Implicating the H63D polymorphism in the *HFE* gene in increased incidence of solid cancers: a meta-analysis

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Genet. Mol. Res. 14 (4): 13735-13745 (2015)
Received May 26, 2015
Accepted August 13, 2015
Published October 29, 2015
DOI http://dx.doi.org/10.4238/2015.October.28.36

ABSTRACT. A number of previous studies have demonstrated that the *HFE* H63D polymorphism is associated with increased risk of incidence multiple types of cancer, including colorectal cancer, breast cancer, liver cancer, pancreatic cancer, and gynecological malignant tumors. However, the clinical outcomes were inconsistent. Therefore, this meta-analysis was conducted to summarize the effect of the H63D variant on the incidence of solid tumor. PubMed and EMBASE databases were searched for articles associating the *HFE* H63D polymorphism with cancer risk. The relationships were evaluated by calculating the pooled odds ratios (ORs) with 95% confidence intervals (CIs). A total of 28 studies, including 7728 cancer cases and 11,895 controls, were identified. Statistically significant associations were identified between the *HFE* H63D polymorphism and solid cancer risk (CG *vs* CC, OR = 1.14, 95%CI = 1.07-1.23, P < 0.001; GG *vs* CC, OR = 1.28, 95%CI = 1.06-1.55, P = 0.010; CG/GG *vs* CC, OR = 1.16, 95%CI = 1.08-1.24, P < 0.001; GG *vs* CC/CG, OR = 1.24, 95%CI = 1.02-1.49, P = 0.027). In the subgroup analysis, we illustrated the effect

of the H63D polymorphism on hepatocellular carcinoma and pancreatic cancer risk, particularly in the Asian and African subgroups; however, this was not observed in gynecological malignant tumors. In summary, this analysis provided strong evidence that the *HFE* H63D polymorphism may play a critical role in the increased aggressiveness of hepatocellular carcinoma and pancreatic cancer.

Key words: *HFE* H63D polymorphism; Solid cancer; Meta-analysis; Molecular epidemiology

INTRODUCTION

Cancer is a serious global public health issue with a high degree of morbidity and mortality. According to reliable records, 1,665,540 new cancer cases and 585,720 cancer deaths were projected to occur in 2014 in the United States of America (Siegel et al., 2014). The progression of cancer is ascribed to several complicated actions, including factors such as the activation of oncogenes, inhibition of tumor suppressors, evasion of apoptosis, unlimited replication, and sustained angiogenesis (Wogan et al., 2004). The presence of mutations is predominantly clustered in important cell signaling pathways across different types of cancer. Therefore, genomic screening for the identification of potential biomarkers is a promising approach for early detection and intervention of cancer. The haemochromatosis (*HFE*) gene, located on chromosome 6p21.3, has been discovered as a candidate oncogene in some solid tumors in many organs, including the colon, breast, and liver.

Previous studies have validated a potential mechanism by which the *HFE* gene mutation regulates iron absorption, by reducing its binding affinity to the cell-surface transferrin receptor (Fleming and Britton, 2006; Fargion et al., 2010). The accumulation of iron eventually functions as a carcinogen, inducing cellular oxidative stress by catalyzing hydroxyl radical formation through the Fenton reaction, as well as inactivating the antioxidant enzymes, resulting in the depletion of antioxidant defenses. Subsequently, iron-catalyzed oxidative stress causes lipid peroxidation, protein modification, and DNA damage.

His63Asp (substitution of aspartic acid to histidine at amino acid 63; H63D; rs1799945), is located in exon 2 of the *HFE* gene (Feder et al., 1996). A number of studies have reported that individuals with the variant rs1799945 tend to store higher levels of body iron (de Valk et al., 2000; Qi et al., 2005). These studies have demonstrated a strong relationship between the H63D polymorphism and tumor susceptibility. A recent study with a case-control has clearly shown that the H63D polymorphism was strongly correlated with the risk of pancreatic cancer (Graff et al., 2014). In order to verify the association between the H63D polymorphism and the development of cancers, we have further examined the published data using a meta-analysis. Based on the statistical data reorganization, our results predict that the H63D polymorphism of the *HFE* gene contributes to cancer progression, as a result of altered cellular iron metabolism.

MATERIAL AND METHODS

Literature search

Electronic databases, including PubMed and EMBASE (up to March 20, 2015), were

searched using several terms and MESH headings, such as HFE/H63D, polymorphism/variant, and cancer/carcinoma/tumor. The search was limited to English-language articles. The PubMed option 'Related Articles' was also used in each research article, in order to search for potentially relevant articles. The cited studies were identified by a manual search of references cited in the original extracted articles. The search strategies are summarized in Figure 1. Studies fulfilling the following selection criteria were included in the meta-analysis: (a) evaluation of H63D polymorphism with solid tumors, (b) use of a case-control design, and (c) the availability of genotype frequency.

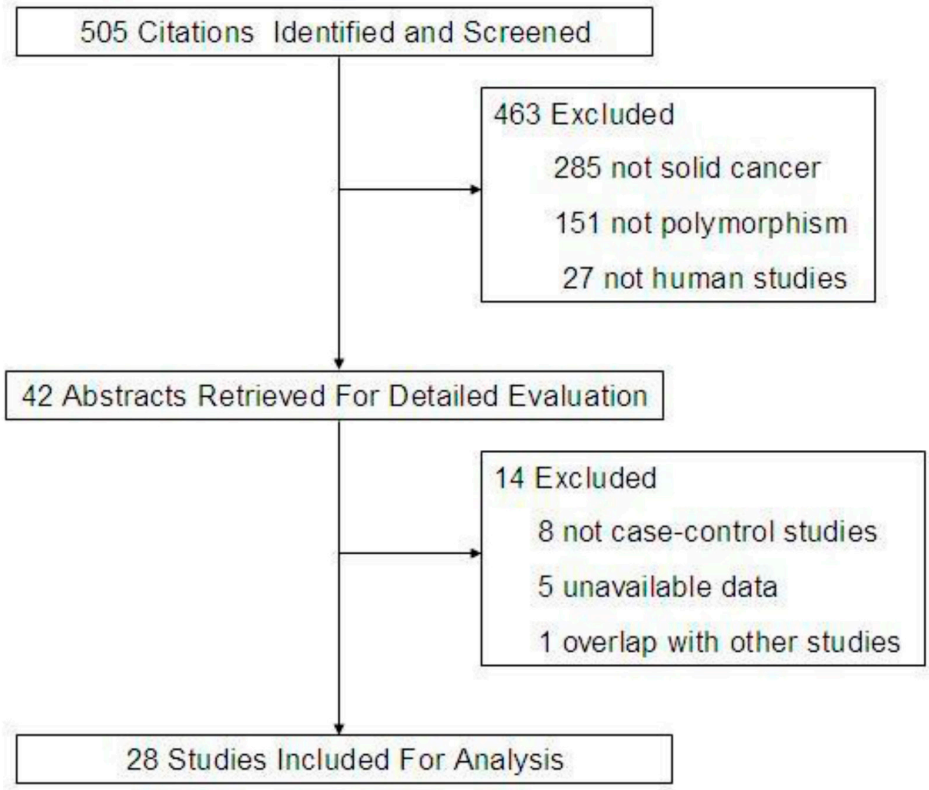

Figure 1. The flow chart of literature search.

Data extraction

The data extraction was performed independently by two individuals (LL Shen and DY Gu) using a standard extraction form. A group consensus was taken, and consultations held with a third reviewer in resolve discrepancies. The following data was retrieved: the name of the first author, publication year, ethnicity, and cancer type, source of controls, numbers of genotyped cases and controls, and value of the Hardy-Weinberg equilibrium (HWE) (Table 1). Different ethnic lines were categorized as European, Asian, and African. The data was extracted separately for each tumor type in studies involving subjects with different tumor types.

Statistical analysis

STATA version 10.0 (Stata Corporation, College Station, Texas, USA) was used for all statistical analysis; two-sided P values were used in this study. The observed genotype frequencies of the *HFE* H63D C>G polymorphism in the control groups of all studies were assessed for HWE using the χ^2 test. The strength of association between the *HFE* H63D C>G polymorphism and solid tumor risk was measured by the Odd's ratio (OR), with 95% confidence interval (95%CI). The risk of H63D genotypes in solid tumors was measured by heterozygote comparison (GC *vs* CC), homozygote comparison (GG *vs* CC), a dominant model (CC/GC *vs* GG), and a recessive model (CC *vs* GC/GG). The significance of pooled ORs was determined using the *Z*-test. Cochran's Q-statistic and I^2-statistic was calculated (to test for heterogeneity and quantify the proportion of the total variation resulting from heterogeneity, respectively) in order to estimate heterogeneity among the included studies (Cochran, 1950). If the P value of the Q-test was < 0.05 (indicating a lack of heterogeneity across studies), the summary OR estimate of each study was calculated by the fixed effects model (the Mantel-Haenszel method) (Mantel and Haenszel, 1959), as described in a previous study (Zhang et al., 2013). In other cases, the random effects model (the DerSimonian and Laird method) was used (DerSimonian and Laird, 1986). Stratified analyses were also performed based on the ethnicity, cancer types (a cancer type analyzed in less than two individual studies was excluded), and source of controls. The healthy controls and liver disease controls within the liver cancer subgroup were further compared. Sensitivity analyses were performed to evaluate the stability of the results by deleting a single study in the meta-analysis each time, in order to show the influence of the individual data set to the pooled OR. Potential publication bias was assessed by Funnel plots and Egger's linear regression test (Egger et al., 1997).

RESULTS

Description of included studies

The study selection process is shown in Figure 1. After reviewing these articles, 28 case-control studies (Racchi et al., 1999; Blanc et al., 2000; Beckman et al., 2000; Willis et al., 2000; Campo et al., 2001; Lauret et al., 2002; Boige et al., 2003; Cauza et al., 2003; Hellerbrand et al., 2003; Shaheen et al., 2003; Robinson et al., 2005; Abraham et al., 2005; Syrjakoski et al., 2006; Cardoso et al., 2006; Kondrashova et al., 2006; Gunel-Ozcan et al., 2006; Hucl et al., 2007; Ropero et al., 2007; Yonal et al., 2007; Ezzikouri et al., 2008; Nahon et al., 2008; Shi et al., 2009; Batschauer et al., 2011; Robertson, 2011; Gannon et al., 2011; Gharib et al., 2011; Ekblom et al., 2012; Motawi et al., 2013; Agudo et al., 2013; Graff et al., 2014; Zhao et al., 2014) investigating the association between H63D polymorphisms and the risk of solid tumor were finally selected for further analyses. The selected published articles included 7,728 cancer cases and 11,895 controls, containing 1,707 breast cancer (BC), 1455 gastrointestinal cancer (GI, including gastric cancer and colorectal cancer), 1,488 hepatocellular carcinoma (HCC), 843 prostate cancer (PC^1), 681 gynecologic malignant tumor (including ovarian cancer and endometrial cancer), and 1554 pancreatic cancer (PC^2) patients. Twenty-two of the 28 selected articles analyzed Caucasian subjects, while 3 studies (each) analyzed Asian and African cancer patients, respectively; nine studies were designed for population-based (PB) investigation, while 19 were designed for hospital-based (HB) investigation. The baseline characteristics of the selected studies are summarized in Table 1.

Table 1. Major characteristics of the articles detailing the association between the H63D variant of the *HFE* gene variant and cancer.

First author	Cancer types	Year	Ethnicity	Source of control	Genotype (case)			Genotype (control)			HWE (P)
					CC	CG	GG	CC	CG	GG	
Graff	BC	2014	European	HB	553	196	16	1008	324	36	0.11
Batschauer	BC	2011	European	PB	49	13	6	57	25	3	0.90
Ozcan	BC	2006	Asian	PB	49	39	0	73	26	1	0.43
Syrjakoski	BC	2006	European	HB	89	26	1	385	88	7	0.45
Abraham	BC	2005	European	PB	421	138	12	457	173	16	0.94
Kondrashova	BC	2005	European	HB	67	30	2	180	75	5	0.38
Syrjakoski	PC	2006	European	HB	649	177	17	385	88	7	0.45
Gannon	GMT	2010	European	HB	415	156	17	60	17	3	0.22
Kondrashova	GMT	2005	European	HB	71	19	3	180	75	5	0.38
Agudo	GC	2013	European	PB	230	82	11	885	249	23	0.27
Ekblom	CRC	2012	European	PB	171	42	5	305	96	13	0.12
Shi	CRC	2009	European	HB	110	33	5	138	43	3	0.87
Robinson	CRC	2005	European	PB	236	83	8	241	73	8	0.39
Shaheen	CRC	2003	European	PB	338	83	10	626	124	12	0.05
Motawi	HCC	2013	African	HB	29	10	0	32	8	0	0.48
Motawi1	HCC	2013	African	HB	29	10	0	30	10	0	0.37
Gharib	HCC	2011	African	HB	52	43	5	72	27	1	0.37
Gharib1	HCC	2011	African	HB	52	43	5	81	18	1	0.99
Ezzkiouri	HCC	2008	African	HB	59	34	3	160	60	2	0.16
Nahon1	HCC	2008	European	HB	75	28	0	149	49	0	0.05
Repero	HCC	2007	European	HB	102	85	9	124	52	5	0.87
Y'onal	HCC	2007	Asian	HB	11	6	2	103	33	2	0.72
Y'onal1	HCC	2007	Asian	HB	11	6	2	73	22	2	0.82
Y'onal1	HCC	2007	Asian	HB	11	6	2	10	6	0	0.36
Boige1	HCC	2003	European	HB	92	41	0	59	40	1	0.04
Cauza	HCC	2003	European	HB	128	31	3	529	133	9	0.85
Hellerbrand	HCC	2003	European	HB	108	27	2	94	29	3	0.67
Hellerbrand1	HCC	2003	European	HB	108	27	2	83	23	1	0.67
Lauret1	HCC	2002	European	PB	44	25	0	125	46	19	<0.05
Campo	HCC	2001	European	HB	16	6	1	65	32	3	0.69
Campo1	HCC	2001	European	HB	16	6	1	65	29	6	0.27
Beckman	HCC	2000	European	HB	37	17	0	229	59	6	0.35
Racchi	HCC	1999	European	HB	9	3	0	85	42	3	0.40

BC, breast cancer; GMT, gynecological malignant tumor (including ovarian cancer and endometrial cancer); CRC, colorectal cancer; GC, gastric cancer; HCC, hepatocellular carcinoma; PC, prostate cancer; HB, hospital-based; PB, population-based; HWE, Hardy-Weinberg Equilibrium in controls; 1 studies with hepatitis or liver cirrhosis controls.

Correlation between H63D polymorphism and the incidence of solid tumor

The results from statistical analyses indicate a significant association between H63D polymorphism and incidence of solid tumor (CG versus CC, OR = 1.14, 95%CI = 1.07-1.23, P < 0.001; GG versus CC, OR = 1.28, 95%CI = 1.06-1.55, P = 0.010; CG/GG versus CC, OR = 1.16, 95%CI = 1.08-1.24, P < 0.001; GG versus CC/CG, OR = 1.24, 95%CI = 1.02-1.49, P = 0.027; Table 2, Figure 2).

Additionally, the 19 studies using hospital-based controls (stratified analysis based on the source of controls) clearly showed a correlation between the H63D polymorphism and cancer risk in all genetic comparisons (heterozygote comparison, CG versus CC: OR = 1.17, 95%CI: 1.07-1.28, P < 0.001, I^2 = 0.0%; homozygote comparison, GG versus CC: OR = 1.42, 95%CI = 1.13-1.79, P = 0.003, I^2 = 0.0%; dominant model, CG/GG versus CC: OR = 1.19, 95%CI = 1.10-1.30, P < 0.001, I^2 = 23.7%; recessive model, GG versus CC/CG: OR = 1.36, 95%CI = 1.08-1.71, P = 0.008, I^2 = 0.0%). Consistently, significantly increased associations were observed in the Asian and African subgroups (P = 0.003 and P = 0.001, respectively).

Table 2. Meta-analysis of the effect of H63D polymorphism on cancer.

Variables	n[a]	CG *vs* CC		GG *vs* CC		CG/GG *vs* CC (dominant)		GG *vs* CG/CC (recessive)	
		OR (95%CI)	P[b]	OR (95%CI)	P[b]	OR (95%CI)	P[b]	OR (95%CI)	P[b]
Total	26	1.15 (1.06-1.24)	0.001	1.19 (0.95-1.49)	0.126	1.15 (1.06-1.24)	<0.001	1.14 (0.91-1.43)	0.238
Ethnicities									
European	21	1.11 (0.93-1.33)	0.238	0.79 (0.46-1.38)	0.414	1.08 (0.90-1.28)	0.416	0.73 (0.42-1.27)	0.268
Asian	2	1.87 (1.19-2.94)	0.007	3.77 (1.14-12.42)	0.029	2.03 (1.31-3.15)	0.002	3.29 (1.02-10.57)	0.046
African	3	1.74 (1.21-2.51)	0.003	5.30 (1.32-21.22)	0.018	1.84 (1.29-2.63)	0.001	4.29 (1.07-17.19)	0.04
Source of controls									
PB	8	1.11 (0.90-1.37)[c]	0.338	1.01 (0.71-1.43)	0.973	1.09 (0.90-1.33)[c]	0.391	0.99 (0.70-1.40)	0.957
HB	18	1.17 (1.06-1.29)	0.003	1.34 (1.00-1.81)	0.056	1.21 (1.03-1.42)[c]	0.024	1.28 (0.95-1.73)	0.106
Cancer type									
BC	6	1.09 (0.85-1.39)[c]	0.494	0.89 (0.59-1.34)	0.572	1.04 (0.91-1.19)	0.561	0.89 (0.59-1.34)	0.576
GMT	2	1.04 (0.75-1.44)	0.819	1.07 (0.48-2.43)	0.864	1.04 (0.76-1.42)	0.807	1.04 (0.46-2.36)	0.919
GI	5	1.12 (0.95-1.31)	0.166	1.35 (0.89-2.04)	0.165	1.14 (0.89-1.33)	0.09	1.32 (0.87-2.00)	0.194
HCC	13	1.30 (1.12-1.51)	<0.001	1.44 (0.94-2.21)	0.097	1.29 (1.03-1.63)[c]	0.03	1.29 (0.84-1.98)	0.253

BC, breast cancer; GMT, gynecologic malignant tumor (including ovarin cancer and endometrial cancer); GI, gastrointestinal cancer; HCC, hepatocellular carcinoma; HB, hospital-based; PB, population-based; OR, Odd's ratio; CI, confidence interval [a]Number of studies; [b]P value of Z-test for pooled OR; [c]Random-effects model was used when P value for heterogeneity test was < 0.05; otherwise, the fixed-effects model was used.

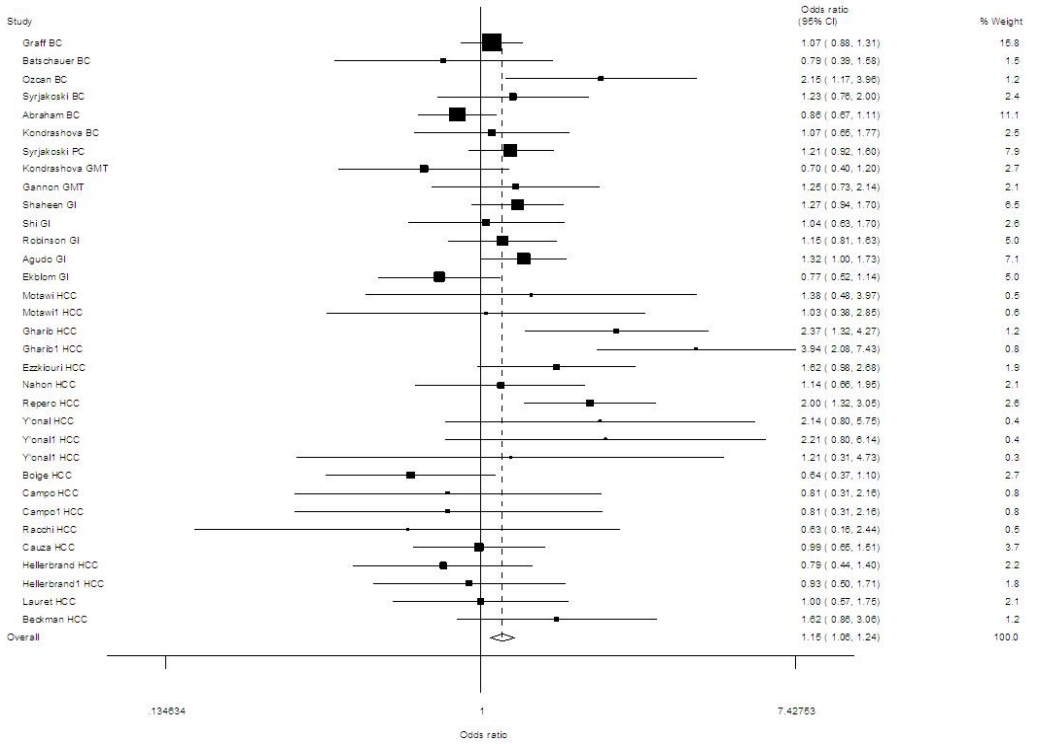

Figure 2. Association between H63D polymorphism and incidence of solid tumor.

In addition, stratification of the studies according to cancer type revealed significantly increased risks in the pancreatic cancer (GG versus CC, OR = 1.54, 95%CI = 1.08-2.20, P =

0.017, I^2 = 0.0%; CG/GG versus CC, OR = 1.18, 95%CI = 1.02-1.37, P = 0.028, I^2 = 0.0%; GG versus CC/CG, OR = 1.49, 95%CI = 1.05-2.13, P = 0.026, I^2 = 0.0%) and hepatocellular carcinoma (CG versus CC, OR = 1.30, 95%CI = 1.12-1.51, P < 0.001, I^2 = 46.4%; CG/GG versus CC, OR = 1.29, 95%CI = 1.03-1.63, P = 0.03, I^2 = 54.3%) subgroups. However, no significant associations were found between the other cancer types and H63D polymorphism in any of the genetic models (for e.g. in a dominant model of gynecological malignant tumor, OR = 1.04, 95%CI = 0.76-1.42; of gastrointestinal cancer, OR = 1.14, 95%CI = 0.98-1.33; of breast cancer, OR = 1.04, 95%CI = 0.91-1.19; and for prostate cancer, OR = 1.21, 95%CI = 0.92-1.60).

Moreover, we observed statistically significant differences between different the physical conditions of controls within the hepatocellular carcinoma subgroup (Figure 3). The results suggested that the association was significant in studies with healthy controls (CG versus CC, OR = 1.35, 95%CI = 1.10-1.67, P = 0.005, I^2 = 42.4%; GG versus CC, OR = 1.81, 95%CI = 1.00-3.25, P = 0.049, I^2 = 0.0%; CG/GG versus CC, OR = 1.37, 95%CI = 1.12-1.68, P = 0.002, I^2 = 49.3%), and not significant in the studies with hepatitis or liver cirrhosis controls.

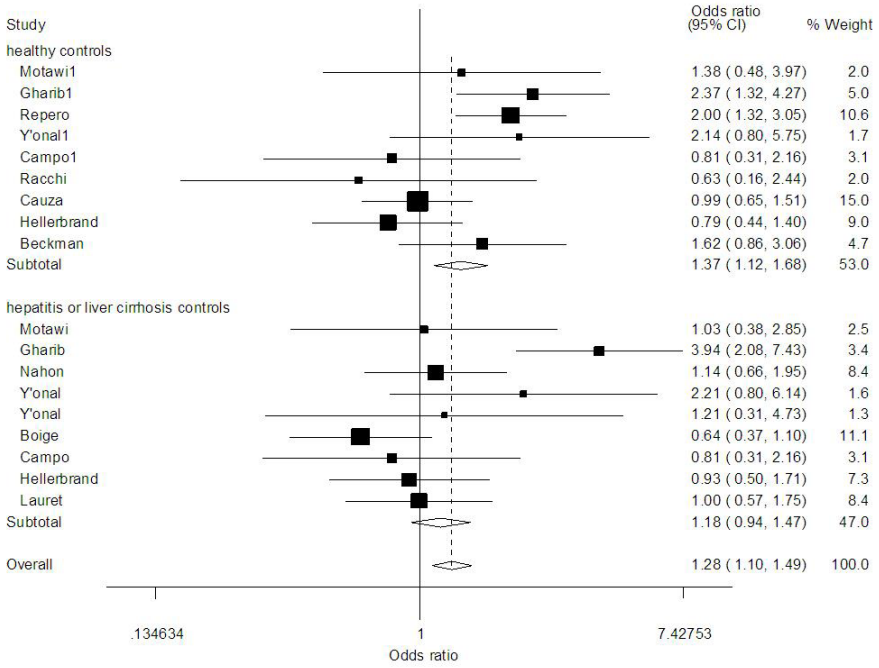

Figure 3. Statistically significant differences between different the physical conditions of controls within the hepatocellular carcinoma subgroup.

Sensitivity analysis and publication bias

A single study included in the meta-analysis was deleted each time to reflect the influence of the individual dataset on the pooled ORs; in addition, the corresponding pooled ORs were not materially altered (data not shown). The publication bias was assessed by the Begg's funnel plot and Egger's test. Evidence of publication bias was detected by plotting funnel plots of HR

(dominant model). The Begg's test showed funnel plot symmetry (z = 0.31 continuity corrected, $Pr>|z|$ = 0.756 continuity corrected), and the Egger's test was also adopted to provide statistical evidence of funnel plot asymmetry (t = 0.79, $P>|t|$ = 0.435). All of the data suggested a lack of publication bias, indicating that our results were statistically robust (Figure 4).

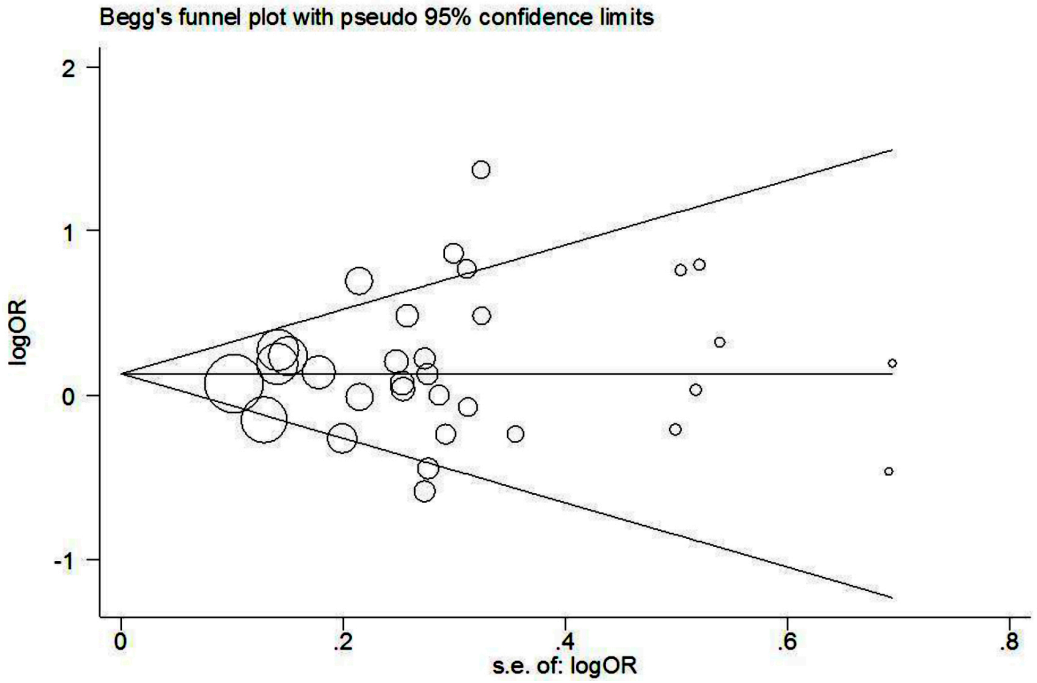

Figure 4. Begg's funnel plot of the publication bias.

DISCUSSION

This study examined the association between H63D variants and multiple types of cancer using a meta-analysis, in order to clarify the possible association between H63D polymorphism and cancer development. Our results clearly showed a strong association between the H63D polymorphism and aggressive cancers, suggesting that the H63D variant significantly increases the incidence of cancer aggressiveness. The subgroup analyses revealed that the H63D polymorphism promoted the malignancy of pancreatic and liver cancers, and significantly increased the risk of incidence of aggressive cancer in the Asian and African subgroups. However, this polymorphism had no significant influence on the development of gynecological malignancy.

Iron is an important participant of energy metabolism in the human body, and abnormalities in iron metabolism are associated with carcinogenesis, because of the oxidative stress generated in cells and tissues by the extra iron stores. Since estrogen-dependent cancers are related to endogenous oxidative stress produced in target tissues by estrogen metabolites, HFE might affect the incidence of estrogen-dependent cancers. So far, numerous studies have reported the relationship between H63D polymorphism and estrogen-dependent cancers, such as breast cancer, ovarian cancer, and endometrial cancer. However, the published data is inconsistent and

controversial. Therefore, we attempted to verify the importance of H63D polymorphism in cancer development, by analyzing the association between gynecological malignant tumor (GMT, including ovarian cancer and endometrial cancer) risk and H63D polymorphism. Unfortunately, we observed no obvious associations between the H63D variant and GMT, excluding a few cases in the Asian subgroup (which cannot be classified as a race difference). Therefore, further studies must include subjects with a wide range of ethnicities; in addition, multicenter studies and those with a larger sample size must be conducted in the future.

In contrast to the results obtained for gynecological cancers, the results of our meta-analysis revealed the H63D variant is strongly correlated with aggressive pancreatic cancer, which is the fourth leading cause of cancer-related deaths in the United States with a 5-year survival rate < 5% (and a poorly-understood etiology). Diabetes is believed to be an independent risk factor for pancreatic cancer; in addition, pancreatic cancer is known to result in diabetic symptoms through the destruction of pancreatic parenchyma. In addition, a recent meta-analysis revealed an association between the H63D variant and a moderately elevated risk of type 2 diabetes mellitus (Ying et al., 2012). Taken together, these findings suggest that the H63D variant-mediated abnormality in iron metabolism plays a causal role in the development of diabetes and pancreatic cancer.

Previous studies have reported that the H63D polymorphism plays an important role in the occurrence and progress of hepatocellular carcinoma. Moreover, hepatitis, cirrhosis, and liver cancer comprise a trilogy of hepatocellular carcinoma progression. Therefore, we theorized that hepatitis and cirrhosis patients present the H63D mutation. In order to confirm this hypothesis, the meta-analysis was stratified based on the physical condition of controls (healthy and liver disease groups). The results suggested that the association between the H63D variant and liver cancer was significantly high in the groups with healthy controls, compared to the groups with hepatitis or liver cirrhosis controls. One potential explanation for this may be that the H63D variant plays a role during the early stages of hepatocarcinogenesis, which would provide the common genetic basis required for hepatitis, liver cirrhosis, and liver cancer.

However, there are some limitations to this study. The overall outcomes were based on individual unadjusted ORs; a more precise evaluation should be adjusted by other potentially suspected factors (including age, sex, family history, environmental factors, cancer stage, and lifestyle), if enough information is available. In addition, some studies have indicated that the interaction between genetic and environmental factors affects cancer development; this was not included in this study. Moreover, the significant association was found to be dependent on the genotypes. However, the source of heterogeneity was not examined in this study. Finally, some of the included studies included P values of HWE < 0.05; this may lead to increased risk of bias.

Despite the limitations, this meta-analysis has certain advantages. Substantial case numbers and qualities of case-control studies significantly increased the statistical power in order to improve the validity of analysis. Importantly, no obvious publication bias was detected, indicating that the results of this study are unbiased and reliable.

In summary, this study demonstrates for the first time that the *HFE* H63D polymorphism increases the aggressiveness of hepatocellular carcinoma and pancreatic cancer, although no such effect was observed in gynecological malignant tumors, breast cancer, or colorectal cancer. Further multicenter studies, including a larger sample size, and multiple genetic and environmental factors, must be conducted in the future to verify our results. These studies could lead to a better and more comprehensive understanding of the role of H63D polymorphisms in cancer development.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research partly supported by grants from the National Natural Science Foundation of China (Grant #81272469), the National “973” Basic Research Program of China (Grant # 2013CB911300), and the Clinical Special Project for the Natural Science Foundation of Jiangsu Province (Grant #BL2012016), and a grant provided by the Nanjing 12th Five-Year key Scientific Project of Medicine to Dr. Jinfei Chen.

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