013	PCR	Brazil	Mix	HCC	109	202	0.075
Teixeira 2013a	PCR	Brazil	Mix	HBV or HCV positive HCC	75	202	0.075
Teixeira 2013b	PCR	Brazil	Mix	HBV or HCV negative HCC	34	202	0.075
Chen 2012	PCR	China-Kazakan	Asian	EC	132	251	0.571
Chen 2012a	PCR	China-Han	Asian	EC	107	211	0.125
Jiang 2011	PCR	China	Asian	HCC	318	599	0.531
Jiang 2011a	PCR	China	Asian	HBV positive HCC	222	60	0.237
Jiang 2011b	PCR	China	Asian	HBV negative HCC	96	539	**0.009**

CRC: Colorectal cancer; GC: Gastric cancer; EC: Esophageal cancer; HCC: Hepatocellular cancer; HBV: Hepatitis B virus; HCV: Hepatitis C Virus; HWE: Hardy Weinberg Equilibrium.
I/D: Insertion/Deletion; Bold: significant P-value (≤0.05).

Table 2. Characteristics of individual studies included in the meta-analysis of sHLA-G dosage in digestive cancer.

Study	Method	Manufacturer	Country	Ethnicity	Sample type	Cases			Controls		Units
						Sample	Number	Mean ± SD	Number	Mean ± SD	
Lázaro-Sánchez 2020	ELISA kit	BioVendor	Spain	Caucasian	Saliva	CRC	15	25.23 ± 16.56	10	6.60 ± 2.03	U/mL
Abu Hassan 2019	ELISA kit	MyBioSource	Saudi-Arabia	Caucasians	Serum	CRC	33	1.42 ± 0.70	30	1.04 ± 0.81	ng/mL
Farjadian 2018	ELISA kit	Exbio	Iran	Caucasian	Plasma	GC	82	85.37 ± 60.83	45	56.99 ± 48.45	U/mL
Kirana 2017	ELISA kit	Exbio	Australia	Oceania	Plasma	CRC	44	NI*	NA	NA	U/mL
Li 2017	ELISA kit	Exbio	China	Asian	Plasma	CRC	178	151.58 ± 88.25	113	37.88 ± 15.58	U/mL
Sun 2017	ELISA kit	Ameko	China	Asian	Ascite	CC	10	17.59 ± 4.69	30	12.47 ± 3.68	μg/L
Sun 2017a	ELISA kit	Ameko	China	Asian	Ascite	GC	8	18.37 ± 4.63	30	12.47 ± 3.68	μg/L
Sun 2017b	ELISA kit	Ameko	China	Asian	Ascite	PC	6	21.42 ± 1.69	30	12.47 ± 3.68	μg/L
Khorrami 2016	ELISA kit	Glory Science	Iran	Caucasian	Serum	GC	50	36.29 ± 1.66	50	11.23 ± 1.47	U/mL
Pan 2016	ELISA kit	Exbio	China	Asian	Plasma	GC	81	55.90 ± 9.23	77	30.53 ± 2.55	U/mL
Xu 2016	ELISA kit	Exbio	China	Asian	Plasma	GC	124	127.93 ± 52.98	130	75.78 ± 22.12	U/mL
Zheng 2014	ELISA kit	BioVendor	China	Asian	Plasma	EC	60	71.10 ± 61.42	28	10.72 ± 7.32	U/mL
Park 2012	ELISA kits	Exbio/ BioVendor	South Korea	Asian	Serum	HCC	80	188.58 ± 24.65	50	16.18 ± 12.03	U/mL
Lin 2011	ELISA kit	Exbio	China	Asian	Plasma	EC	41	143.28 ± 52.68	153	21.48 ± 9.82	U/mL
Zhu 2011	ELISA kit	Exbio	China	Asian	Serum	CRC	144	124.30 ± 19.17	60	25.83 ± 6.43	U/mL
Wang 2011	ELISA kits	Exbio/ BioVendor	China	Asian	Serum	HCC	36	132.60 ± 31.40	25	47 ± 15.5	U/mL
Lin 2010	ELISA kits	Exbio	China	Asian	Plasma	HCC	19	175.26 ± 126.67	86	18.44 ± 7.74	U/mL

CC: Colon cancer; CRC: Colorectal cancer; EC: Esophageal cancer; GC: Gastric cancer; HCC: Hepatocellular cancer; NA: Not applicable; NI: Not indicated; PC: Pancreatic cancer; SD, Standard deviation, sHLA-G, soluble HLA-G. *No data for the total CRC cohort. Data are given only in CRC patients after stratifications.

Table 3. Main results of the meta-analysis of HLA-G 14bp I/D polymorphism with digestive cancers.

Cancer type	Ethnicity	Genetic model	N	Odds ratio			Heterogeneity			P_{Egger}	P_{Begg}
				OR	*95% CI*	P_{OR}	I^2 *(%)*	Tau^2	P_H		
DC	Overall	*D vs. I*	10	1.127	0.918–1.383	0.254	73.86	0.075	**0.000**	0.998	0.858
		DD + ID vs. II		1,115	0.813–1.530	0.500	58.18	0.136	**0.011**	0.983	0.721
		DD vs DI + II		1,215	0.939–1.,572	0,138	66.62	0.105	**0.001**	0.764	0.858
	Asian	*D vs. I*	4	1.040	0.724–1.495	0.832	81.3	0.109	**0.001**	0.255	0.497
		DD + ID vs. II		1.083	0.524–2.239	0.830	73.3	0.379	**0.010**	0.404	1
		DD vs DI + II		1.162	0.956–1.411	0.131	71.6	0.112	**0.014**	0.293	**0.042**
CRC	Overall	*D vs. I*	3	1,015	0.698–1.477	0.937	81.58	0.089	0.004	0.934	1
		DD + ID vs. II		0,891	0.607–1.308	0.555	53.56	0.061	0.116	0.635	1
		DD vs DI + II		1,179	0.641–2.167	0.597	83.52	0.238	0.002	0.693	1
EC	Asian	*D vs. I*	2	1.107	0.8820–1.389	0.382	88	0.198	**0.004**	NA	NA
		DD + ID vs. II		1.179	0.735–1.890	0.495	85.5	0.682	**0.009**	NA	NA
		DD vs DI + II		1.121	0.806–1.558	0.497	80.4	0.236	**0.024**	NA	NA
HCC	Overall	*D vs. I*	4	1.152	0.827–1.605	0.404	66.2	0.071	**0.031**	0.571	0.174
		DD + ID vs. II	4	1.291	0.753–2.211	0.353	37.2	0.109	0.189	0.109	**0.042**
		DD vs DI + II	4	1.213	0.836–1.760	0.310	54.3	0.072	0.087	0.860	0.174
	Asian	*D vs. I*	2	1.164	0.956–1.418	0.131	86.9	0.202	**0.006**	NA	NA
		DD + ID vs. II		1.349	0.805–2.263	0.256	76.4	1.050	**0.040**	NA	NA
		DD vs DI + II		1.184	0.931–1.506	0.169	81.5	0.186	**0.020**	NA	NA

bp: base pairs, CI: Confidence interval, CRC: Colorectal cancer, DC: Digestive cancers, EC: Esophageal cancer, HCC: Hepatocellular cancer, I/D: insertion/deletion, N: number of studies, NA: Not applicable, OR: odds ratio, P_{Begg}: P-value associated to Begg and Mazumdar rank correlation test (Two-tailed) without continuity correction, P_{Egger}: P-value associated to Egger's test (Two-tailed), P_H: P-value associated to heterogeneity, P_{OR}: P-value associated to OR, Bold: significant P-value (≤0.05).

2.2. Statistical analyses

We evaluated the implication of HLA-G 14-bp Insertion (I)/Deletion (D) polymorphism and levels of soluble HLA-G (sHLA-G) in the initiation and prognosis of DC. Concerning HLA-G gene polymorphism, we meta-analyzed studies through the calculation of odds ratios (OR) and its corresponding 95% confidence interval (CI). The pooled OR was calculated under three genetic models: allelic, recessive, and dominant models.

Concerning sHLA-G meta-analysis, standardized mean differences (SMDs) were calculated. When median and range were reported, we calculated the mean ± SD (standard deviation) according to Hozo et al. [15]. Heterogeneity between studies was assessed by I^2 and Tau^2 value. I^2 values were interpreted according to the Cochrane guidelines [16]. Tau^2 test reflected the variance of the true effect sizes [17].

The Funnel plot measured the study size [18]. Egger's test of the intercept estimated the sample size effect [19]. Publications bias was evaluated through funnel plot and Begg and Mazumdar rank correlation test [20]. Two-tailed P_{Egger}, and P_{Begg} values without continuity correction, were reported.

The random-effects model assuming significant variation in different studies and testing sampling errors and variances between studies [21, 22]. When homogeneity among studies was assumed, we used fixed effects model. Comprehensive meta-analysis software (Biostat, Englewood, NJ, USA) was used to perform statistical analysis. $P \leq 0.05$ was considered statistically significant.

3. Results

3.1. Studies included in the meta-analysis

For HLA-G polymorphisms, we identified 67 studies. Of these articles, duplicates, non relevant papers based on titles, reviews and meta-analysis were excluded leaving 14 articles for full screening. Additional 5 articles were omitted due to lack or irrelevant data. Therefore, in total, 9 articles met our inclusion criteria [23, 24, 25, 26, 27, 28, 29, 30, 31] (Table 1, Figure 1).

For sHLA-G, we identified 212 studies. Of these, duplicate and irrelevant papers were excluded based on titles. After excluding reviews and meta-analyses, 69 were selected for full-text screening based on the title and abstract. Of which, 30 articles were excluded based on abstract, and 24 were omitted due to lack or irrelevant data. Therefore, in total, 15 articles met our inclusion criteria for sHLA-G [25, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45], (Table 2, Figure 2). When there were at least two comparisons, the meta-analysis of HLA-G-related polymorphisms or sHLA-G was performed.

3.2. Meta-analysis of HLA-G 14-bp I/D polymorphism and digestive cancers susceptibility

The HLA-G 14-bp I/D was not associated with the risk of DC neither under allelic, dominant or recessive genetic models (Table 3, Figure 3). After stratifications according ethnicity or type of cancer, we did not reach significant association between HLA-G 14-bp I/D and DC under allelic (D vs. I; OR = 1.127, 95% CI = 0.918–1.383, p = 0.254; Figure 3A), dominant (DD + ID *vs.* II; OR = 1.115, 95% CI = 0.813–1.530, p = 0.500; Figure 3B), and recessive (DD *vs* DI + II; OR = 1.214, 95% CI = 0.939–1.572, p = 0.138; Figure 3C) genetic models (Table 3).

In the subset of HBV or HCV (HBV/HCV) positive HCC patients, we demonstrated a clear association of HLA-G 14-bp I/D with the disease initiation under allelic (D vs. I; OR = 1.698, 95% CI = 1.263–1.650, p = 0.000, Table 4), dominant (DD + ID vs, II; OR = 2.321, 95% CI = 1.277–4.218, p = 0.006, Table 4), and recessive (DD vs. DI + II; OR = 1.739, 95% CI = 1.173–2.577, p = 0.006, Table 4) genetic models. As expected, HLA-G 14-bp I/D was not associated to the disease initiation in the subset of HBV or HCV negative HCC patients (Table 4). Interestingly, the infection by HBV/HCV seems to

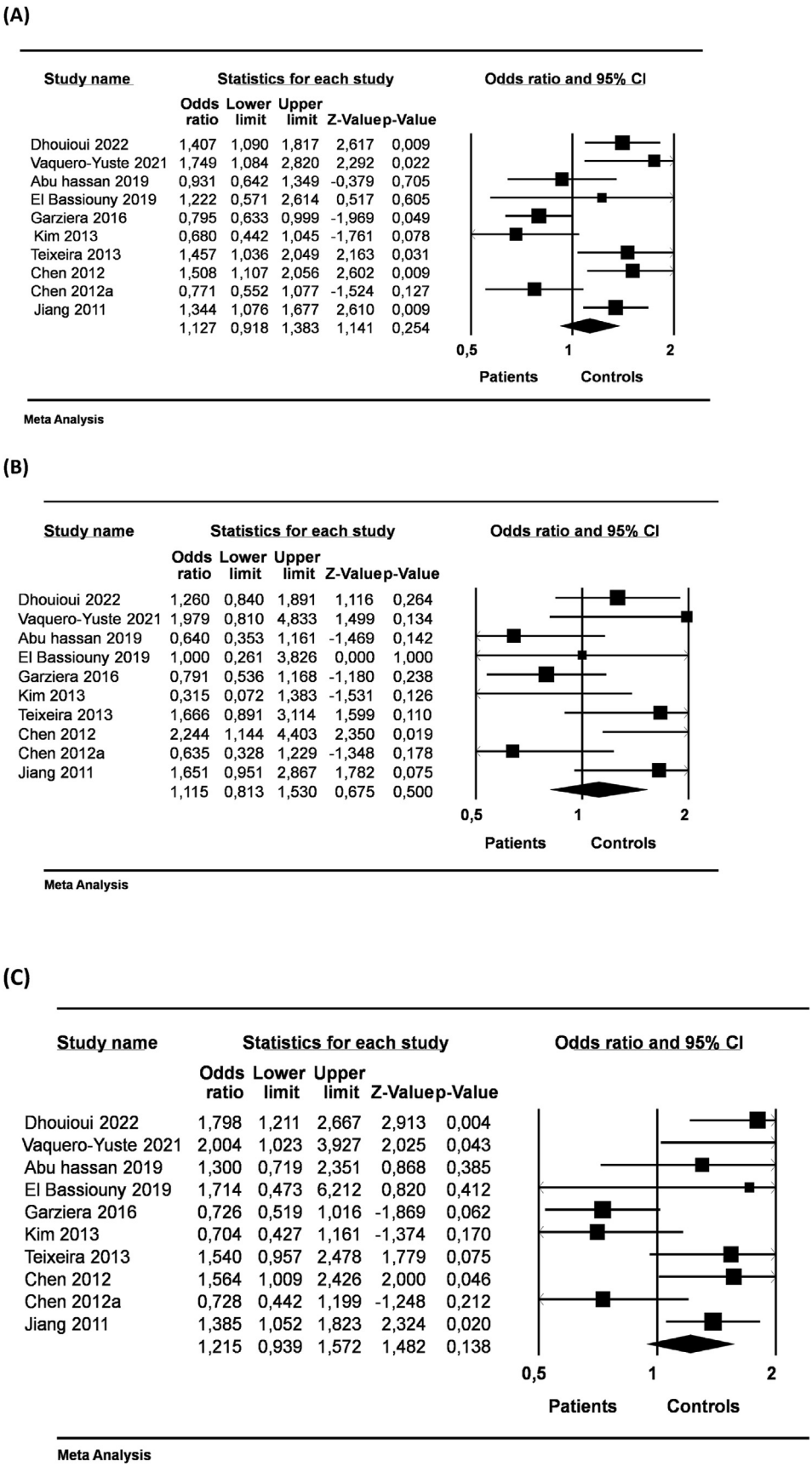

Figure 3. Forest plot of the association between HLA-G 14-bp I/D polymorphism and digestive cancer risk with the random effects model. (A) Allelic model (D vs. I) alleles, (B) Dominant genotype (DD + ID vs. II) and (C) Recessive model (DD vs DI + II) in the overall population.

Table 4. Main results of the meta-analysis on two studies of HLA-G 14bp I/D polymorphism with Hepatocellular cancer.

	HLA-G 14bp I/D polymorphism		
	D vs. I	DD + ID vs. II	DD vs DI + II
Subset comparison	N cases (N controls) OR (95% CI) P_{OR}	N cases (N controls) OR (95% CI) P_{OR}	N cases (N controls) OR (95% CI) P_{OR}
HBV/HCV positive vs. control	594 (524) 1.698 (1.263–2.282) P_{OR} =0.000	297 (262) 2.321 (1.277–4.218) P_{OR} =0.006	297 (262) 1.739 (1.173–2.577) P_{OR} =0.006
HBV/HCV negative vs. control	260 (1482) 1.238 (0.929–1.650) P_{OR} = 0.145	130 (741) 1.188 (0.684–2.064) P_{OR} = 0.541	130 (741) 1.342 (0.921–1.955) P_{OR} = 0.126
HBV/HCV positive vs. HBV/HCV negative	594 (260) 1.429 (1.029–1.983) P_{OR} =0.033	297 (130) 1.981 (1.002–3.916) P_{OR} =0.049	297 (130) 1.385 (0.910–2.107) P_{OR} = 0.128

bp: base pairs, CI: Confidence interval, HBV: Hepatitis B virus, HCV: Hepatitis C virus, I/D: insertion/deletion, N: number of studies, NA: Not applicable, OR: odds ratio, P_{OR}: P-value associated to OR, Bold: significant P-value (≤0.05).

be implicated in the HCC development when we compared HBV/HCV positive patients to HBV/HCV negative patients under allelic (D vs. I; OR = 1.429, 95% CI = 1.029–1.983, p = 0.033, Table 4) and dominant (DD + ID vs.II; OR = 1.981, 95% CI = 1.002–3.916, p = 0.049, Table 4) genetic models.

3.3. Meta-analysis of soluble HLA-G levels in digestive cancer patients and controls

Overall analysis of DC cancers including CRC and GC showed significant increased sHLA-G in patients compared to healthy controls (SMD = 3.341, 95% CI = 2.415–4.267, p = 0.000; Table 5, Figure 4A). sHLA-G were significantly increased in both CRC and GC compared to healthy controls with sHLA- G mean was 2 points higher in GC patients (SMD = 4.043, 95% CI = 2.28–5.858, p = 0.000; Table 5) than in CRC group (SMD = 2.165, 95% CI = 0.506–3.824, p = 0.011; Table 5) (Figure 4B and C). sHLA-G levels were also increased in the serum/plasma in DC patients (SMD = 3.994, 95% CI = 2.793–5.195, p = 0.000; Table 5) and in the CRC patients subgroup (SMD = 2.686, 95% CI = 00.159–5.214, p = 0.037; Table 5).

sHLA-G were increased in both Caucasian and Asian patients with sHLA-G found to be 2 fold higher in Asians (SMD = 2.292, 95% CI = 2.150–2.434, p = 0.000; Table 5) than in Caucasians (SMD = 0.831, 95% CI = 0.551–1.111, p = 0.000; Table 5). Of note, in Asians, sHLA-G was 2 fold higher in serum/plasma (SMD = 2.266, 95% CI = 2.016–2.516, p = 0.000; Table 5) than in ascites (SMD = 1.666, 95% CI = 1.163–2.169, p = 0.000; Table 5).

In Asians, more types of DC were investigated and all had significant increased sHLA-G compared to healthy controls (Table 5). HCC patients presented the highest level of sHLA-G (SMD = 4.058, 95% CI = 3.608–4.508, p = 0.000; Table 5) compared to CRC, EC and GC. Due to few meta-analysed studies, subgroups results should be taken with caution.

We further investigated differences between grades in relation to sHLA-G levels. We found that in GC Asian patients, sHLA-G was significantly increased in high grade (Grade 3) compared to low grades (Grade 1 or Grade 2) (SMD = 0.448, 95% CI = 0.109–0.787, p = 0.000; Table 6). Further analysis showed that sHLA-G was significantly increased in grade 3 of DC when compared to grade 1 (SMD = 0.464, 95% CI = 0.150–0.778, p = 0.004; Table 6). Positive vascular invasion

Table 5. Meta-analysis results of sHLA-G significance in digestive cancers initiation*.

Ethnicity	Fluidics	Cancer type	Effects Models	N	Standardized mean differences				Heterogeneity			P_{Egger}	P_{Begg}
					SMD	*SEM*	*95% CI*	P_{SMD}	I^2 *(%)*	*Tau*^{2}	P_H		
Overall	All	DC	R	16	3.341	0.472	2.415–4.267	**0.000**	98	3.392	**0.000**	**0.011**	**0.015**
		CRC	R	5	2.165	0.847	0.506–3.824	**0.011**	97.9	3.473	**0.000**	0.686	0.327
		GC	R	5	4.043	0.926	2.28–5.858	**0.000**	98.46	4.011	**0.000**	0.142	0.142
	Serum/Plasma	DC	R	11	3.994	0.613	2.793–5.195	**0.000**	98.6	3.952	**0.000**	**0.004**	**0.016**
		CRC	R	3	2.686	1.289	0.159–5.214	**0.037**	98.9	4.923	**0.000**	0.636	0.602
Caucasian	All	DC	F	4	0.831	0.143	0.551–1.111	**0.000**	98.3	6.448	**0.000**	0.113	**0.042**
	Serum/Plasma	GC	F	2	0.905	0.186	0.541–1.269	**0.000**	99.4	119.190	**0.000**	NA	NA
Asian	All	DC	F	12	2.292	0.072	2.150–4.434	**0.000**	97.6	2.849	**0.000**	**0.034**	0.11
	Ascite	DC	F	3	1.666	0.257	1.163–2.169	**0.000**	48.7	0.193	0.143	0.083	0.117
	Serum/Plasma	CRC	F	2	2.266	0.127	2.016–2.516	**0.000**	99.3	9.349	**0.000**	NA	NA
		EC	F	2	2.625	0.190	2.253–2.997	**0.000**	98.8	6.328	**0.000**	NA	NA
		GC	F	2	1.818	0.122	1.579–2.058	**0.000**	98.5	2.861	**0.000**	NA	NA
		HCC	F	3	4.058	0.230	3.608–4.508	**0.000**	97.3	6.115	**0.000**	0.217	0.217

CI: Confidence interval, CRC: Colorectal cancer; DC: digestive cancer; EC: Esophageal cancer; F:Fixed effects model, GC: Gastric cancer; HCC: Hepatocellular cancer; N: number of studies, NA: Not applicable, P_{Begg}: P-value associated to Begg and Mazumdar rank correlation test (Two-tailed) without continuity correction, P_{Egger}: P-value associated to Egger's test (Two-tailed), P_H: P-value associated to heterogeneity, P_{SMD}: P-value associated to SMD, R: Random effects model, SEM: Standard errors of the mean, SMD: standardized mean differences, Bold: significant P-value (≤0.05). * Cases vs. healthy controls.

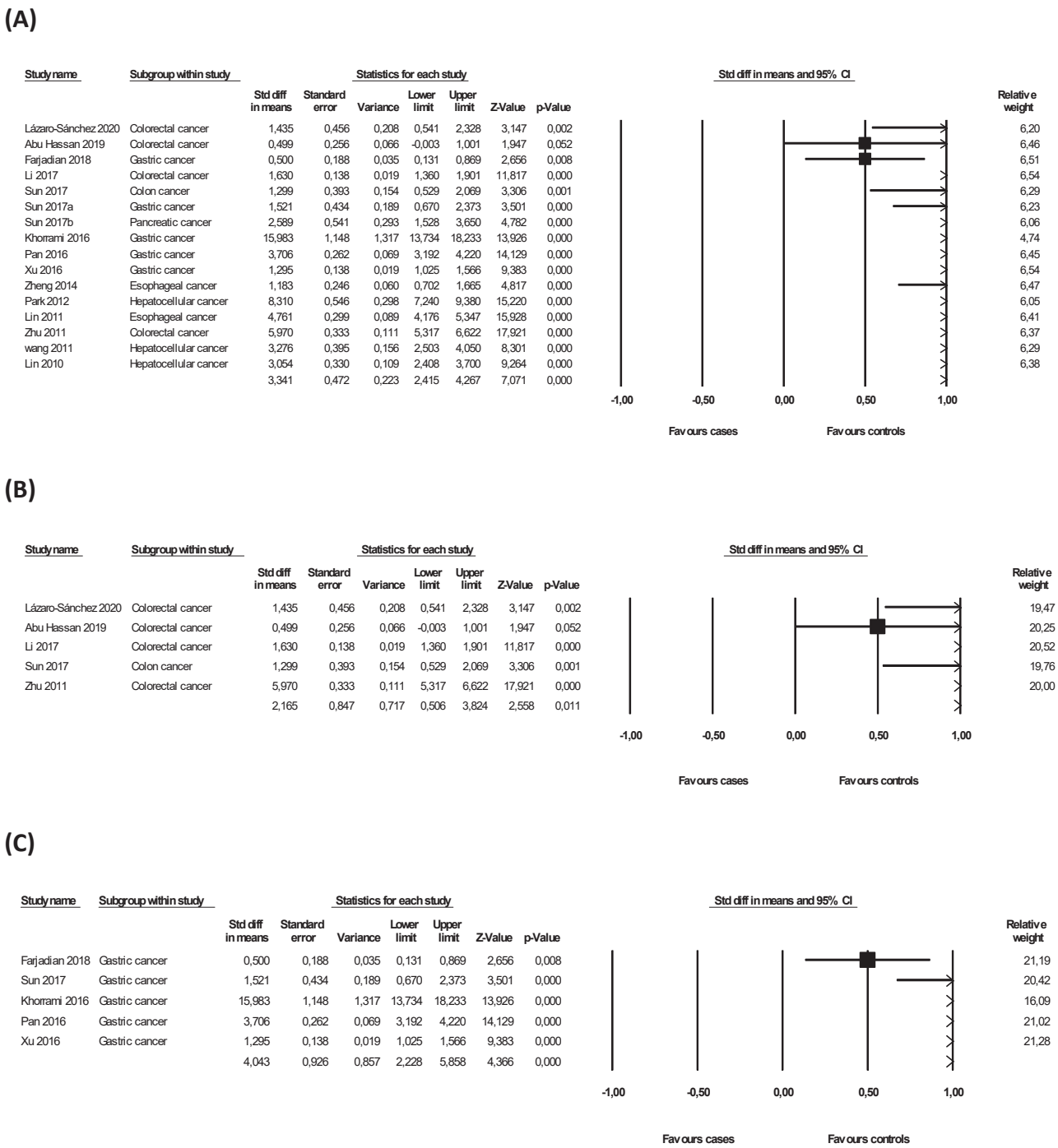

Figure 4. Forest plot of the association between sHLA-G dosage and digestive cancers susceptibility in overall population (A) All digestive cancers, (B) Colorectal cancer, (C) Gastric cancer.

presented significant increase in sHLA-G compared to negative vascular invasion in overall analysis (SMD = 0.743, 95% CI = 0.385–1.100, p = 0.000; Table 6) and in Asians (SMD = 0.721, 95% CI = 0.336–1.107, p = 0.000; Table 6). Accordingly, sHLA-G was associated to a poor prognosis in DC.

3.4. Heterogeneity and publication bias

We detected substantial heterogeneity in meta-analyses investigating the genetic risk of 14-bp I/D for overall analysis and different DC subtypes ($p < 0.05$, Table 3). In meta-analyses investigating sHLA-G, heterogeneity was also detected. Clinical features could be major source of heterogeneity. Meta-analysis of genetic risk did not present a publication bias by means of Begg' test ($p_{Begg} = 0.457$) and symmetric funnel plot (Figure 5A). However, meta-analysis of sHLA-G presented a bias of publication ($p_{Begg} = 0.015$) and asymmetric funnel plot (Figure 5B).

4. Discussion

In recent years, more and more studies are investigating the implication of HLA-G in DC, but in some cases results are conflicting. Our

Table 6. Meta-analysis results of sHLA-G significance in digestive cancers according to histoprognostic parameters.

Subset comparison	Ethnicity	Cancer type	Effect models	N	Standardized mean differences				Heterogeneity			P_{Egger}	P_{Begg}
					SMD	*SEM*	*95% CI*	P_{SMD}	I^2 *(%)*	Tau^2	P_H		
Grade 3 vs (Grade 1 or Grade 2)	Asian	DC	F	4	0.133	0.113	-0.089–0.356	0.239	88.7	0.406	**0.000**	0.565	1
		CRC	F	2	-0.104	0.150	-0.399–0.190	0.487	95	0.868	**0.000**	NA	NA
		GC	F	2	0.448	0.173	0.109–0.787	**0.000**	0	0	0.488	NA	NA
Grade 3 vs. Grade 1	Asian	DC	F	2	0.464	0.160	0.150–0.778	**0.004**	0	0	0.463	NA	NA
Grade 3 vs. Grade 2	Asian	DC	F	2	-0.201	0.161	-0.516–0.115	0.212	94.2	0.864	**0.000**	NA	NA
N+ * vs. N0	Overall	DC	R	3	-0.078	0.228	-0.525–0.369	0.732	53.7	0.085	0.116	0.524	0.117
		CRC	R	2	0.105	0.138	-0.165–0.376	0.444	0	0	0.876	NA	NA
	Asian	DC	F	2	-0.023	0.143	-0.303–0.257	0.873	75.4	0.255	**0.044**	NA	NA
PVI vs. NVI	Overall	DC	R	3	0.743	0.182	0.385–1.100	**0.000**	0	0	0.866	0.208	0.602
PVI vs. NVI	Asian	HCC	F	2	0.721	0.197	0.336–1.107	**0.000**	0	0	0.650	NA	NA

CI: Confidence interval, CRC: Colorectal cancer; DC: digestive cancer; F:Fixed effects model, GC: Gastric cancer; HCC: Hepatocellular cancer; N: number of studies, NA: Not applicable, NVI: Negative vascular invasion, PVI: Positive vascular invasion, P_{Begg}: P-value associated to Begg and Mazumdar rank correlation test (Two-tailed) without continuity correction, P_{Egger}: P-value associated to Egger's test (Two-tailed), P_H: P-value associated to heterogeneity, P_{SMD}: P-value associated to SMD, R: Random effects model, SEM: Standard errors of the mean, SMD: standardized mean differences, Bold: significant P-value (≤0.05), *N+= (N1 or N2 or N1+N2).

meta-analysis pooled published studies and examined the relationships between both HLA-G 14-bp genetic polymorphism and sHLA-G with the risk of DC. We further investigated the significance of sHLA-G according to clinicopathological features of DC.

Our results did not support a significant implication of HLA-G 14-bp I/D in DC susceptibility. A previous meta-analysis restricted to only HCC did not show a significant implication of 14-bp I/D [46], which confirm our finding. Further analysis revealed a clear association of HLA-G 14bp I/D in HCC patients infected with either HBV or HCV. Expression of HLA-G has been associated with HBV/HCV infection, via increased viral load [47, 48]. In early stages of HCV associated liver infection, both soluble and membrane bound HLA-G protein production are increased [7]. It is also possible that the presence of HLA-G expression in the context of HBV/HCV infections could favour the escape of cancerous cells from immune-surveillance. Because only few studies were meta-analysed, further investigations are still needed to clearly establish the role of HCV/HBV infection and the influence of HLA-G 14-bp I/D in HCC. In the context of sHLA-G, overall analysis showed significant increased sHLA-G in patients with DC cancers. In fact, sHLA-G has been suggested as a good diagnostic factor to distinguish benign colorectal related disease from CRC [43]. Because the invasive nature of the disease and the tumour microenvironment are different across multiple DC, the expression of HLA-G varies among different cancer types. Therefore, we conducted a stratified analysis to investigate the relationship between sHLA-G and DC cancers by cancer type. sHLA-G was significantly increased in either CRC or GC, with sHLA- G mean was 2 points higher in GC patients than in CRC patients. A previous study indicated that plasma sHLA-G level was a potential biomarker for GC diagnosis [38]. A large-scale genomewide association (GWAS) study of East Asians (22,775 CRC patients and 47,731 controls) revealed that HLA-G is one of the leading loci associated with the risk of CRC [49]. Group analysis by ethnicity revealed that sHLA-G was increased in either Caucasians or Asians, with sHLA-G mean 2 fold higher in Asians. This result may be explained by differences in genetic backgrounds between ethnicities.

Analysis by disease grades showed that sHLA-G was significantly increased in high grade compared to low grades. In addition, positive vascular invasion presented significantly elevated sHLA-G compared to negative vascular invasion. Accordingly, our results suggest that sHLA-G is associated with a poor prognosis in DC. These findings support the conclusion drawn from previous studies that tumour HLA-G expression is closely related to tumour progression and poor clinical outcomes in patients with cancer. A recent meta-analysis by Peng et al, performed on immunohistochemical and ELISA dosage [50], showed significant association of HLA-G with poor prognosis in gastric cancer. In addition, this meta-analysis showed that there was a significant correlation between HLA-G expression and TNM stage, lymph node status, and histological grade [50]. Interestingly, subgroup metaanalysis showed that HLA-G expression was only associated with clinic-pathological features in ESCC [50]. Interestingly, enhanced HLA-G expression has been found to be greatly correlated with weak anti-tumour immune response, disease progression, and poor survival in CRC [51]. Thus, HLA-G has been suggested as an independent prognostic predictor of CRC [51]. Du et al. indicated that HLA-G expression was strongly associated with tumour progression and involved in tumour evasion by raising the frequency of infiltrating Tregs locally. Therefore, HLA-G expression is a factor that should be taken into account when considering immunotherapy as treatment option, and is also a promising predictor for worse prognosis in DC patients [52]. HLA-G expression can be affected by ILT4 that regulates the cell proliferation, invasion and migration of CRC. HLA-G fusion protein treatment also increased ILT4 expression in a dose-dependent manner, thereby activating protein kinase B (AKT) and extracellular signal-regulated kinase (ERK) signaling, and facilitating the proliferation, migration and invasion of CRC cells. HLA-G interacts with ILT4 to promote CRC progression through AKT and ERK signal activation [2]. Accordingly, ILT4 and HLA-G could be prognostic factors to predict poor clinical response and survival time in patients with CRC providing a novel strategy of blocking ILT4/HLA-G for the treatment of CRC [2]. Therefore, HLA-G has the potential to serve as a biomarker for DC cancers prognosis, and screening for this marker could allow for the early diagnosis and treatment.

Our meta-analysis presents substantial limitations related to heterogeneity and size data. We also detected a marginal publication bias in meta-analysis on sHLA-G, which is due to unpublished studies and language restriction. We particularly acknowledge that the number of included studies was relatively small in subgroups; which might weaken the statistical power of the results. All the studies included were observational studies, so substantial heterogeneity was inevitable in this meta-analysis due to the various regimens, populations and sample sizes. Therefore, more clinical studies are needed to give definitive conclusions.

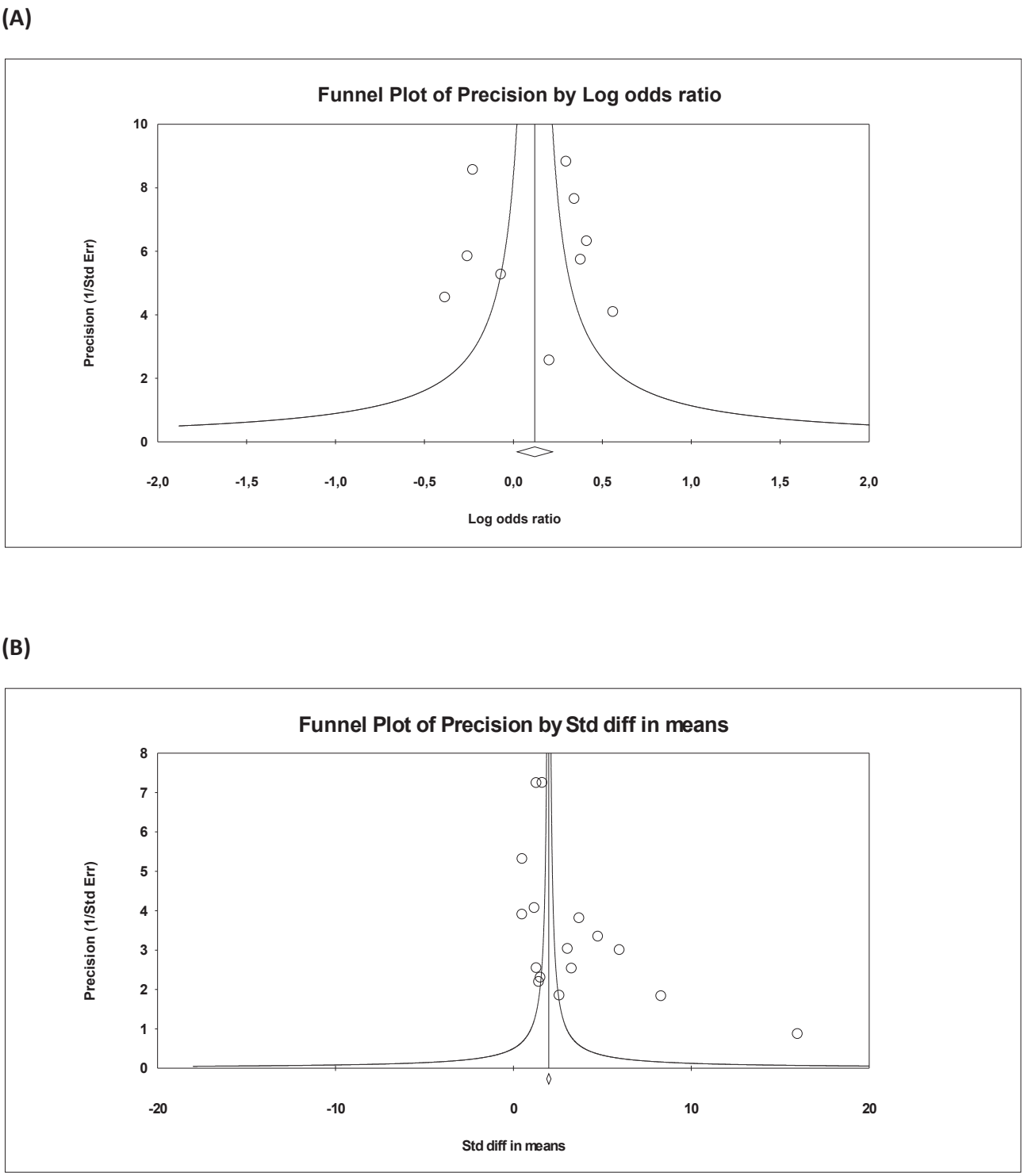

Figure 5. Funnel plot assessing presence/absence of publication bias. (A) In the allelic models of HLA-G 14bp Ins/Del polymorphism, (B)sHLA-G meta-analysis in digestive cancers.

5. Conclusion

The current meta-analysis suggests that HLA-G 14-pb I/D polymorphism is associated with HCC in the case of HBV/HCV infections. Increased sHLA-G indicates a poor prognosis for DC patients. sHLA-G is likely associated with prognostic clinical features of DC. Further larger studies are warranted to consolidate current findings.

Declarations

Author contribution statement

Sabrine DHOUIOUI, Kalthoum TIZAOUI: Data collection, Data analysis and interpretation, Drafting of manuscript.

Nadia BOUJELBENE: Medical validation, Critical data interpretation, Critical revision, Hadda-imene OUZARI: Critical data interpretation,

Critical revision. Inès ZIDI: Study conception and design, supervision, Data analysis, Drafting of manuscript, Critical revision.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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References

[1] R. Liang, Y. Lin, C.L. Yuan, Z.H. Liu, Y.Q. Li, X.L. Luo, et al., High expression of estrogen-related receptor alpha is significantly associated with poor prognosis in patients with colorectal cancer, Oncol. Lett. 15 (2018) 5933.
[2] Z. Cai, L. Wang, Y. Han, W. Gao, X. Wei, R. Gong, et al., Immunoglobulinlike transcript 4 and human leukocyte antigenG interaction promotes the progression of human colorectal cancer, Int. J. Oncol. 54 (2019) 1943.
[3] M. Loustau, F. Anna, R. Drean, M. Lecomte, P. Langlade-Demoyen, J. Caumartin, HLA-G Neo-expression on tumors, Front. Immunol. 11 (2020) 1685.
[4] E.D. Carosella, N. Rouas-Freiss, D. Tronik-Le Roux, P. Moreau, J. LeMaoult, HLA-G: an immune checkpoint molecule, Adv. Immunol. 127 (2015) 33.
[5] W.H. Yan, Human leukocyte antigen-G in cancer: are they clinically relevant? Cancer Lett. 311 (2011) 123.
[6] S.M. Yie, H. Yang, S.R. Ye, K. Li, D.D. Dong, X.M. Lin, Expression of human leukocyte antigen G (HLA-G) correlates with poor prognosis in gastric carcinoma, Ann. Surg Oncol. 14 (2007) 2721.
[7] G. Amodio, R. Sales de Albuquerque, S. Gregori, New insights into HLA-G mediated tolerance, Tissue Antigens 84 (2014) 255.
[8] L. Amiot, S. Ferrone, H. Grosse-Wilde, B. Seliger, Biology of HLA-G in cancer: a candidate molecule for therapeutic intervention? Cell. Mol. Life Sci. 68 (2011) 417.
[9] A. Lin, W.H. Yan, Heterogeneity of HLA-G expression in cancers: facing the challenges, Front. Immunol. 9 (2018) 2164.
[10] E. Alegre, R. Rizzo, D. Bortolotti, S. Fernandez-Landazuri, E. Fainardi, A. Gonzalez, Some basic aspects of HLA-G biology, J Immunol Res 2014 (2014), 657625.
[11] X.Y. Chen, W.H. Yan, A. Lin, H.H. Xu, J.G. Zhang, X.X. Wang, The 14 bp deletion polymorphisms in HLA-G gene play an important role in the expression of soluble HLA-G in plasma, Tissue Antigens 72 (2008) 335.
[12] P. Rousseau, M. Le Discorde, G. Mouillot, C. Marcou, E.D. Carosella, P. Moreau, The 14 bp deletion-insertion polymorphism in the 3' UT region of the HLA-G gene influences HLA-G mRNA stability, Hum. Immunol. 64 (2003) 1005.
[13] Z. Tan, G. Randall, J. Fan, B. Camoretti-Mercado, R. Brockman-Schneider, L. Pan, et al., Allele-specific targeting of microRNAs to HLA-G and risk of asthma, Am. J. Hum. Genet. 81 (2007) 829.
[14] D. Moher, A. Liberati, J. Tetzlaff, D.G. Altman, P. Group, Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement, PLoS Med. 6 (2009), e1000097.
[15] S.P. Hozo, B. Djulbegovic, I. Hozo, Estimating the mean and variance from the median, range, and the size of a sample, BMC Med. Res. Methodol. 5 (2005) 13.
[16] J.P.T. Higgins, J. Thomas, J. Chandler, M. Cumpston, T. Li, M.J. Page, V.A. Welch. Cochrane Handbook for Systematic Reviews of Interventions, 2nd Edition, John Wiley & Sons, Chichester (UK), 2019.
[17] L.V.H. Michael Borenstein, Julian P.T. Higgins, Hannah R. Rothstein, Introduction to Meta-Analysis, Copyright © 2009 John Wiley & Sons, Ltd, 2009.
[18] J. Anzures-Cabrera, J.P. Higgins, Graphical displays for meta-analysis: an overview with suggestions for practice, Res. Synth. Methods 1 (2010) 66.
[19] M. Egger, G.D. Smith, M. Schneider, C. Minder, Bias in meta-analysis detected by a simple, graphical test, BMJ 315 (1997) 629.
[20] C.B. Begg, M. Mazumdar, Operating characteristics of a rank correlation test for publication bias, Biometrics 50 (1994) 1088.
[21] R. DerSimonian, N. Laird, Meta-analysis in clinical trials revisited, Contemp. Clin. Trials 45 (2015) 139.
[22] M. Egger, G.D. Smith, A.N. Phillips, Meta-analysis: principles and procedures, BMJ 315 (1997) 1533.
[23] S. Dhouioui, A.-B. Laaribi, N. Boujelbene, R. Jelassi, H. Ben Salah, H. Bellali, et al., Association of HLA-G 3′UTR polymorphisms and haplotypes with colorectal cancer susceptibility and prognosis, Hum. Immunol. 83 (2022) 39.
[24] C. Vaquero-Yuste, I. Juarez, M. Molina-Alejandre, E.M. Molanes-López, A. López-Nares, F. Suárez-Trujillo, et al., HLA-G 3'UTR polymorphisms are linked to susceptibility and survival in Spanish gastric adenocarcinoma patients, Front. Immunol. (2021) 12.
[25] M. Abu Hassan, S. Al Omar, H. Halawani, M. Arafah, S. Alqadheeb, J. Al-Tamimi, et al., Relationship of HLA-G expression and its 14-bp insertion/deletion polymorphism with susceptibility to colorectal cancer, Genet. Mol. Res. 18 (2019) gmr18324.
[26] M.A. El Bassiouny, A.A. Elshaarawy, G.A. Tawfeek, M.I. Elashmawy, Association of HLA-G gene polymorphism with hepatocellular carcinoma in Egyptian population, Menoufia Med. J. 32 (2019) 255.
[27] M. Garziera, E. Catamo, S. Crovella, M. Montico, E. Cecchin, Lonardi S, et al., Association of the HLA-G 3'UTR polymorphisms with colorectal cancer in Italy: a first insight, Int. J. Immunogenet. 43 (2016) 32.
[28] S.K. Kim, J.H. Chung, J.W. Jeon, J.J. Park, J.M. Cha, K.R. Joo, et al., Association between HLA-G 14-bp insertion/deletion polymorphism and hepatocellular carcinoma in Korean patients with chronic hepatitis B viral infection, Hepato-Gastroenterology 60 (2013) 796.
[29] A.C. Teixeira, C.T. Mendes-Junior, F.F. Souza, L.A. Marano, N.H.S. Deghaide, S.C. Ferreira, et al., The 14bp-deletion allele in the HLA-G gene confers susceptibility to the development of hepatocellular carcinoma in the Brazilian population, Tissue Antigens 81 (2013) 408.
[30] Y. Chen, X-j Gao, Y-c Deng, H-x Zhang, Relationship between HLA-G gene polymorphism and the susceptibility of esophageal cancer in Kazakh and Han nationality in Xinjiang, Biomarkers 17 (2012) 9.
[31] Y. Jiang, S. Chen, S. Jia, Z. Zhu, X. Gao, D. Dong, et al., Association of HLA-G 3' UTR 14-bp insertion/deletion polymorphism with hepatocellular carcinoma susceptibility in a Chinese population, DNA Cell Biol. 30 (2011) 1027.
[32] A.D. Lazaro-Sanchez, P. Salces-Ortiz, L.I. Velasquez, D. Orozco-Beltran, N. Diaz-Fernandez, A. Juarez-Marroqui, HLA-G as a new tumor biomarker: detection of soluble isoforms of HLA-G in the serum and saliva of patients with colorectal cancer, Clin. Transl. Oncol. 22 (2020) 1166.
[33] S. Farjadian, M. Tabebordbar, M. Mokhtari, A. Safaei, M. Malekzadeh, A. Ghaderi, HLA-G expression in tumor tissues and soluble HLA-G plasma levels in patients with gastrointestinal cancer, Asian Pac. J. Cancer Prev. APJCP : Asian Pac. J. Cancer Prev. APJCP 19 (2018) 2731.
[34] C. Kirana, A. Ruszkiewicz, R.S. Stubbs, J.E. Hardingham, P.J. Hewett, P.J. Maddern, et al., Soluble HLA-G is a differential prognostic marker in sequential colorectal cancer disease stages, Int. J. Cancer 140 (2017) 2577.
[35] J.B. Li, Y.Y. Ruan, B. Hu, S.S. Dong, T.N. Bi, A. Lin, et al., Importance of the plasma soluble HLA-G levels for prognostic stratification with traditional prognosticators in colorectal cancer, Oncotarget 8 (2017), 48854.
[36] J. Sun, Y.-X. Chang, C.-Y. Niu, Evaluation of ascitic soluble human leukocyte antigen-G for distinguishing malignant ascites from benign ascites, Tumor Biol. 39 (2017), 1010428317726840.
[37] S. Khorrami, R. Rahimi, H. Mohammadpour, S. Bahrami, F. Yari, H. Poustchi, et al., Association of HLA-G*01:01:02:01/G*01:04:01 polymorphism with gastric adenocarcinoma, Hum. Immunol. 77 (2016) 153.
[38] Y.Q. Pan, Y.Y. Ruan, J.B. Peng, Q.Y. Han, X. Zhang, A. Lin, et al., Diagnostic significance of soluble human leukocyte antigen-G for gastric cancer, Hum. Immunol. 77 (2016) 317.
[39] D.P. Xu, W.W. Shi, T.T. Zhang, H.Y. Lv, J.B. Li, A. Lin, et al., Elevation of HLA-G-expressing DC-10 cells in patients with gastric cancer, Hum. Immunol. 77 (2016) 800.
[40] J. Zheng, C. Xu, D. Chu, X. Zhang, J. Li, G. Ji, et al., Human leukocyte antigen G is associated with esophageal squamous cell carcinoma progression and poor prognosis, Immunol. Lett. 161 (2014) 13.
[41] A. Lin, X. Zhang, W.J. Zhou, Y.Y. Ruan, D.P. Xu, Q. Wang, et al., Human leukocyte antigen-G expression is associated with a poor prognosis in patients with esophageal squamous cell carcinoma, Int. J. Cancer 129 (2011) 1382.
[42] Y. Park, Y. Park, H.S. Lim, Y.S. Kim, D.J. Hong, H.S. Kim, Soluble human leukocyte antigen-G expression in hepatitis B virus infection and hepatocellular carcinoma, Tissue Antigens 79 (2012) 97.
[43] C.B. Zhu, C.X. Wang, X. Zhang, J. Zhang, W. Li, Serum sHLA-G levels: a useful indicator in distinguishing colorectal cancer from benign colorectal diseases, Int. J. Cancer 128 (2011) 617.
[44] Y. Wang, Z. Ye, X.Q. Meng, S.S. Zheng, Expression of HLA-G in patients with hepatocellular carcinoma, Hepatobiliary Pancreat. Dis. Int. 10 (2011) 158.
[45] A. Lin, H.X. Chen, C.C. Zhu, X. Zhang, H.H. Xu, J.G. Zhang, et al., Aberrant human leucocyte antigen-G expression and its clinical relevance in hepatocellular carcinoma, J. Cell Mol. Med. 14 (2010) 2162.
[46] A.V. Coelho, R.R. Moura, S. Crovella, F. Celsi, HLA-G genetic variants and hepatocellular carcinoma: a meta-analysis, Genet. Mol. Res. 15 (2016).
[47] F.J. Souto, J.C. Crispim, S.C. Ferreira, A.S. da Silva, C.L. Bassi, Soares CP, et al., Liver HLA-G expression is associated with multiple clinical and histopathological forms of chronic hepatitis B virus infection, J. Viral Hepat. 18 (2011) 102.
[48] P.J. Weng, Y.M. Fu, S.X. Ding, D.P. Xu, A. Lin, W.H. Yan, Elevation of plasma soluble human leukocyte antigen-G in patients with chronic hepatitis C virus infection, Hum. Immunol. 72 (2011) 406.

[49] Y. Lu, S.S. Kweon, C. Tanikawa, W.H. Jia, Y.B. Xiang, Q. Cai, et al., Large-scale genome-wide association study of East Asians identifies loci associated with risk for colorectal cancer, Gastroenterology 156 (2019) 1455.
[50] Y. Peng, J. Xiao, W. Li, S. Li, B. Xie, J. He, et al., Prognostic and clinicopathological value of human leukocyte antigen G in gastrointestinal cancers: a meta-analysis, Front. Oncol. 11 (2021), 642902.
[51] R.L. Zhang, X. Zhang, S.S. Dong, B. Hu, Q.Y. Han, J.G. Zhang, et al., Predictive value of different proportion of lesion HLA-G expression in colorectal cancer, Oncotarget 8 (2017), 107441.
[52] L. Du, X. Xiao, C. Wang, X. Zhang, N. Zheng, L. Wang, et al., Human leukocyte antigen-G is closely associated with tumor immune escape in gastric cancer by increasing local regulatory T cells, Cancer Sci. 102 (2011) 1272.