Review Article
Association between H63D polymorphism and alcoholic liver disease risk: a meta-analysis

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Abstract: Objectives: Gene plays an important role in alcoholic liver disease (ALD). The H63D polymorphism (rs1799945, C>G) of hemochromatosis (HFE) gene has been found to be related to alcoholic liver disease in various studies. To classify the association between H63D polymorphism and alcoholic liver disease susceptibility, we performed a meta-analysis. Methods: We retrieved published studies on the association between H63D and ALD by electronic database (Embase, PubMed, Cochrane and Web of Science). Related data was extracted. Pooled odds ratios (ORs) and 95% confidence intervals (CIs) were performed with fixed effect model or random effect model. Sensitivity analysis and Publication bias have also been presented. Results: Nine eligible studies were included, with a total of 2720 controls and 1200 cases. The pooled results showed that a significantly increased risk of ALD susceptibility was observed in homozygote model (GG versus CC: OR=2.28, 95% CI 1.39-3.75, I^2=0%, P_H=0.999) and recessive model (GG versus GC+CC: OR=2.22, 95% CI 1.35-3.65, I^2=0%, P_H=0.999). Ethnic subgroup analysis showed similar results in Caucasian: homozygote model (GG versus CC: OR=2.28, 95% CI 1.39-3.75, I^2=0%, P_H=0.999), recessive model (GG versus GC+CC: OR=2.22, 95% CI 1.35-3.65, I^2=0%, P_H=0.999). In the subgroup analysis by genotyping method and quality, the effects remained in high quality studies and PCR-RFLP (restriction fragment length polymorphism). Conclusions: This meta-analysis suggested that H63D polymorphism (rs1799945) is associated with ALD susceptibility, especially for GG genotype in Caucasian. H63D polymorphism of HFE gene may be a potential target in gene therapy for alcoholic liver disease patients.

Keywords: Alcoholic, liver diseases, membrane proteins, polymorphism, meta-analysis

Introduction

Alcoholic liver disease (ALD) seriously jeopardized the health of human beings, which involves in various hepatic lesions. Although the exact mechanisms of alcoholic liver disease are not clear, several studies have showed that iron may be the core of ALD [1-4]. More than half of ALD patients with advanced cirrhosis [5] and approximately 33% of ALD patients presented with excessive liver iron stores [6]. Similarly, iron plays a central role in oxidative stress which may precede the development of ALD [7]. Due to the vital role of iron in ALD, elevation of total body iron stores and iron overload might be one of the risks of ALD.

Hereditary hemochromatosis (HH) is an autosomal recessive disease of iron metabolic disorder, leading to an increased iron absorption and excessive iron accumulation [8]. A study has reported that patients with hemochromatosis had a 9-fold risk to develop cirrhosis when they uptook more than 60 g alcohol daily [9] which approved the association between HH and ALD. H63D polymorphism in hemochromatosis (HFE) gene is prevalent in patients with hereditary hemochromatosis by TfR [10] which may affect iron level in the body. Furthermore, some studies have proved that there is a positive association between HFE mutations and risk of ALD [11, 12]. It's traditionally considered that excessive alcohol ingestion is the main reason to

Table 1. Quality evaluation tabulation

Criteria	Score
Source of control	
Population-based.	3
Hospital-based.	2
Blood donors or volunteers.	1
No described.	0
Source of cases	
ALD diagnosed according to acknowledged criteria.	1
Mentioned the diagnosed criteria but no specially described.	0
Hardy-Weinberg equilibrium in controls	
Hardy-Weinberg equilibrium.	1
Hardy-Weinberg disequilibrium.	0
Case-control match	
Gender and age matching	1
Gender and age no matching	0
Sample size	
>300	2
200-300	1
<200	0
Genotyping methods	
Detecting samples by different methods	2
Detecting samples by the same method	1
No describing the genotyping methods	0

result in ALD, however, only about 20% of subjects with heavy alcohol consumption will develop alcoholic liver disease [13]. It suggests that gene has a significant contact with alcoholic liver disease. Thus, H63D polymorphism may have a positive relationship with ALD.

Notwithstanding, many studies have represented the connection between H63D and ALD risk [11, 12, 14-20], the sample size in every study is limited and results were inconsistent [11, 12, 15-17]. Thus, we performed a meta-analysis to confirm the association between H63D and risk of ALD.

Materials and methods

Search strategy

Two investigators independently performed a systematic search using PubMed, Cochrane, Excerpta Medica Database (Embase) and Web of Science with the last search updated on August 25, 2016. The following search terms were combined: "(SNP or SNPs or single nucleotide polymorphism or polymorphism or genetic polymorphism or mutation or variant or varia-

tion)" and "(alcoholic liver disease or alcoholic liver cirrhosis or alcoholic hepatitis or alcoholic liver fibrosis)" and "(HFE or H63D or His63Asp or rs1799945)". Language and publication years are not restrictive in our search. Finally, 119 articles were retrieved using the aforementioned terms.

Inclusion and exclusion criteria

Studies in the meta-analysis must be conformed to the following inclusion criteria: (i) ALD as the outcome of study; (ii) Assessed the association between ALD and HFE rs1799945; (iii) Presenting sufficient genotype data of cases and controls with risk of ALD to calculate odds rations (ORs) and 95% confidence interval (CIs); (iv) Case-control design for human; (v) Only full-text manuscripts were included. Exclusion criteria included: (i) Deficient genotype frequency; (ii) Duplicate literatures; (iii) Letter, comment, review; (iv) Evaluated other HFE SNPs, no rs1799945; (v) Case-only study; (vi) No human study. Two investigators separately selected the potential literatures according to these criteria. When divergences appear, the third investigator makes the final decision.

Data extraction

Two investigators independently extracted information from all eligible literatures. Discrepancies were verdict by a third investigator until all investigators are unanimous. The following data was collected: name of first author, ethnicity, Hardy-Winberg equilibrium, sample sizes, Genotyping method, Histological types of ALD, genotype frequency in cases and controls and the quality of studies. Ethnicity was classified as Asian and Caucasian. We will send a request to corresponding author for additional data when primary data cannot be obtained from relevant articles.

Quality score assessment

The qualities of the included literatures were accessed by two authors respectively accord-

Figure 1. Flow chart of study selection.
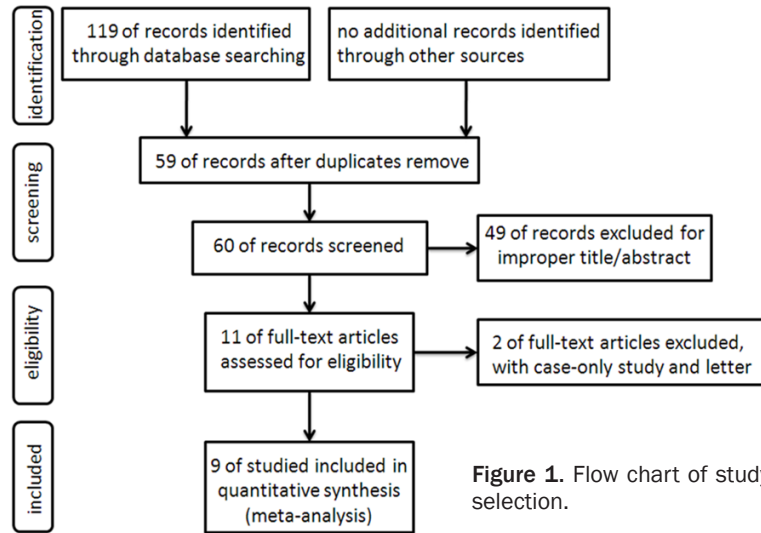
effect model (Mantel-Haenszel method) to evaluate the summary OR and 95% CI, if not, the random effect model (the DerSimonian and Laird method) was performed to assess the summary OR and 95% CI. Sensitivity analysis was performed by examining the effect of omitting individual studies. Begg's funnel plot and the Egger' test were performed to check the publication bias ($P < 0.05$ was suggested that the consequence was significant). STATA software (version 12.0; StataCorp, College Station, Texas USA) was used to perform all the tests with two-sided *P*-values in our meta-analysis.

ing to the predetermined criteria (**Table 1**) which was adjusted and revised from previous articles [7, 21] and the Newcastle-Ottawa Scale (NOS). The adjusted criteria cover the items of source of control, source of cases, case-control match, sample size, genotyping methods and the Hardy-Weinberg equilibrium in controls. Two authors respectively grade the included studies and any divergence was determined by the third author. Scores ranged from zero to nine. A study quality score ≥6 was defined as a "High quality", while a study quality score <6 was defined as a "Low quality" [22].

Statistical methods

The meta-analysis was performed according to the PRISMA checklist and followed the guideline [23]. The control in every included study was assessed the Hardy-Weinberg equilibrium (HWE) by Chi-square test and it was considered a Hardy-Weinberg disequilibrium when the P<0.05. OR and 95% CIs were calculated to evaluate the strength of the association between H63D and the susceptibility to ALD. The pooled ORs were used to assess allelic comparison (G versus C), heterozygote model (CG versus CC), homozygote model (GG versus CC), dominant model (GG+GC versus CC), recessive model (GG versus GC+CC), respectively. Heterogeneity was evaluated by Q statistic (significance level of P<0.1) and I² statistic (greater than 50% as evidence of significant inconsistency) [24]. When the heterogeneity was not significant we carried out the fixed

Results

Characteristics of studies

A total of 119 studies were acquired from PubMed, Cochrane, Embase and and Web of Science databases. The flow chart in **Figure 1** showed the literature screening process. 108 articles were excluded, of which 59 were duplicate ones and 49 with no relation to this topic. The remained 11 articles were full-text. Then 2 studies were excluded, among which, one was letter [25] and the other was not a case-control study [26]. Eventually, 9 eligible case-control studies [11, 12, 14-20], conforming to the inclusion criteria, were included in our meta-analysis.

Nine independent studies were included in our meta-analysis (1200 cases and 2720 controls) [11, 12, 14-20]. The characteristics of each study were showed in the **Table 2**. Only one study was based on Asian population [20] while other studies were based on Caucasian population [11, 12, 14-19]. The consequences of Hardy-Weinberg equilibrium test for the distribution of the genotype in control population are shown in **Table 2**. The controls in all studies meet the HWE. The quality scores for all studies were ranged from 4 to 8, among which 56% (5 of 9) studies were fallen into high quality subgroup (≥6).

Table 2. Characteristics of the studies included in the meta-analysis

First author	Genotyping method	Ethnicity	HT of ALD	Case			Control			HWE p-value	Quality
				CC	GC	GG	CC	GC	GG		
Ropero [11]	PCR-RFLP	Caucasian	ALD	70	46	9	124	52	5	0.871	8
Dhillon [14]	PCR-RFLP	Caucasian	ALD	19	3	0	88	11	1	0.342	5
Grove [15]	PCR-RFLP	Caucasian	ALC	192	58	7	82	34	1	0.209	7
Raszeja [16]	PCR-RFLP	Caucasian	ALD	86	27	6	1077	401	38	0.926	7
Gleeson [17]	Non-RFLP	Caucasian	ALD	182	68	4	90	39	1	0.140	8
Costa [18]	Non-RFLP	Caucasian	ALD	41	20	2	37	14	1	0.806	5
Machado [12]	PCR-RFLP	Caucasian	ALD	42	33	3	54	20	2	0.928	5
Dostalikova [19]	PCR-RFLP	Caucasian	ALD	154	56	8	334	139	8	0.130	8
Sohda [20]	Non-RFLP	Asian	ALD	62	2	0	30	1	0	0.927	4

ALD: alcoholic liver disease; ALC: alcohol-related liver cirrhosis; HT: Histological types.
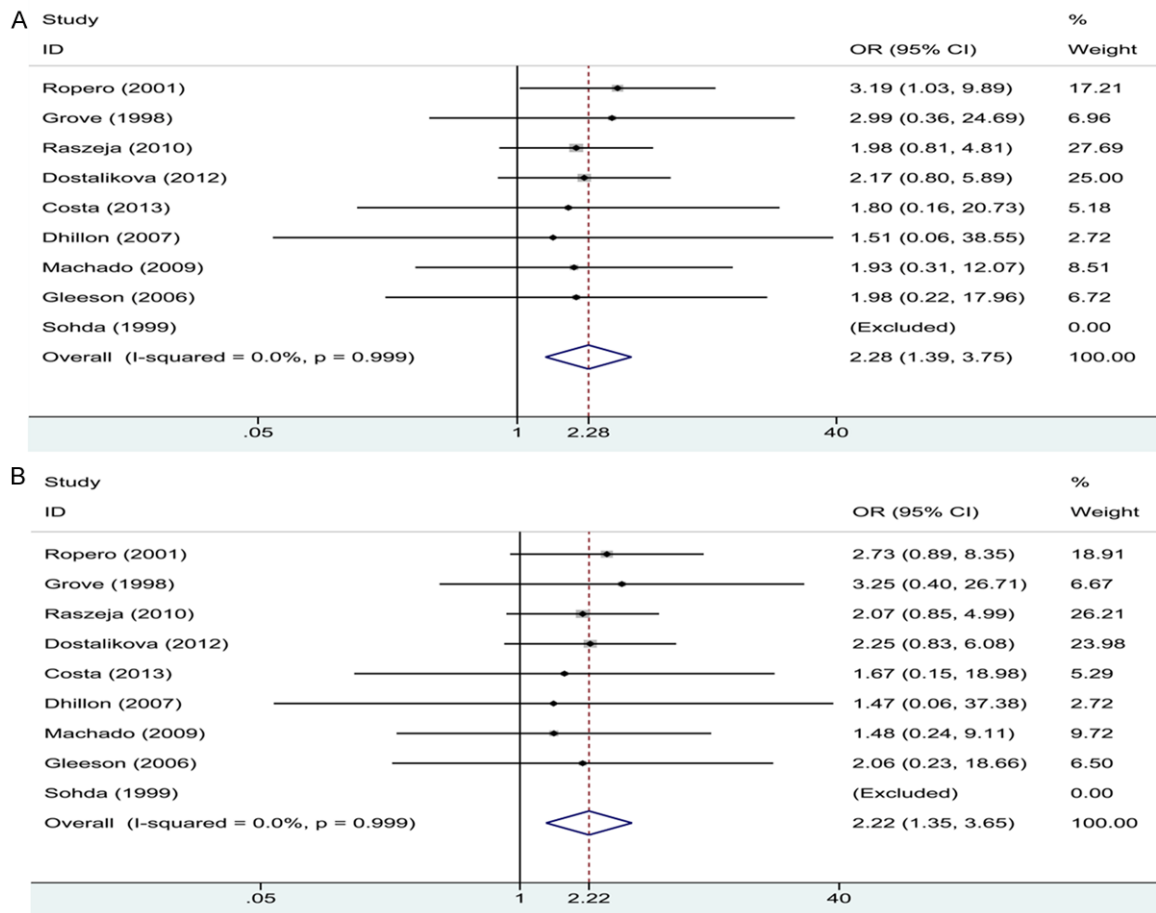

Figure 2. A: Forest plot about the homozygote model of H63D for overall comparison (GG vs CC), fixed effect model; B: Forest plot about the recessive model of H63D for overall comparison (GG vs GC+CC), fixed effect model. The size of the black squares represents the weight of the study in the meta-analysis. The rhombus represents the combined OR.

Meta-analysis results

The pooled result showed that a significantly increased risk of ALD susceptibility was observed in homozygote model (GG versus CC: OR=2.28, 95% CI 1.39-3.75, I^2=0%, P_H=0.999) and recessive model (GG versus GC+CC: OR= 2.22, 95% CI 1.35-3.65, I^2=0%, P_H=0.999) (**Figure 2**). No significant association between ALD susceptibility and H63D polymorphism
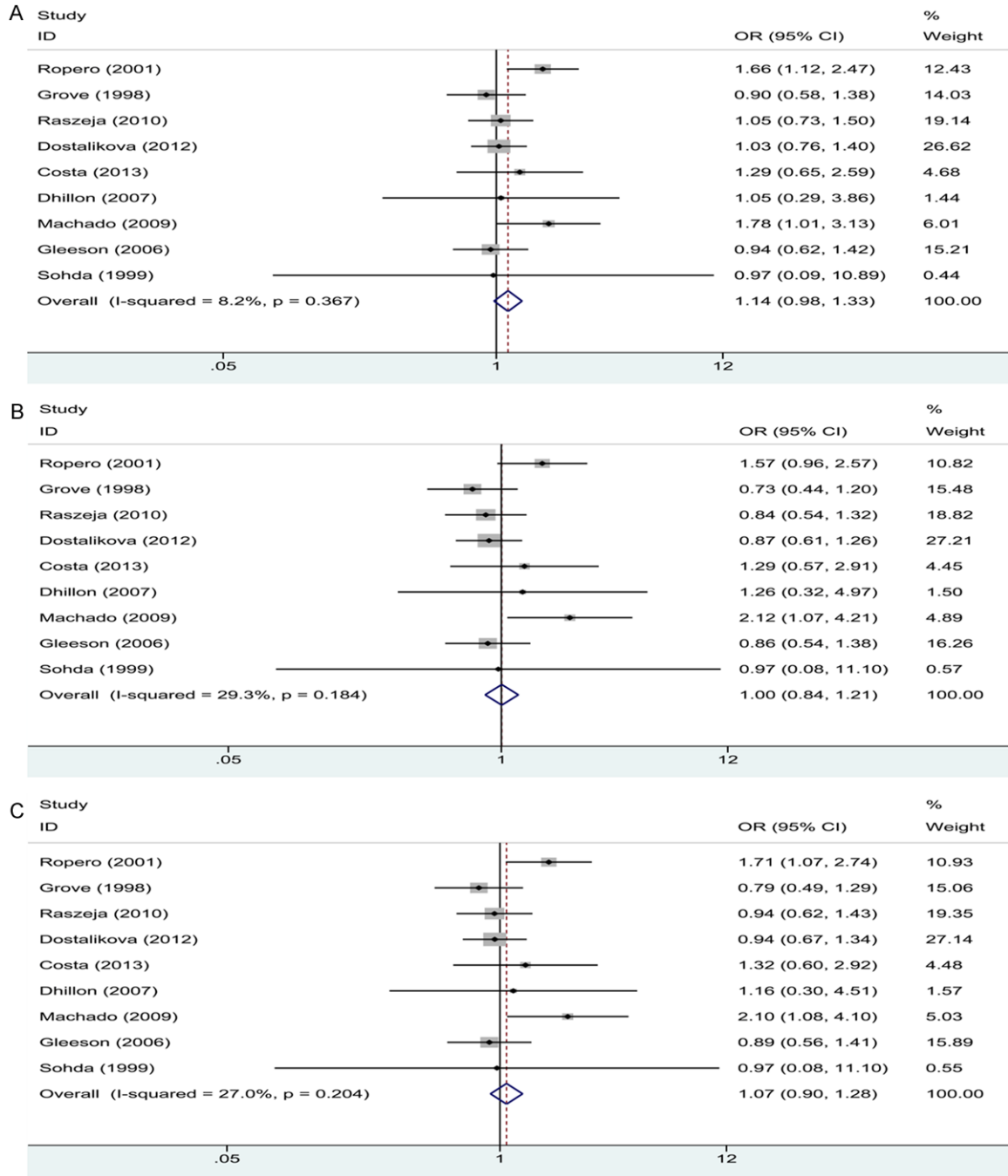
Figure 3. A: Forest plot about the allelic comparison of H63D for overall comparison (G versus C), fixed effect model; B: Forest plot about the heterozygote model for overall comparison (GC versus CC), fixed effect model; C: Forest plot about the dominant model of H63D for overall comparison (GC+GG versus CC), fixed effect model. The size of the black square represents the weight of the study in the meta-analysis. The rhombus represents the combined OR.

was observed in dominant model (GC+GG versus CC: OR=1.07, 95% CI 0.90-1.28, I^2=27%, P_H=0.204), allelic comparison (G versus C: OR= 1.14, 95% CI 0.98-1.33, I^2=8.2%, P_H=0.367) and heterozygote model (GC versus CC: OR= 1.00 95% CI 0.84-1.21, I^2=29.3%, P_H=0.184) (**Figure 3**).

Subgroup analysis

Subgroup analysis based on ethnicity showed the same effects in Caucasian. A significant risk of ALD susceptibility in homozygote model (GG versus CC: OR=2.28, 95% CI 1.39-3.75, I^2=0%, P_H=0.999) and recessive model (GG ver-

Table 3. Summary of polled ORs in the meta-analysis

Subgroup		No. of Studies	OR (95% CI)	I^2	P_H
Overall	G vs C	9	1.14 (0.98-1.33)	8.2%	0.367
	GC vs CC	9	1.00 (0.84-1.21)	29.3%	0.184
	GG vs CC	8	2.28 (1.39-3.75)	0%	0.999
	GC+GG vs CC	9	1.07 (0.90-1.28)	27%	0.204
	GG vs GC+CC	8	2.22 (1.35-3.65)	0%	0.999
Caucasian	G vs C	8	1.14 (0.98-1.33)	19.6%	0.275
	GC vs CC	8	1.00 (0.84-1.21)	38.2%	0.125
	GG vs CC	8	2.28 (1.39-3.75)	0%	0.999
	GC+GG vs CC	8	1.08 (0.90-1.28)	36.1%	0.141
	GG vs GC+CC	8	2.22 (1.35-3.65)	0%	0.999
High quality	G vs C	5	1.09 (0.92-1.28)	32.1%	0.208
	GC vs CC	5	0.92 (0.76-1.13)	28.9%	0.229
	GG vs CC	5	2.37 (1.39-4.04)	0%	0.971
	GC+GG vs CC	5	1.00 (0.83-1.21)	36%	0.181
	GG vs GC+CC	5	2.37 (1.39-4.04)	0%	0.992
Low quality	G vs C	4	1.49 (0.99-2.23)	0%	0.819
	GC vs CC	4	1.63 (1.01-2.62)	0%	0.755
	GG vs CC	3	1.82 (0.48-6.90)	0%	0.992
	GC+GG vs CC	3	1.62 (1.02-2.58)	0%	0.742
	GG vs GC+CC	3	1.54 (0.41-5.79)	0%	0.997
PCR-RFLP	G vs C	6	1.17 (0.98-1.38)	34.8%	0.175
	GC vs CC	6	1.07 (0.78-1.48)	52.5%	0.061
	GG vs CC	6	2.28 (1.35-3.58)	0%	0.988
	GC+GG vs CC	6	1.14 (0.85-1.55)	50%	0.075
	GG vs GC+CC	6	2.23 (1.33-3.74)	0%	0.991
Non-RFLP	G vs C	3	1.02 (0.72-1.45)	0%	0.739
	GC vs CC	3	0.95 (0.64-1.42)	0%	0.703
	GG vs CC	3	1.90 (0.37-9.75)	0%	0.956
	GC+GG vs CC	3	0.99 (0.66-1.46)	0%	0.698
	GG vs GC+CC	3	1.88 (0.37-9.59)	0%	0.900

P_H is p-value of Q test for heterogeneity; OR: odds ratio; vs: versus.

the subgroup of high quality studies, increased ALD risk was observed in homozygote model (GG versus CC: OR=2.37, 95% CI 1.39-4.04, I^2=0%, P_H=0.971) and recessive model (GG versus GC+CC: OR=2.37, 95% CI 1.39-4.04, I^2=0%, P_H=0.992). No significant association was found in allelic comparison (G versus C: OR=1.09, 95% CI 0.92-1.28, I^2=32.1%, P_H=0.208), dominant model (GC+GG versus CC: OR=1.00, 95% CI 0.83-1.21, I^2=36%, P_H=0.181) and heterozygote model (GC versus CC: OR=0.92, 95% CI 0.76-1.13, I^2=28.9%, P_H=0.229). As for the subgroup of low quality studies, different results are presented. No association between H63D polymorphism and the risk of ALD was observed in allelic comparison (G versus C: OR= 1.49, 95% CI 0.99-2.23, I^2=0%, P_H=0.819), homozygote model (GG versus CC: OR=1.82, 95% CI 0.48-6.90, I^2=0%, P_H=0.992) and recessive model (GG versus GC+CC: OR=1.54, 95% CI 0.41-5.79, I^2=0%, P_H=0.997). Increased ALD risk was observed in dominant model (GC+GG versus CC: OR=1.62, 95% CI 1.02-2.58, I^2=0%, P_H=0.742) and heterozygote model (GC versus CC: OR=1.63, 95% CI 1.01-2.62, I^2=0%, P_H=0.755) (**Table 3**).

sus GC+CC: OR=2.22, 95% CI 1.35-3.65, I^2=0%, P_H=0.999). Nevertheless, no prominent association was found in dominant model (GC+GG versus CC: OR=1.08, 95% CI 0.90-1.28, I^2=36.1%, P_H=0.141), allelic comparison (G versus C: OR=1.14, 95% CI 0.98-1.33, I^2=19.6%, P_H=0.275) and heterozygote model (GC versus CC: OR=1.00, 95% CI 0.84-1.21, I^2=19.6%, P_H=0.125) (**Table 3**). Since only one study was based on Asian race, the subgroup analysis was not carried out in Asian population.

Then we performed another subgroup analysis to investigate the effect of quality of studies. In

When stratifying by Genotyping method, the similar effects remained in PCR-RFLP subgroup (G versus C: OR=1.17, 95% CI 0.98-1.38, I^2= 34.8%, P_H=0.175; GG versus CC: OR=2.28, 95% CI 1.35-3.58, I^2=0%, P_H=0.988; GG versus GC+CC: OR=2.23, 95% CI 1.33-3.74, I^2=0%, P_H=0.991; GC+GG versus CC: OR=1.08, 95% CI 0.90-1.28, I^2=50%, P_H=0.075; GC versus CC: OR=1.07, 95% CI 0.78-1.48, I^2=52.5%, P_H= 0.061). However, no significant effect was found in Non-RFLP subgroup (**Table 3**).

Sensitivity analysis

We detected the influence of individual study on the pooled ORs for H63D by sensitivity anal-
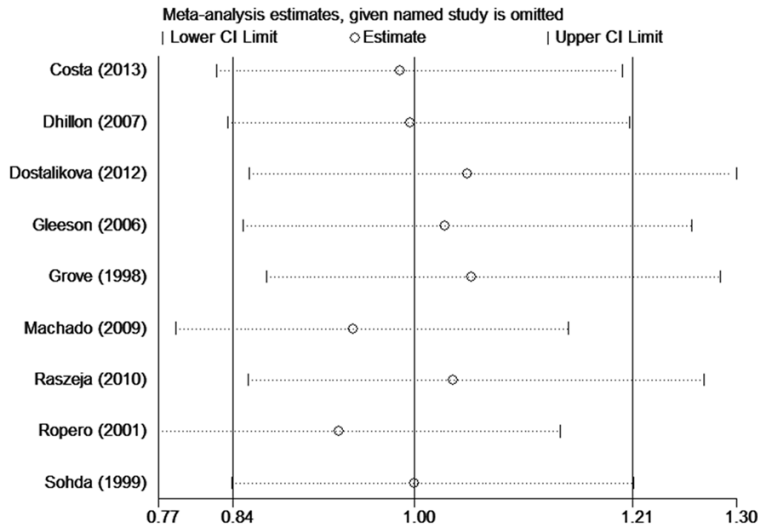
Figure 4. Sensitivity analysis for H63D polymorphism (rs1799945) in heterozygote model (GC versus CC).
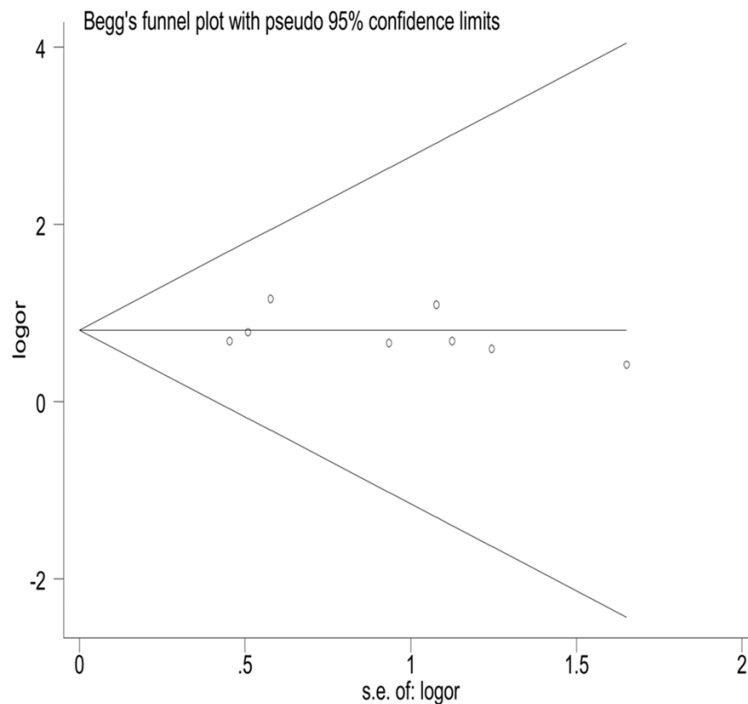

Figure 5. Begg's funnel plot for publication bias analysis for H63D (rs1799945) polymorphism (GG versus CC).

ysis in each genetic model. Consistently, the pooled estimate remained no significant change when any single study was omitted at a time from each meta-analysis. The sensitivity analysis in heterozygote model (GC versus CC) was showed in **Figure 4**.

Publication bias

We evaluated the publication bias of the literatures by Funnel plot and Egger's test. The result showed that no significant publication bias in all genetic models. **Figure 5** showed the Begg's funnel plot in homozygote model (GG versus CC, P=0.536). Information concerning the Egger's funnel plot is listed in **Table 4**.

Discussion

In our meta-analysis, nine eligible studies [11, 12, 14-20], including 1200 cases and 2720 controls, were identified and analyzed. The pooled results showed that H63D polymorphism (rs1799945) is significantly associated with ALD susceptibility in homozygote model (GG versus CC: OR=2.28, 95% CI 1.39-3.75, I^2=0%, P_H=0.999) and recessive model (GG versus GC+CC: OR=2.22, 95% CI 1.35-3.65, I^2=0%, P_H=0.999). The similar consequences were observed in Caucasian (GG versus CC: OR=2.28, 95% CI 1.39-3.75, I^2=0%, P_H=0.999; GG versus GC+CC: OR=2.22, 95% CI 1.35-3.65, I^2=0%, P_H=0.999) and the subgroups of high quality studies (GG versus CC: OR=2.37, 95% CI 1.39-4.04, I^2=0%, P_H=0.971; GG versus GC+CC: OR=2.22, 95% CI 1.35-3.65, I^2=0%, P_H=0.999) and PCR-RFLP (GG versus CC: OR=2.28, 95% CI 1.35-3.58, I^2=0%, P_H=0.988; GG versus GC+CC: OR=2.23, 95% CI 1.33-3.74, I^2=0%, P_H=0.991). The sensitivity analysis and publication bias all supported our results.

In subgroup analysis by quality of studies and genotyping method, the subgroups of high quality studies and PCR-RFLP remained the same

Table 4. Egger's funnel plot

H63D	95% CI	$P_{Egger'\ test}$
G versus C	-1.76-2.45	0.710
GC versus CC	-1.38-3.36	0.358
GG versus CC	-0.82-0.57	0.687
GC+GG versus CC	-5.83-1.88	0.265
GG versus GC+CC	-0.84-0.44	0.470

$P_{Egger'\ test}$ is the *p*-value of Egger's test.

effects in homozygote model and recessive model. However, as for the low quality studies and Non-RFLP subgroups, different results were observed in all genetic models. The differences may be due to the relatively smaller sample size in these low quality studies and Non-RFLP subgroup which may cover the potential association.

Excessive iron deposition in individual with chronic alcohol consumption has been observed for a long time [27]. Iron and alcohol can both lead to liver injury through their combined effects on damaged hepatocytes, kupffer cell, hepatic stellate cells, and extracellular matrix. Several studies have demonstrated that ALD was usually related to hepatic iron overload [3, 4]. Brittenham [28] also showed that iron chelation may offer new approaches to the treatment and prevention of alcoholic liver disease. Moreover, iron appears to be as a vital prognostic factor for overall survival in patients with alcoholic liver disease [29]. The iron metabolism is normally controlled by the HFE (haemochromatosis) protein. H63D is a C-to-G transversion at nucleotide 187 of the HFE gene and is widely distributed in different populations, Especially in Caucasian. The H63D mutation on the HFE gene can weaken the ability of the HFE protein to bind to transferrin, hence contributing to iron overload. The above results reveal a potential association among ALD, iron and H63D.

In our meta-analysis, the H63D heterozygote was not significantly associated with ALD, which was coincident with previous studies [17, 20]. But though the GC genotype has been appreciated for a long time in various disease [30, 31] and illustrated the influence in iron metabolism [32], Maybe the mild iron overload in GC carrier should not promotes the development and progression of ALD. However, Ropero [11] et al and Machado et al [12] demonstrated

that the G allele of H63D (rs1799945) may increase the risk of developing advanced liver alcoholic disease and alcoholics with liver disease had increased frequency of H63D HFE mutation. Furthermore, Some studies have also found that H63D homozygosis could lead to greater iron overload [33, 34]. All above studies support our meta-analysis that H63D homozygote significantly increases ALD risk. Thus, the individual with GG genotype might increase their susceptibility to ALD as the more severe iron overload. Our results are similar to previous liver disease researches, such as HCC (Hepatocellular Carcinoma) [35, 36], NAFLD (Non Alcoholic Fatty Liver Disease) [37] and liver fibrosis [38]. All these results strongly implicate that H63D polymorphism is a common risk factor for chronic liver disease, including ALD and emphasize the essential effect of H63D in various types of liver disease.

Compared with traditional research, our meta-analysis has several strengths. To begin with, this is the first meta-analysis focused on the association between H63D polymorphism and susceptibility to alcoholic liver disease; moreover, we utilize a much larger total sample size to evaluate its effect in our meta-analysis. Thus, our results are more reliable. Additionally, we performed a sensitivity analysis to evaluate the effect of each study on the overall assessment, which suggested that our result was stable. Thus, we confirm that our results are more reliable than the previous studies' consequences.

Our meta-analysis also has some limitations. Firstly, several factors were not clear in included studies, such as living habit, occupational history and environment; thus, we cannot properly assess the association between H63D and ALD according to stratification of these factors. Secondly, although we have performed a systematic search to access to relevant literatures as much as possible, it is possible to miss some studies. Finally, only one study involved in Asian was included and its quality is low, thus, we cannot completely analysis the subgroup of ethnicity.

In conclusion, The H63D polymorphism (rs1799945) may be association with the risk of ALD, especially for the GG genotype in Caucasian. H63D polymorphism may be a potential therapeutic target for ALD patients.

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Disclosure of conflict of interest

None.

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