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BRIEF ARTICLE

Relationship between *HLA-DR* gene polymorphisms and outcomes of hepatitis B viral infections: A meta-analysis

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Abstract

AIM: To assess the rigorous relationship between human leukocyte antigens (HLA)-DR alleles and outcomes of hepatitis B virus (HBV) infections by means of meta-analysis.

METHODS: Medline/PubMed, EMBASE, CNKI and VIP were searched to identify relevant studies. Study quality was evaluated using the Newcastle-Ottawa Scale. Odds ratios (OR) and 95% confidence interval (95% CI) were pooled using Stata 11.0. Subgroup analyses were performed by ethnicity. Heterogeneity and publication bias analyses were performed to validate the credibility.

RESULTS: A total of 2609 patients with chronic hepatitis B and 2606 controls spontaneously recovering from prior HBV infection were included. Meta-analysis showed that HLA-DR*04 (OR = 0.72, 95% CI: 0.60-0.85) and DR*13 (OR = 0.27, 95% CI: 0.19-0.37) alleles were significantly associated with HBV clearance while patients carrying HLA-DR*03 (OR = 1.47, 95% CI: 1.16-1.87) or DR*07 (OR = 1.59, 95% CI: 1.24-2.03) alleles had a significantly increased risk of chronic HBV persistence. For the HLA-DR*01 polymorphism, a significantly association with HBV clearance was found in Chinese Han group (OR = 0.48, 95% CI: 0.26-0.86), but not found in other ethnic groups (P = 0.191). For other polymorphisms, no association with the HBV infection outcome was found.

CONCLUSION: HLA-DR*04 and DR*13 alleles may be the protective factors for HBV clearance and HLA-DR*03, and DR*07 alleles may be the risk factors for HBV persistence.

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Key words: Hepatitis B virus; Human leukocyte antigens; Meta-analysis; Polymorphism

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INTRODUCTION

Hepatitis B virus (HBV) infection is a serious public health problem affecting more than 400 million people worldwide. The clinical features of HBV infection vary from clearance of the virus to fulminate hepatitis. Although some HBV carriers spontaneously eliminate the virus, 2%-10% of the individuals with chronic hepatitis B

(CHB) are estimated to develop liver cirrhosis, and a subset of these individuals eventually suffer from liver failure or hepatocellular carcinoma[1]. A complex combination of environmental, pathogenic and host genetic factors plays a role in determining both susceptibility to HBV and the course of the infection[2]. Several epidemiological factors, such as age at infection, sex, chronic alcohol abuse and co-infection with other hepatitis viruses, were suspected to affect viral persistence. In addition, host immunological factors and genetic background were also considered to influence the susceptibility to and outcome of persistent HBV infection[3]. Family studies in China provide some evidences that the host genetic factors affect viral persistence, as concordance rates of HBeAg persistence were higher in identical twins than in non-identical twins[4].

Although genetic variants in *IFNG*[5], *TNF*[6], *VDR*[7], *MBP*[8], *ESR1*[9], *CXCL10*[10], *IL-10*[11] and several human leukocyte antigen (HLA) loci[12,13] have been shown to associate with CHB, none of the associations has been proven to be conclusive. The mechanism of susceptibility to chronic persistent HBV infection is not well clarified. Since the outcome of HBV infection mainly depends on the host immune response, and HLA, an integral component of the immune response, plays an important role in immunological reaction to HBV infection[14], the highly polymorphic *HLA* gene has been considered as an appropriated biological candidate susceptibility gene that is associated with the development and the progression of chronic HBV infection. Indeed, previous studies have highlighted that *HLA-DR* polymorphisms influence individual immune responses, thus affecting the outcome of diseases, and that many different *HLA* alleles play a role in HBV infection[15]; however, this relationship between *HLA-DR* polymorphisms and HBV infection is not universal for all investigated populations. As for Caucasians[16,17] and Koreans[18], *HLA-DRB1*1301-02* has been found to be associated with acute self-limited hepatitis B. For Taiwanese people, *HLA-DRB1*0406* is associated with recovery from HBV infection in the Han Chinese, as is *HLA-DRB1*4001* in indigenous Taiwanese people[19]. Han Chinese with HLA-DR12 (especially one of its alleles, *DRB1*1201*) or *HLA-DRB1*1101/1104* are able to resist HBV infection, while those with *HLA-DR9*, *DQ9*, *HLA-DRB1*0301*, *HLA-DQB1*0301*[20,21], and *DRB1*10* are susceptible to chronic HBV infection[22].

In spite of the pivotal role that the polymorphic HLA antigens play in immune surveillance and immune response and the multitude of studies performed, there is still a lack of conclusive evidence of the association between polymorphisms and the outcomes of HBV infection; the relationship between them is not universal for all the investigated populations. In this meta-analysis, the identification of common *HLA-DR* alleles causing CHB susceptibility was examined through a systematic review of the literature followed by a meta-analysis of all case-control studies. Meta-analysis is a powerful method for quantitatively summarizing the results from different studies. One of the advantages is to enhance the statistical power of outcomes in ethnically- and ancestrally-rich populations and to enlarge the sample sizes, which may reduce the possibility of producing false-positive or false-negative association by random error[23].

MATERIALS AND METHODS

Literature search strategy

A systematic search was conducted by two investigators independently (Yan ZH and Fan Y). All articles were retrieved from four main databases: Medline/PubMed, EMBASE, CNKI (China National Knowledge Infrastructure) and VIP database (Chinese Journal of Science and Technology of VIP). The latest search was updated on April 20, 2011, using the search terms: "hepatitis B" or "HBV", "polymorphism", "human leukocyte antigen" or "*HLA*" or "Major Histocompatibility Complex" or "MHC". The search was not confined to articles written in a certain language, but focused on studies conducted on human subjects. All searched studies were retrieved and the relevant publications in the bibliographies were checked simultaneously. Abstracts and unpublished reports were not considered; only published studies with full-text articles were included. When the same patient population was included in several publications, only the most recent or complete study was used in this meta-analysis. When a study reported results of different subpopulations, we treated each subpopulation as a separate comparison in the meta-analysis. This work has been carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. This study was approved ethically by University Hospital Birmingham Trust (LREC 2002/166). All patients provided informed written consent.

Inclusion and exclusion criteria

The inclusion and exclusion criteria were drawn up on the basis of discussion. The articles to be included in this meta-analysis should meet these inclusion criteria: (1) the full text was available for the published study; (2) the study was designed as an unrelated case-control study; (3) the study provided the number of persistent chronic HBV infection cases and controls; (4) the study provided the number of individual *HLA-DR* genotypes in cases and controls; (5) the study provided enough information for odd ratio (OR) and 95% confidence interval (95% CI) calculation; and (6) the diagnosis for CHB was based on the following criteria: seropositive for HBsAg for at least 6 mo; serum HBV DNA $> 1 \times 10^5$ copies/mL for HBeAg-positive and $> 1 \times 10^4$ copies/mL for HBeAg-negative patients; and persistent or intermittent elevation in alanine aminotransferase (ALT) level, or liver biopsy showing chronic hepatitis with moderate or severe necroinflammation. The criteria for spontaneous recovery from infection were: positive for both anti-HBs and anti-HBc antibodies, negative for HBsAg, normal liver function tests and no history of dominant hepatitis B and HBV vaccination.

The exclusion criteria were: (1) the study was not a case-control study; (2) the control group was only with

healthy patients and without those who recovered from prior HBV infection; (3) the study did not fit the diagnosis criteria; (4) the individuals included in the case-control study had serologic evidence for co-infection with hepatitis C virus, hepatitis D virus, or human immunodeficiency virus (HIV); and (5) the studies were duplicated (only the most recent or complete study was used in this meta-analysis when the same patient population was included in several publications[24]). In addition, interim analyses and comparisons of laboratory methods were all excluded.

Data extraction and validity assessment

In order to retrieve articles as completely and correctly as possible, two investigators extracted data independently using a standardized form and they reached a consensus on all items. Disagreement was resolved by discussion among the whole study groups. For each study, the following data were extracted: name of the first author, year of publication, ethnicity, ethnic origin of the studied population, number of patients with persistent HBV infection (including asymptomatic carriers and patients with chronic liver diseases), number of spontaneously recovered controls, number of individual genotypes, source of controls, genotype frequency of cases and controls, genotyping methods, statistical methods and the results of the study.

The quality of the primary studies was evaluated using the Newcastle-Ottawa Scale (NOS)[25]. This scale judges the study on three broad perspectives: the selection of the study groups; the comparability of the groups; and the ascertainment of either the exposure or outcome of interest for case-control.

Statistical analysis

We compared persistent HBV infection cases with spontaneously recovered controls to assess the relationship between *HLA-DR* polymorphisms and HBV infection clearance. Subgroup analyses were mainly performed by ethnicity. Ethnic groups were mainly defined as Chinese Han and other ethnic group. Statistical analysis was conducted using the Stata 11.0 (StataCorp, College Station, TX, United States). The association between *HLA-DR* polymorphisms and the CHB risk was measured by OR with 95% CI. The pooled OR was determined by the *Z* test and statistical significance was set at $P < 0.05$. All *P* values were two-sided. To ensure the reliability and the accuracy of the results, two investigators uploaded the data into the statistic software programs independently and obtained the same results. In our study, two models of meta-analysis for dichotomous outcomes were constructed: the random-effects model and the fixed-effects model. The random-effects model was constructed using the DerSimonian and Laird's method[26], which assumed that studies were conducted in populations with varying effect sizes and calculated the study weights from both in-study and between-study variances. The fixed-effects model was constructed using the Mantel-Haenszel's method[27], which assumed that studies were conducted in populations with the same effect sizes and made an adjustment to the study weights according to the in-study variances.

To assess the between-study heterogeneity more precisely, both the χ^2-based Cochran's Q-statistic[28] (to test the heterogeneity) and the Higgins (I^2) test[29] (to quantify the proportion of the total variation due to heterogeneity) were calculated. Because of the low power of Cochran's Q statistic, heterogeneity was considered significant at $P_h < 0.10$, and the random-effects model was used to pool the results. When the *P* value of Cochran's Q statistic was greater than 0.10, the fixed-effects model was used to pool the results. Besides, the Galbraith plot was used to spot the outliers as the possible major sources of heterogeneity[30]. To better investigate the possible sources of between-study heterogeneity, meta-regression analysis was also applied to both general analyses and subgroup analyses, when heterogeneity was observed. To confirm the effect of clinical heterogeneity between studies on the conclusions of the meta-analysis, subgroup analysis was conducted based on races. To validate the credibility of outcomes in this meta-analysis, a sensitivity analysis was performed by sequential omission of individual studies or by omitting studies plotted by the Galbraith plot method as the possible major source of heterogeneity. The potential publication bias was estimated by funnel plot, in which the standard error of logOR of each study was plotted against its logOR. An asymmetric plot suggests a possible publication bias. Visual inspection of asymmetry in funnel plots was conducted to assess the potential for publication bias. In addition, Begg's rank correlation method and the Egger weighted regression method were also used to statistically assess the publication bias (publication bias with a $P \leqslant 0.05$ was considered statistically significant)[31].

RESULTS

Characteristics of studies

A flow diagram of the study selection process is shown in Figure 1. We identified a total of 313 potentially relevant articles to our search criteria. After carefully reviewing the titles, abstracts and full text of the literature, we identified 72 articles with analysis on the association between *HLA-DR* polymorphisms and persistent HBV infection. However, according to the exclusion criteria, 54 publications were excluded including 21 articles involving the same study subjects as other included articles, nine papers not about case-control study, 22 publications without spontaneously recovered patients in the control group, and two papers in which the individuals included in the case-control study had serologic evidence for co-infection with HIV. Finally, 18 articles evaluating *HLA-DR* polymorphisms met the inclusion criteria and were chosen for this systematic review and meta-analysis. All the selected studies presented original data on independent samples.

The characteristics of each study are presented in Table 1. These 18 articles were published from 1995

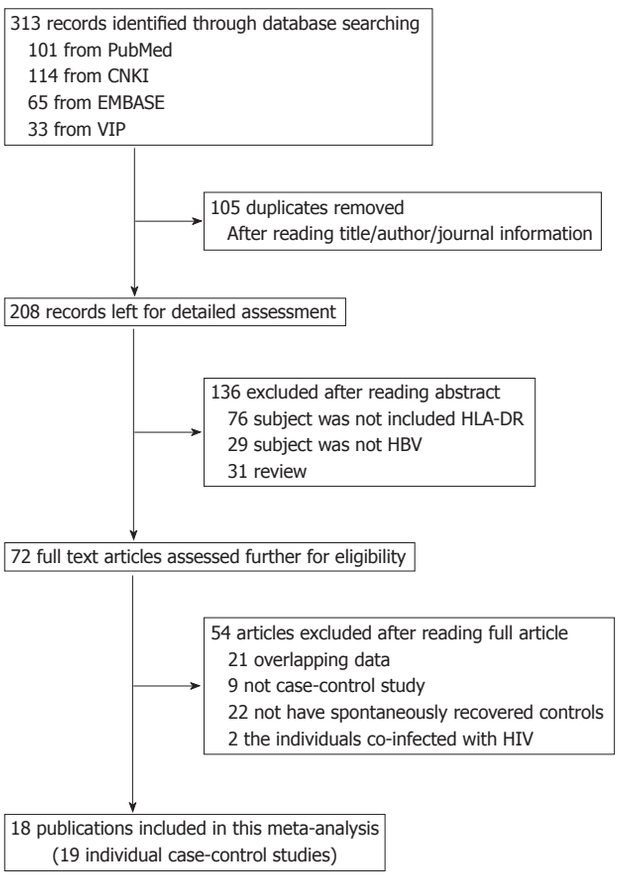


Figure 1 Results of literature search. This figure describes the whole process of searching for articles for inclusion in this systematic review and meta-analysis. HLA: Human leukocyte antigens; HBV: Hepatitis B virus; HIV: Human immunodeficiency virus.

to 2011 with 15 in English[16,18-21,32-41] and three in Chinese[42-44]. Of them, nine studies were on Han Chinese populations[19-21,34-36,42-44] and 10 studies on other race (Gambia[32], Caucasian[16], Korean[18,37,39], Iran[33,40], Thai[38] and Turkish[41]). Besides, one publication contained two individual case-control studies from two different ethnic populations (Chinese Han and Taiwanese aborigines[19]). All studies used the individuals who spontaneous recovered from prior HBV infection as control subjects. The sample size in the individual case-control studies ranged from 60 to 638. A total of 2609 patients with CHB and 606 spontaneously recovered controls prior to HBV infection, were included from 19 studies (cases and controls were counted only once for each study). Genotyping methods used in the studies included PCR-restriction fragment length polymorphism, PCR-sequence specific primer, PCR-sequence specific oligonucleotide probe and sequence-specific oligonucleotide probe hybridization.

The NOS results showed that the median overall score was 7 (range, 5-8), which indicated that the methodological quality was generally good. We defined studies that scored a 7 or above as having high methodological quality, and judged that 2 out of the 19 studies to be of low quality (one study[44] scored 5 and another study[43] scored 6) primarily due to either no description of case selection, no definition of control, a lack of adjusted analysis, or no description of the method of ascertainments for case-controls.

Meta-analysis of relationship between HLA-DR gene polymorphisms and clearance of persistent HBV infection

Table 2 lists the main results of meta-analysis of the relationship between *HLA-DR* polymorphisms and the clearance of persistent HBV infection. We found no evidence of associations between the clearance of persistent HBV infection and *HLA-DR*08, DR*09, DR*10, DR*11, DR*12, DR*14, DR*15, DR*16* alleles (P_{OR} > 0.05). For these negatively associated alleles, the results of subgroup analyses by ethnicity also showed no associations in either Chinese Han group or other ethnic group (P_{OR} > 0.05).

For the *HLA-DR*01* polymorphism, the fixed-effects model was used to pool the meta-analysis result, as the between-study heterogeneity was not significant (I^2 = 0%, P_H = 0.826) when all eligible studies were pooled into meta-analysis. The results of pooling all 13 eligible studies showed that *HLA-DR*01* allele was associated with an increased clearance rate of HBV infection (P_{OR} = 0.016, $OR_{fixed\text{-}effects}$ = 0.69, 95% CI: 0.52-0.93). In the subgroup analyses by ethnicity, the association between the *DR*01* allele and HBV clearance was found in Chinese Han group (P_{OR} = 0.014, $OR_{fixed\text{-}effects}$ = 0.48, 95% CI: 0.26-0.886), but not found in other ethnic groups (P_{OR} = 0.191) (Figure 2A).

For the *HLA-DR*03* polymorphism, the between-study heterogeneity was also not significant when all 12 eligible studies were pooled into meta-analysis (I^2 = 16.0%, P_H = 0.287). The results of the fixed-effects model meta-analysis which pools all studies showed that the *HLA-DR*03* allele was associated with a significantly increased risk of chronic HBV persistence (P_{OR} = 0.002, $OR_{fixed\text{-}effects}$ = 1.47, 95% CI: 1.16-1.87). The subgroup analyses by ethnicity showed that the *DR*03* allele was associated with a significantly increased risk of chronic HBV persistence in both Chinese Han group (P_{OR} = 0.020, $OR_{fixed\text{-}effects}$ = 1.57, 95% CI: 1.07-2.30) and other ethnic groups (P_{OR} = 0.029, $OR_{fixed\text{-}effectss}$ = 1.41, 95% CI: 1.03-1.91) (Figure 2B).

For the *HLA-DR*04* polymorphism, the between-study heterogeneity was also not significant when all 16 studies were pooled into meta-analysis (I^2 = 0%, P_H = 0.757). The results of pooling all studies using the fixed-effects model meta-analysis showed that the *HLA-DR*04* allele was associated with the clearance of HBV infection (P_{OR} = 0.000, $OR_{fixed\text{-}effects}$ = 0.72, 95% CI: 0.60-0.85). The subgroup analyses by ethnicity showed that the *DR*04* allele was associated with an increased clearance rate of HBV infection in both Chinese Han group (P_{OR} = 0.002, $OR_{fixed\text{-}effects}$ = 0.63, 95% CI: 0.48-0.84) and other ethnic groups (P_{OR} = 0.016, $OR_{fixed\text{-}effectss}$ = 0.77, 95% CI: 0.62-0.95) (Figure 2C).

For the *HLA-DR*07* polymorphism, the fixed-effects model was used to pool the meta-analysis result, since the between-study heterogeneity was not significant (I^2 = 14.0%, P_H = 0.304) when all 13 studies were pooled into

Table 1 Main characteristics of case-control studies included in this systematic review and meta-analysis

No.	Study[ref.]	Yr	Language	Ethnicity	Genotyping	Spontaneously recovered control		Chronic hepatitis B case		NOS score
						n	Characteristics	*n*	Characteristics	
1	Thursz *et al*[32]	1995	English	Gambia	PCR-RFLP	413	218 children; 195 adults	225	185 children; 40 adults	7
2	Hohler *et al*[16]	1997	English	Caucasian	PCR-SSP	24	NA	70	Outpatients	7
3	Ahn *et al*[18]	2000	English	Korean	PCR-SSP	243	156 males, mean age: 41 yr	83	53 males, mean age: 35 yr	8
4	Chen *et al*[42]	2002	Chinese	Chinese Han	PCR-SSP	56	43 males, mean age: 47.6 yr	30	22 males, mean age: 38.7 yr	7
5	Akcam *et al*[33]	2002	English	Iran	PCR-SSP	30	20 males, mean age: 35.9 yr	30	7 males, mean age: 31.0 yr	7
6	Jiang *et al*[21]	2003	English	Chinese Han	PCR-SSP	30	24 males, mean age: 33.2 yr	52	43 males, mean age: 33.46 yr	7
7	Meng *et al*[20]	2003	English	Chinese Han	PCR-SSP	56	22 males, mean age: 38.7 yr	30	43 males, mean age: 47.6 yr	7
8	Wu *et al*[19]	2004	English	Chinese Han	SSOPH	324	169 males, mean age: 39.1 yr	98	66 males, mean age: 50.9 yr	8
9	Wu *et al*[19]	2004	English	Taiwanese aborigines	SSOPH	229	90 males, mean age: 52.1 yr	138	60 males, mean age: 45.7 yr	8
10	Lu *et al*[43]	2006	Chinese	Chinese Han	PCR-SSP	148	70 males, mean age: 37.75 yr	417	289 males; 207 CHB, 212ASC ASC	6
11	Zhang *et al*[34]	2006	English	Chinese Han	PCR-SSP	32	20 males, mean age: 38.4 yr	61	40 males, mean age: 38.4 yr	7
14	Yang *et al*[35]	2007	English	Chinese Han	PCR-SSP	108	Age and sex matched	108	90 males, 58 LC, 24 HCC	7
15	Song *et al*[44]	2007	Chinese	Chinese Han	PCR-SSP	102	63 males, mean age: 33.9 yr	276	168 males, 77 LC, 106 ASC	5
14	Zhu *et al*[36]	2007	English	Chinese Han	PCR-SSP	133	63 males, mean age: 37 yr	151	120 males, mean age: 40 yr	7
15	Hwang *et al*[37]	2007	English	Korean	PCR-SSP	438	286 man, mean age: 39 yr	198	148 males, 76 LC, 28 HCC	7
16	Kummee *et al*[38]	2007	English	Thai	PCR-SSP	100	48 males, mean age: 51.0 yr	150	80 males, mean age: 30.9 yr	8
17	Cho *et al*[39]	2008	English	Korean	PCR-SSP	80	60 males, mean age: 47.9 yr	384	283 males, mean age: 41.0 yr	8
18	Remezani *et al*[40]	2008	English	Iran	PCR-SSP	30	17 males, mean age: 32.2 yr	33	20 males, mean age: 38 yr	8
19	Albayrak *et al*[41]	2011	English	Turkish	PCR-SSP	30	15 males, mean age: 33.9 yr	75	48 males, mean age: 33.0 yr	8

NA: Relative data were not available in original studies; PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism; PCR-SSP: Polymerase chain reaction sequence specific primers; PCR-SSO: Polymerase chain reaction sequence specific oligonucleotide probe; SSOPH: Sequence-specific oligonucleotide probe hybridization: CHB: Chronic hepatitis B; LC: Liver cirrhosis; HCC: Hepatocellular carcinoma; ASC: Asymptomatic hepatitis B virus carriers; NOS: Newcastle-Ottawa Scale.

meta-analysis. The results showed that *HLA-DR*07* allele was associated with a significantly increased risk of chronic HBV persistence (P_{OR} = 0.000, $OR_{fixed\text{-}effects}$ = 1.59, 95% CI: 1.24-2.03). In the subgroup analyses by ethnicity, the results showed that the *DR*07* allele was associated with a significantly increased risk of chronic HBV persistence among Chinese Han group (P_{OR} = 0.017, $OR_{fixed\text{-}effects}$ = 1.50, 95% CI: 1.07-2.10) and other ethnic groups (P_{OR} = 0.005, $OR_{fixed\text{-}effectss}$ = 1.69, 95% CI: 1.17-2.44) (Figure 2D).

For the *HLA-DR*13* polymorphism, the fixed-effects model was used to pool the meta-analysis result, since the between-study heterogeneity was not significant (I^2 = 0.00%, P_H = 0.699). The results showed that *HLA-DR*13* allele was associated with an increased clearance rate of HBV infection (P_{OR} = 0.000, $OR_{fixed\text{-}effects}$ = 0.27, 95% CI: 0.19-0.37). In the subgroup analyses by ethnicity, the results showed that the *DR*13* allele was associated with HBV clearance among Chinese Han group (P_{OR} = 0.003, $OR_{fixed\text{-}effects}$ =0.36, 95% CI: 0.18-0.70) and other ethnic groups (P_{OR} = 0.000, $OR_{fixed\text{-}effectss}$ = 0.24, 95% CI: 0.17-0.35) (Figure 2E).

Heterogeneity analysis and sensitivity analysis

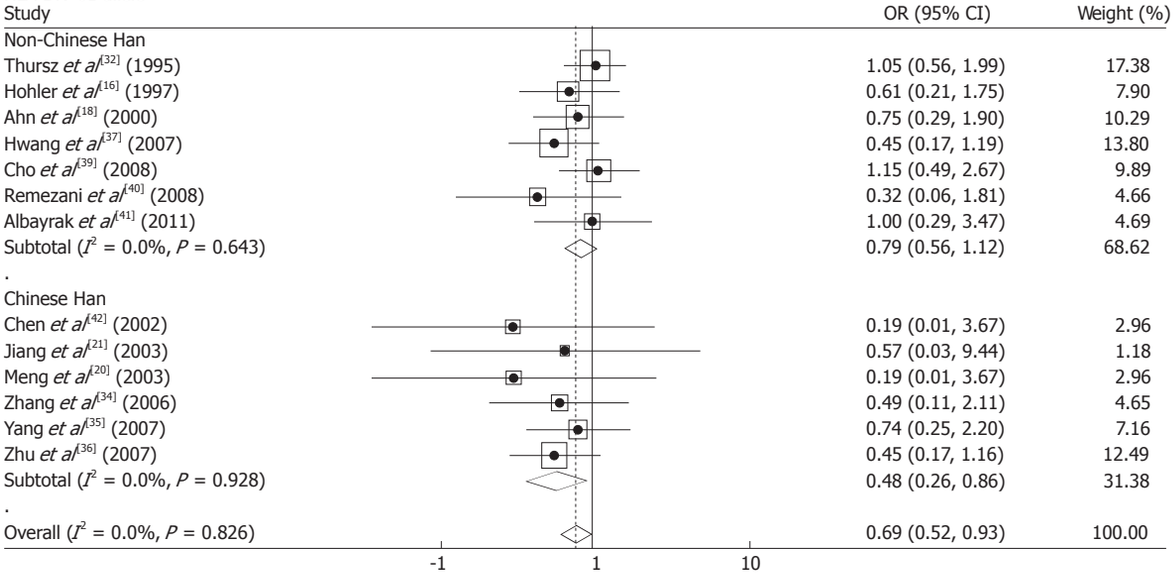
The heterogeneity was calculated among all studies using the Q-statistic and the I^2 test. As shown in Table 2, for the *HLA-DR*11* polymorphism, the between-study heterogeneity was significant (I^2 = 41.3%, P_H = 0.053) when all 14 studies were pooled into meta-analysis. In the subgroup analyses by ethnicity for this allele, the between-study heterogeneity was also significant in Chinese Han subgroups (I^2 = 59.1%, P_H = 0.023), while heterogeneity was not found in other ethnic groups (I^2 = 20.0%, P_H = 0.277). Except for the meta-analysis for DR**13* allele, heterogeneity in the all meta-analysis for other *HLA-DR* alleles was not found (P_H > 0.10).

Univariate analysis of meta-regression suggested that the publishing year and language were not important sources of between-study heterogeneity in both generate analyses and subgroup analyses. We carried out a sensitivity analysis for each *HLA-DR* allele interaction analysis by sequential omission of individual studies. A single study involved in the meta-analysis was deleted each time to investigate the influence of the individual dataset on the pooled ORs. The corresponding pooled ORs were not materially altered, indicating that our results were statistically robust.

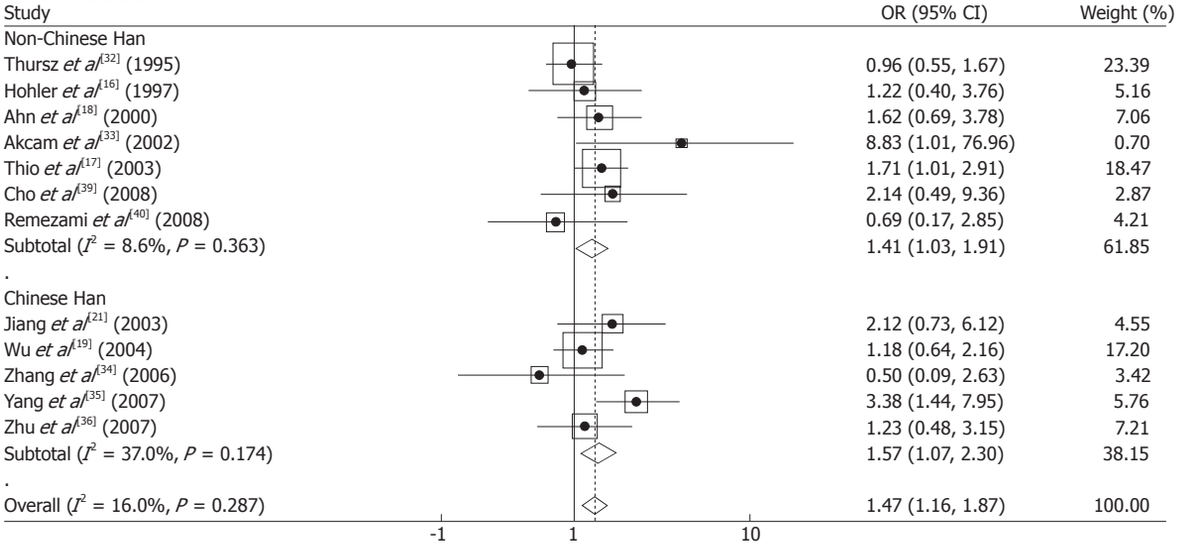
Publication bias

Begg's funnel plot and Egger's test were performed to assess the publication bias of the literature in this meta-analysis. All the *P* values of Begg's test ($P_{beggr's}$) and Egger's tests ($P_{Egger's}$) are shown in Table 2. The $P_{Egger's}$ with more than 0.05 was considered as statistical evidence of the funnel plots' asymmetry. The Egger's test results suggested that publication bias in our meta-analyses of *HLA-DR* alleles was not remarkable except for the three subgroup meta-analyses. As shown in Table 2, the publication bias was borderline significant in the analysis of HLA-DR**10 allele ($P_{Egger's}$ = 0.040) and Chinese Han subgroups analysis of HLA-DR**16 ($P_{Egger's}$ = 0.042). The publication bias was evident in other ethnic group analysis of *HLA-DR*13* allele ($P_{Egger's}$ = 0.017). Then, the Duval and Tweedie nonparametric "trim and fill" method was used to adjust

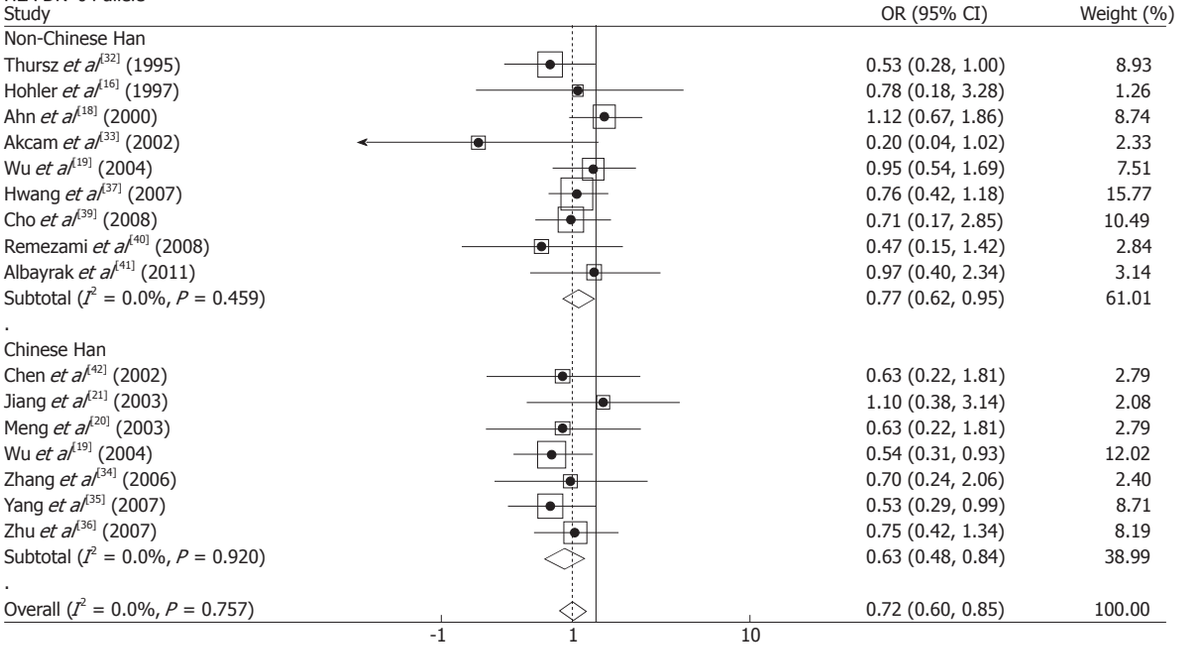
A HLA-DR*01 allele



B HLA-DR*03 allele



C HLA-DR*04 allele



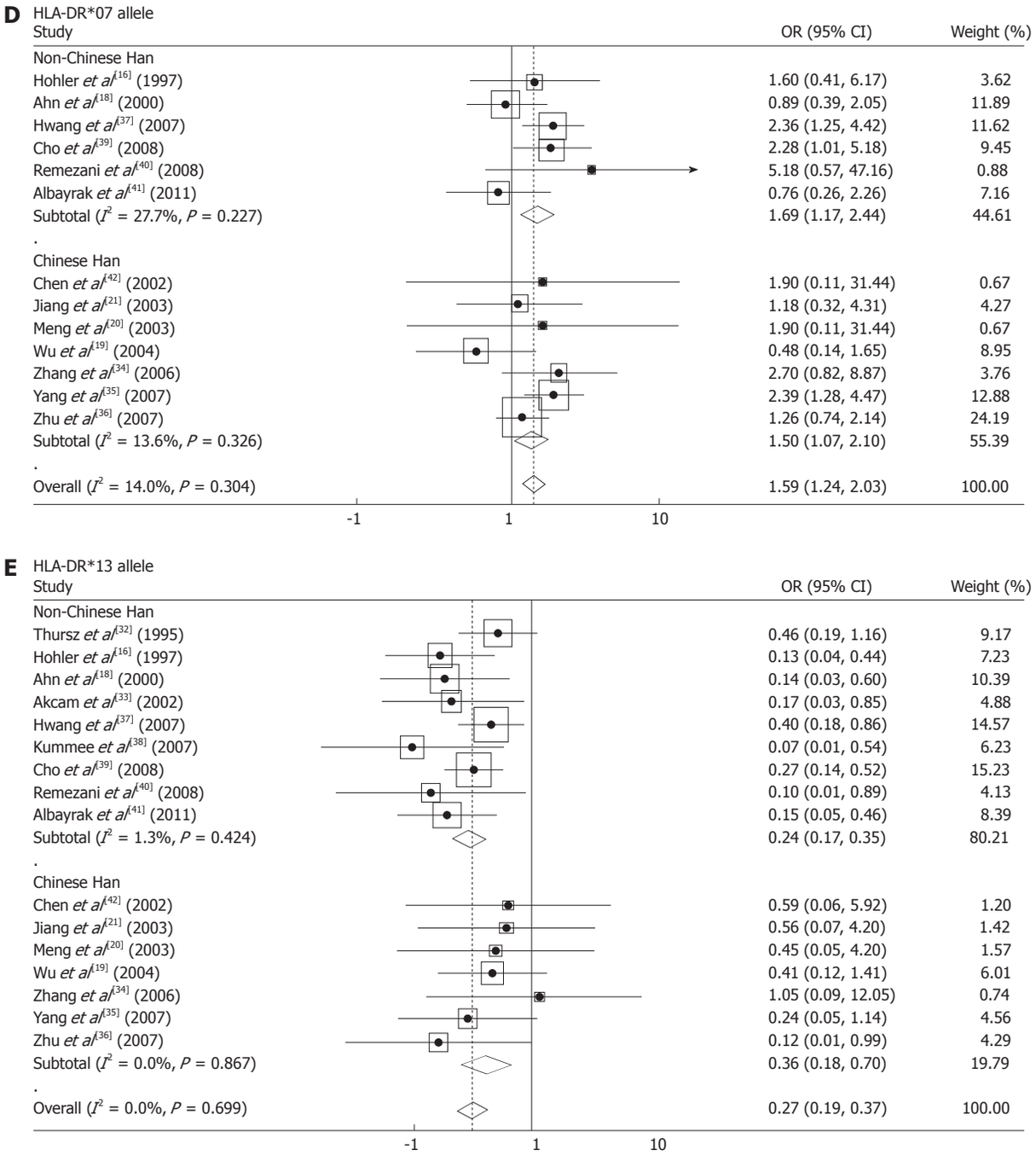


Figure 2 Forest plots of pooled odd ratio with 95% confidence interval for associations between human leukocyte antigens-*DR* polymorphisms and clearance of chronic hepatitis B virus infection. The fixed-effects model was constructed using the Mantel-Haenszel's method. Subgroup meta-analysis was conducted based on ethnicity (Chinese Han group and other ethnic groups). The squares and horizontal lines correspond to the study-specific odd ratio (OR) and 95% confidence interval (95% CI); the box size is proportional to the meta-analysis study weight, the diamonds represent the pooled OR and 95% CI. HLA: Human leukocyte antigens.

publication bias[45]. Meta-analysis with and without using the "trim and fill" method did not draw different conclusions, indicating that our results were statistically robust.

DISCUSSION

It is believed that the host genetic factors involved in genetic polymorphisms are responsible for the susceptibility to and clinical outcomes of infectious diseases. In recent years, genetic susceptibility to chronic HBV infection has been a research focus, and it has been identified that the polymorphisms of a number of immune-response-associated genes, including *HLA* loci, affected the susceptibility to and clearance of persistent chronic HBV infection among different populations. HLA plays an essential role in the pathogenesis of virus-associated hepatitis. The HLA class Ⅱ molecules are expressed as cell surface glycol-proteins that bind short peptide epitope to CD4+ T cells. HLA-DR, a subtype of HLA class Ⅱ molecule, has a particular binding motif that dictates

Table 2 Meta-analysis of relationship between human leukocyte antigens-DR gene polymorphism and clearance of persistent hepatitis B virus infection

Allele	Present *vs* null	Studies No.	SRC (*n*/N)	CHB (*n*/N)	Heterogeneity		M	OR		Publication bias	
					I^2 (%)	PH		OR (95% CI)	POR	Pbeggr's	PEgger's
DR*01	Total studies	13	109/1500	132/1673	0	0.826	F	0.69 (0.52-0.93)	0.016	0.143	0.221
	Chinese Han group	6	18/432	34/415	0	0.928	F	0.48 (0.26-0.86)	0.014	0.348	0.219
	Other ethnic groups	7	91/1068	98/1258	0	0.643	F	0.79 (0.56-1.12)	0.191	0.133	0.104
DR*03	Total studies	12	180/1489	177/1789	16.0	0.287	F	1.47 (1.16-1.87)	0.002	0.945	0.572
	Chinese Han group	5	72/470	74/627	37.0	0.174	F	1.57 (1.07-2.30)	0.020	0.806	0.838
	Other ethnic groups	7	108/1019	103/1162	8.6	0.363	F	1.41 (1.03-1.91)	0.029	0.548	0.444
DR*04	Total studies	16	358/1766	549/2256	0	0.757	F	0.72 (0.60-0.85)	0.000	0.207	0.255
	Chinese Han group	7	104/530	215/739	0	0.920	F	0.63 (0.48-0.84)	0.002	0.453	0.299
	Other ethnic groups	9	254/1236	334/1517	0	0.459	F	0.77 (0.62-0.95)	0.016	0.175	0.178
DR*07	Total studies	13	239/1373	146/1584	14	0.304	F	1.59 (1.24-2.03)	0.000	0.625	0.634
	Chinese Han group	7	111/530	82/739	13.6	0.326	F	1.50 (1.07-2.10)	0.017	0.881	0.601
	Other ethnic groups	6	128/843	64/845	27.7	0.227	F	1.69 (1.17-2.44)	0.005	0.452	0.924
DR*08	Total studies	15	290/1736	301/2226	0	0.541	F	1.19 (0.98-1.44)	0.083	0.255	0.250
	Chinese Han group	7	105/530	124/739	0	0.850	F	1.30 (0.96-1.77)	0.086	0.293	0.188
	Other ethnic groups	8	185/1206	177/1487	27.7	0.207	F	1.11 (0.86-1.43)	0.409	0.711	0.447
DR*09	Total studies	14	344/1703	385/2196	33.3	0.108	F	1.17 (0.98-1.40)	0.075	0.661	0.740
	Chinese Han group	7	157/530	206/739	32.3	0.182	F	1.10 (0.85-1.42)	0.467	0.230	0.241
	Other ethnic groups	7	187/1173	179/1457	40.7	0.120	F	1.25 (0.98-1.59)	0.077	0.764	0.786
DR*10	Total studies	11	62/955	96/1447	0	0.921	F	1.08 (0.76-1.53)	0.682	0.815	0.343
	Chinese Han group	6	9/469	24/707	0	0.871	F	0.68 (0.29-1.49)	0.314	0.039	0.040
	Other ethnic groups	5	51/1206	70/1487	0	0.860	F	1.22 (0.82-1.81)	0.325	0.806	0.687
DR*11	Total studies	14	221/1459	229/1630	41.3	0.053	R	1.03 (0.75-1.42)	0.839	0.477	0.915
	Chinese Han group	7	73/530	107/739	59.1	0.023	R	1.08 (0.59-1.97)	0.806	0.652	0.848
	Other ethnic groups	7	148/929	122/891	20.0	0.277	F	1.00 (0.74-1.35)	0.993	0.548	0.953
DR*12	Total studies	12	336/1478	360/1590	30.1	0.152	F	1.08 (0.90-1.31)	0.406	0.945	0.948
	Chinese Han group	6	142/500	173/683	33.7	0.183	F	1.23 (0.93-1.62)	0.149	0.707	0.642
	Other ethnic groups	6	194/978	187/978	27.2	0.231	F	0.98 (0.76-1.26)	0.845	1.000	0.709
DR*13	Total studies	16	69/1779	211/2127	0	0.699	F	0.27 (0.19-0.37)	0.000	0.964	0.473
	Chinese Han group	7	12/531	48/739	0	0.867	F	0.36 (0.18-0.70)	0.003	0.368	0.510
	Other ethnic groups	9	57/1248	163/1388	1.3	0.424	F	0.24 (0.17-0.35)	0.000	0.175	0.017
DR*14	Total studies	14	281/1511	335/739	12.2	0.319	F	1.17 (0.94-1.46)	0.151	0.071	0.073
	Chinese Han group	7	73/530	97/739	0.2	0.422	F	1.11 (0.79-1.57)	0.552	0.176	0.108
	Other ethnic groups	7	208/981	238/1074	29.7	0.201	F	1.22 (0.92-1.62)	0.172	0.022	0.076
DR*15	Total studies	12	248/1230	236/1132	0	0.525	F	0.84 (0.67-1.05)	0.127	0.075	0.052
	Chinese Han group	7	113/530	161/739	13.2	0.329	F	0.92 (0.69-1.22)	0.556	0.293	0.155
	Other ethnic groups	5	135/700	75/393	0	0.717	F	0.73 (0.51-1.04)	0.084	0.221	0.133
DR*16	Total studies	12	41/876	78/1082	22.4	0.224	F	0.71 (0.47-1.06)	0.097	0.170	0.140
	Chinese Han group	7	24/530	59/739	12.7	0.333	F	0.60 (0.42-1.13)	0.137	0.652	0.042
	Other ethnic groups	5	17/346	19/343	44.8	0.124	F	0.76 (0.37-1.54)	0.447	0.221	0.764

M: Model of meta-analysis; F: Fixed-effects model; R: Random-effects model; PH: The *P* value for heterogeneity test; POR: The *P* value for OR test; Pbeggr's: The *P* value for Egger's test; PEgger's: The *P* value for Egger's test; CHB: Chronic hepatitis B; SRC: Spontaneously recovered control; OR: Odds ratio.

a specific range of peptides that can physically bind in a groove on the surface of the HLA molecule[14].

Wide variations have been documented in the frequencies of *HLA-DR* gene polymorphisms which have been most widely investigated in healthy populations and been demonstrated to influence TNF-α expression. The association between *HLA-DR* polymorphisms and outcome of HBV infection has been investigated by several research groups. However, the previous studies have yielded conflicting results, and included no more than a few hundred CHB cases, which are too few to assess the genetic effects reliably. Meta-analysis has been recognized as an important tool to precisely assess the effect of the selected genetic polymorphisms on the risk of diseases and to identify potentially important sources of between-study heterogeneity. In our present meta-analysis, a total of 2609 patients with CHB and 2606 controls spontaneously recovering prior to HBV infection were included from 19 case-control studies which were evaluated using the NOS. It could provide the most comprehensive assessment to draw reliable conclusions.

Heterogeneity is a potential problem when interpreting the results of all meta-analyses, and finding the sources of heterogeneity is one of the most important goals of meta-analysis[46]. In this present meta-analysis, we assessed the between-study heterogeneity by different methods, including the χ^2 based Q statistic test (Cochran's Q statistic)[28] (to test for heterogeneity) and the I^2 statistic (to quantify the between-study heterogeneity)[29]. For the meta-analyses comparing persistent HBV infection cases with spontaneously recovering controls, there was significant between-study heterogeneity in pooled meta-analyses of total eligible studies on *HLA-DR*11* allele, which suggested obvious consistency of effects across those

included studies. Subgroup analyses by ethnicity showed that the heterogeneity was still significant in subgroup analyses in Chinese Han populations for *HLA-DR*11* polymorphism. Univariate analysis of meta-regression suggested that the publishing year and language were not important causes of between-study heterogeneity in both general analyses and subgroup analyses. We presumed that the quality of the primary studies would be the main cause of heterogeneity. Interesting, we found no significant between-study heterogeneity in pooled meta-analyses of total eligible studies and subgroup analyses by ethnicity for other alleles.

Some limitations still exist in this meta-analysis. First, meta-analysis essentially remains with observational study that was subject to the methodological deficiencies of the included studies. Since only published studies written in English and Chinese were included in the meta-analysis, publication bias may occur, even though it was not found by statistical tests. Second, the associations were investigated in all kinds of cases (asymptomatic carriers, patients with CHB, patients with liver cirrhosis), and there may be specific genetic effects among these cases, but we could not obtain enough information to further estimate these effects. It is necessary to conduct large trials using standardized unbiased methods on homogeneous CHB patients and well matched controls, with the assessors blinded to the data. Third, our results were based on unadjusted estimates. A more precise analysis should be conducted with individual data, which would allow the adjustment by other co-varieties including age, ethnicity, family history, environmental factors and lifestyle. Finally, gene-gene and gene-environment interactions were not addressed in this meta-analysis due to the lack of sufficient data. For instance, the major genotypes of HBV in Chinese are B and C, but most of the studies did not analyze them separately, which could not be solved because of the methodological limitations of the meta-analysis.

Despite these limitations, this meta-analysis suggests that *HLA-DR*04* and *DR*13* alleles may be the protective factors for HBV clearance, and *HLA-DR*03* and *DR*07* alleles may be the risk factors for HBV persistence. For the *HLA-DR*01* polymorphism, a significantly association with HBV clearance was found in Chinese Han group, but not found in other ethnic groups. In summary, ethnicity may play an important role in HBV infection outcome, leading to conflicting results. More studies on individuals from various ethnic groups and large and carefully designed case-control studies will be necessary to determine the role of *HLA-DR* polymorphisms in the outcome of HBV infection.

COMMENTS

Background

Chronic hepatitis B virus (HBV) infection is a serious public health problem worldwide. Host genetic factors play a role in determining both susceptibility to HBV and the outcome of the infection. A large number of studies on the association between human leukocyte antigens (*HLA*)-*DR* gene polymorphisms and the risk of chronic hepatitis B (CHB) have been conducted, but their conclusions are different or even contradictory.

Research frontiers

The polymorphic HLA antigens play an important role in immune surveillance and immune response. However, the relationships HLA-DR polymorphisms and the outcomes of HBV infection are not universal for all the investigated populations and no meta-analysis has been conducted.

Innovations and breakthroughs

This meta-analysis systemically assessed the associations of *HLA-DR* gene polymorphisms with the outcomes of HBV infections, and concluded that *HLA-DR*03*, *DR*04*, *DR*07* and *DR*13* alleles have significant associations with HBV clearance in both Chinese Han population and other ethnic groups.

Applications

The results of meta-analysis in this study show that *HLA-DR*04* and *DR*13* alleles may be the protective factors for HBV clearance, while *HLA-DR*03* and *DR*07* alleles may be the risk factors for HBV persistence, which may benefit early prevention and treatment of CHB.

Peer review

This meta-analysis of *HLA-DR* gene polymorphisms and HBV infection outcomes is well written, addressed an important in the field. However, there are still some problems.

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