Association of HLA and post-schistosomal hepatic disorder: A systematic review and meta-analysis

Running title: HLA and Post-schistosomal Hepatic Disorder

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Authors' contributions

Conceived and designed the experiments: NTH KH. Analyzed the data: NTH MH MK NTPL JZ KH. Contributed reagents/materials/analysis tools: NTH MH MK NTPL MY JZ KH. Wrote the paper: NTH MH NTPL JZ KH.

Keywords

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ABSTRACT

Several human genetic variants, HLA antigens and alleles are reportedly linked to post-schistosomal hepatic disorder (PSHD), but the results from these reports are highly inconclusive. In order to estimate overall associations between human genetic variants, HLA antigens, HLA alleles and PSHD, we systematically reviewed and performed a meta-analysis of relevant studies in both post-schistosomal hepatic disorder and post-schistosomal non-hepatic disorder patients. PubMed, Scopus, Google Scholar, The HuGE Published Literature database, Cochrane Library, and manual search of reference lists of articles published before July 2009 were used to retrieve relevant studies. Two reviewers independently selected articles and extracted data on study characteristics and data regarding the association between genetic variants, HLA antigens, HLA alleles and PSHD in the form of 2×2 tables. A meta-analysis using fixed-effects or random-effects models to pooled odds ratios (OR) with corresponding 95% confidence intervals were calculated only if more than one study had investigated particular variation. We found 17 articles that met our eligibility criteria. *Schistosoma mansoni* and *Schistosoma japonicum* were reported as the species causing PSHD. Since human genetic variants were only investigated in one study, these markers were not assessed by meta-analysis. Thus, only HLA-genes (a total of 66 HLA markers) were conducted in the meta-analysis. Our meta-analysis showed that human leucocyte antigens HLA-DQB1*0201 (OR = 2.64, *P* = 0.018), DQB1*0303 (OR = 1.93, *P* = 0.008), and DRB1*0901 (OR = 2.14, *P* = 0.002) alleles and HLA-A1 (OR = 5.10, *P* = 0.001), A2 (OR = 2.17, *P* = 0.005), B5 (OR = 4.63, *P* = 0.001), B8 (OR = 2.99, *P* = 0.02), and B12 (OR = 5.49, *P* = 0.005) serotypes enhanced susceptibility to PSHD, whereas HLA-DQA1*0501 (OR = 0.29, *P* = <0.001) and DQB1*0301 (OR = 0.58, *P* = 0.007)

were protective factors against the disease. We further suggested that the DRB1*0901-DQB1*0201, DRB1*0901-DQB1*0303 and A1-B8 haplotypes enhanced susceptibility to PSHD, whereas DQA1*0501-DQB1*0301 linkage decreased the risk of PSHD. The result improved our understanding of the association between the HLA loci and PSHD with regard to pathogenic or protective T-cells and provided novel evidence that HLA alleles may influence disease severity.

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1. Introduction

Schistosomiasis is well established as a major health problem in Africa, Asia, and South America [1]. Each year, more than 250,000 deaths are reported from an estimated 200 million individual sufferers [2,3] due to complications of chronic schistosomiasis-related liver disease [4]. Chronic schistosomiasis-related liver disease is caused by *Schistosoma mansoni* and *Schistosoma japonicum*. The disease is characterized by periodic activation of the host immune system against fluke eggs that were deposited in the intestinal wall veins and then delivered to the liver by the blood flow, resulting in granuloma formation and peri-portal fibrosis [5]. Peri-portal fibrosis can cause portal blood hypertension, venous obstruction, splenomegaly, esophageal varices, ascites, hematemesis, and congestive heart failure resulting in death in the absence of proper treatment [5].

Schistosomicides are generally administered with the aim of killing the parasite and preventing against re-infections, while waiting the excretion of tissue-dwelling eggs. However, only one schistosomicide, praziquantel, has been used in large-scale controlled programs, raising concerns about the development of drug resistance [6]. Therefore, it is necessary to study the molecular, biochemical, and immunological aspects of schistosomiasis to develop vaccines and new treatments.

The pathogenesis of peri-portal fibrosis is not yet completely understood. Host immunity, including suppressed Th1/predominant Th2 profiles [7,8], changes in cytokine production [9,10], T-cell responses [11,12], and B-cell responses [13], has been extensively analyzed as a factor that is potentially responsible for fibrotic development. In addition, host genetic background is considered a potential risk factor

contributing to disease development [14-16]. Although associations between post-schistosomal hepatic disorder (PSHD) and HLA antigens, HLA alleles, and genetic variations have been reported in individual studies, the associations are not observed consistently across studies. Therefore, the present study was conducted to estimate overall associations between human genetic variants, HLA antigens, HLA alleles and PSHD by systemic review and meta-analysis of relevant studies in both post-schistosomal hepatic disorder and post-schistosomal non-hepatic disorder patients.

2. Methods

2.1. Search strategy and study selection

A protocol was designed before this study was performed as recommended by the PRISMA statement (http://www.prisma-statement.org/statement.htm). In July 2009, five electronic databases: PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez), Scopus (http://www.scopus.com/scopus/home.url), Google Scholar (http://scholar.google.com/), The HuGE Published Literature database (http://hugenavigator.net/HuGENavigator/startPagePubLit.do), and the Cochrane Library (http://www.cochrane.org) were searched for suitable studies. The search terms used for PubMed and Scopus were as follows: “("gene variant" OR polymorphism OR SNP OR genotyp* OR "Case control study" OR Haplotype OR "linkage disequilibrium" OR "genetic risk") AND schistosom*” (OR was used in the literature search as a connect word). We used “schistosoma OR schistosomiasis OR schistosome” to search in Cochrane Library and The HuGE Published Literature database. For the “Advanced Scholar Search”, we used “schistosoma OR schistosomiasis OR schistosome” to fill in the field “with all of the words”, “genetic polymorphism variant SNP genotype Haplotype disequilibrium” to fill in the field “with at least one of the words”, and “where my words occur” in the field “title of article”. We further supplemented these searches with a manual search of reference lists and citation list using the Scopus databases. For each identified gene, we performed gene-specific searches by replacing the genetic terms with gene name terms.

We sought only articles that evaluated the association between post-schistosomal hepatic disorder (PSHD) and human genetic variants, HLA antigens, or HLA alleles. No restrictions were made with

respect to language, patient age (children or adult), gender, or study design (family-based association studies or population designs that use un-related individuals). Since a number of zero event were found in several variants from included studies, and a meta-analysis method of adding 0.5 to cells with zero event creates estimation problems if the sample size is too small. We excluded studies with fewer than 20 participants (at least 10 cases and 10 controls were required for inclusion) to limit selection bias [17] and reduce the effect of adding 0.5 to cells with zero event on the result less than 5%. Furthermore, studies were also excluded for any of the following reasons: (1) studies that were reported as animal studies, case reports, scientific correspondence, or reviews; (2) studies from which data could not be reliably extracted; (3) studies that used healthy individuals or other severe forms of schistosomiasis as the control group.

Initially, two independent reviewers (Huy and Hamada) scanned primary titles and abstracts (when available) to select potential full text articles for further scrutiny. When the title and abstract could not be rejected by any reviewer, the full text of the article was obtained and carefully reviewed for inclusion by the two reviewers. Inclusion or exclusion of each study was determined by discussion and consensus between the two reviewers.

2.2. Data management

Full-text versions of all papers eligible for inclusion were obtained and, after the inclusion was confirmed, data were extracted by two independent investigators (Huy and Hamada). The data extracted included the first author, year of publication, study design (family-based or case-control), country of

origin, source of the samples (clinic-, hospital-, or community-based), number of included individuals, gender ratio, and age at examination of included individuals.

In cases where genotype or allele distributions were not provided or data contained obvious errors in the original publication (such as typographical errors, switched allele frequencies, or incorrect allele designations), we attempted to clarify the discrepancy directly with study authors, generally by contacting the first and last authors twice via e-mail. Studies for which we could not obtain clarification or genotype information (after at least two attempts by e-mail) are listed as “no data available”.

2.3. Duplicate publications

Papers published by same research group and studying the same genetic variation or HLA types were checked for potential duplicate data. Whenever data overlap was suspected, authors were contacted via e-mail and asked for clarification. If no clarification could be obtained (for example, if we received no answer after at least two attempts by e-mail), data sets were considered as overlapping, and the largest data set was used for meta-analysis.

2.4. Meta-analysis

Meta-analyses for population designs and for family studies were performed separately [18,19] using Comprehensive Meta-analysis software version 2.0 (http://www.meta-analysis.com). For each genetic variation or HLA types, 2×2 tables were generated, and the odds ratio (OR) for particular allele was

computed. Heterogeneity between studies was evaluated using the *Q* statistic and I^2-test. Heterogeneity was considered statistically significant if *P* was less than 0.10 [20]. I^2 values >25%, 50%, or 75% are considered as low, moderate, or high heterogeneity, respectively [21]. Pooled OR with the corresponding 95% confidence intervals (95%CI) was calculated only if more than one study had investigated a particular allele. A fixed-effects model with weighting of the studies was used when there was a lack of significant heterogeneity ($P > 0.10$), while a random-effects model with weighting of the studies was used when there was heterogeneity between studies ($P \leq 0.10$) [22]. Adjustment of *P* value for multiple comparisons was not conducted because it may increase the likelihood of type II errors [23,24]. In order to reduce the false discovery rate, a confidence interval and interpretation of across studies are proposed to give complement information to P value. Therefore, in the present study, statistical significance was defined as *P* value was <0.05 (two-tailed test) and the 95%CI of OR did not overlap 0.9-1.1, in combination with replicated direction in results across studies.

Finally, to assess the presence of publication bias statistically, we performed Egger's regression test where there were three or more studies assessing the effect of a particular allele on the development of PSHD [25,26]. Publication bias was considered significant when the *P* value was <0.1. Publication bias was further evaluated by Begg's modified funnel plot where there were five or more studies assessing the effect of a particular allele [27].

3. Results

3.1. Study characteristics

Our literature searches identified 395, 578, 303, and 33 publications in the initial searches of PubMed, Scopus, Google Scholar, and HuGE Published Literature, respectively (Fig. 1). After screening the title and/or abstract, 79 articles were selected for full text reading. We further identified additional studies by searching reference lists and tracking articles citing relevant publications using the Scopus databases from the selected full text studies, review articles, and textbook chapters. A total of 62 articles were excluded from the 79 articles that were read in full for one of the following reasons: (1) Leishmaniasis association (n=1), (2) review/conference/book/thesis (n=15), (3) genetic studies of parasite (n=6), (4) no genetic association (n=2), (5) animal research (n=1), (6) no patient with hepatic disorder (n=25), (7) negative control was intestinal or cerebral schistosomiasis (n=2), (8) negative control was healthy group or non-schistosomiasis (n=2), (9) co-infection with hepatitis C or HIV (n=2), (10) unable to extract data and no response after contacting the authors via email (n=3) [28-30], and (11) overlapping studies (n=3) [31-33]. Finally, 17 studies were selected for final analysis [16,34-49].

The characteristics of the studies in this meta-analysis are outlined in Table 1. Most of the studies were case-control studies of un-related individuals (n=16); only one was a family-based study. More studies were performed among Africans (n=9) and Asians (n=7) than among South Americans (n=1). Ten studies investigated *S. mansoni*, while other 7 studies did on *S. japonicum*. Ten studies did not stated the gender information of subjects, all remained studies (n=7) showed dominant male in both PSHD and control

groups. Nine studies included adults [16,35-37,40-43,48], three studies enrolled children subjects [44,46,49], one study recruited both adults and children [34], and four studies did not mention this information [38,39,45,47]. A total of three different methods for PSHD diagnosis (ultrasound: n = 10, biopsy: n = 2, clinical diagnosis n = 4) were performed in 16 of 17 studies. One study did not mention the method for PSHD diagnosis [35]. Concerning the ultrasound for PSHD diagnosis, one study did not describe in detail [16], while the remaining studies used the WHO ultrasound criteria [50]**.** Only two studies took in consideration the prevalence of each variant in the general population [34,36]. The human leucocyte antigens (HLA) loci were investigated in 11 studies, while cytokines and other signal protein genes were investigated in six studies.

3.2. Meta-analysis

Since only one study investigated cytokine and other related protein genes, these markers were not assessed by meta-analysis. Thus, only HLA-types (a total of 66 HLA markers) were included in the meta-analysis as shown in Table 2. Pooled ORs showed that eight HLA variants—DQB1*0201, DQB1*0303, DRB1*0901, A1, A2, B5, B8, and B12—were associated with a significant increase in risk for PSHD, while DQA1*0501 and DQB1*0301 were associated with a significant decrease in risk for PSHD ($P < 0.05$) (Fig. 2). The negative association of DQA1*0501 and PSHD was consistent among two studies (P value for heterogeneity = 0.48, $I^2 = 0$) (Table 2 and Fig. 2A), whereas A1 and B5 were found associated with PSHD among two and three of five studies, respectively (Table 2 and Fig. 2B). The positive association DQB1*0201 and DRB1*0901 with PSHD were found to be significant in one study

but not significant in another study with no evidence of significant heterogeneity between two studies (Table 2 and Fig. 2A). Similarly, DQB1*0301, DQB1*0303, A2, B8, and B12 were found to be significant in one study but not in two or three other studies. Significant heterogeneity among studies of the A1 and B5 antigens was found; however, based on the results of analysis using the random effect model, the risk of PSHD was significantly higher in carriers of these variants.

We further evaluate the effect of different schistosomal species on the significant association between HLA types and PSHD. All HLA variants—DQB1*0201, DQB1*0303, and DRB1*0901 were associated with *S. japonicum*-induced PSHD in all studies, while HLA antigens-A1, A2, B5, B8, and B12 were correlated with *S. mansoni*-induced PSHD in all studies except one study by Wang *et al*. (Table 1 and Fig. 2). Removing the study by Wang *et al*. had little effect on the significant association ($P < 0.05$), pooled ORs and 95%CIs (data not shown).

4. Discussion

Our pooled results suggested that HLA-A1, -B8, DQB1*0201 are associated with increased risk of PSHD (Table 2 and Fig. 2B). Furthermore, these alleles are reportedly associated with the severity of other granulomatous diseases [51-53], suggesting that the mechanism of PSHD development shares some similar part of other granulomatous diseases.

The frequency of the HLA-DQB1*0201 allele was higher in PSHD patients in Zhang's study (OR = 5.83, $P = 0.009$) and Hirayama's study (OR = 1.68, $P = 0.31$) when compared to the control patients (Fig. 2A). This trend was also observed in the study by Secor *et al*. [29], which was not included in this analysis due to un-extractable data, further supporting this association. The DQB1*0201 allele has been also linked to the progression of cirrhosis due to hepatitis C virus (HCV) [54], the elevation of alanine aminotransferase levels in serum (a marker of the hepatic damage) [55], the risk of anti-tuberculosis drugs-hepatotoxicity [56], the severity of the intestinal mucosal damage [57], and the risk of several autoimmune disorders [58,59]. Furthermore, autoimmunity has also been proposed as a factor in the development of schistosomiasis-associated hepatic disease [60]. It has also been shown that human papillomavirus-16 E7 (aa 71-85) peptide presented to some pathogenic T cells resulted in dysplastic cervical lesions in individuals carrying HLA-DQB1*0201 [61]. Therefore, HLA-DQB1*0201 may play a role in the antigen presentation to some pathogenic T-cells that could enhance the development of PSHD.

Another HLA-DQB1 allele, DQB1*0303, has also been associated with PSHD (OR = 1.93, 95%CI = 1.19-3.14, $P = 0.008$). There may be a closely linked variant (possibly in the HLA-DRB1 locus) that is primarily responsible for the PSHD susceptibility and associated with these DQB1 alleles. A number of

studies have demonstrated DRB1*0901-DQB1*0201 [62-64] and_DRB1*0901-DQB1*0303 linkage [41,65] are common in African and Asian populations. The DRB1*0901 allele was also associated with susceptibility to PSHD (OR = 2.14, 95%CI = 1.31-3.48, *P* = 0.002). The possibility that the HLA-DRB1*0901 allele is primarily responsible for PSHD susceptibility is an interesting point to be considered because IgG4 elevation has been found in individuals with the HLA-DRB1*0901 allele [66] and is positively linked to several systemic fibrosis conditions [67] and schistosomiasis peri-portal fibrosis [68]. It is probable that patients with HLA-DRB1*0901 are prone to produce B cells for specific IgG4 and Th2 cells that are reactive to schistosomal antigens.

The HLA-DQA1*0501 (OR = 0.29, 95%CI = 0.17-0.50, $P < 0.001$) allele was identified as a resistant allele for PSHD in two studies [37,42]. Furthermore, *Trypanosoma cruzi* B13 protein was reportedly recognized by T-cells in individuals bearing HLA-DQA1*0501 [69], suggesting that individual with HLA-DQA1*0501 may clear the parasite better than those without this allele. Another allele, HLA-DQB1*0301, correlated with PSHD resistance (OR = 0.58, 95%CI = 0.39-0.86, *P* = 0.007), but results on this allele were inconsistent. Two studies indicated that DQB1*0301 had a protective effect against PSHD [42,43], while other two studies did not find a significant association [37,41]. The DQA1*0501-DQB1*0301 linkage is also a common haplotype in several studied populations [42,55,70] and may enhance resistance to PSHD development.

In class I, HLA- A1, -A2, -B5, -B8, and -B12 serotypes were significantly associated with an increased risk for PSHD. There was significant linkage disequilibrium of the A1-B8 haplotype in many prior studies of autoimmune disease risk (http://www.absoluteastronomy.com/topics/HLA-A1), further

supporting the hypothesis that autoimmunity is an important mechanism in the pathogenesis of PSHD.

The results of our pooled OR suggested that DQA1*0101/4 was possibly associated with increased risk of PSHD after pooling analysis (OR = 2.59, 95%CI = 0.87-7.72, *P* = 0.087) (Table 2), though the two separate studies of DQA1*0101/4 did not identify an increased risk of PSHD. Similarly, DQA1*0103 had a pooled OR of 1.76 with a 95%CI of 0.95-3.25 and *P* value of 0.073 even though it was not found to be significantly associated with PSHD in three separate studies. Therefore, further studies are required to clarify these variants.

DQA1*0601, DRB1*1202, and DRB1*1302 were determined to have conflicting effects on PSHD in previous studies, and we found no significant association between these alleles and PSHD in our meta-analyses (Fig. 2C). Other HLA markers, including DQB1*0503.1, DQB1*0601, and DRB1*1101, were found to have a significant association with PSHD in at least one study, but we did not identify a significant relationships between any of these alleles and PSHD after pooling the data for our meta-analysis (Table 2). This variation can be attributed to low statistical power and variability in study designs, diagnoses, population selection, and phenotype definitions.

Table 2 also summarizes the results of Egger's test for publication bias. No evidence of publication bias was found for the following significant alleles, DQB1*0303, A1, A2, B5, B8, and B12 ($P > 0.1$). The funnel plot analysis was further performed to detect publication bias of each study for HLA-A1 and HLA-B5, respectively. The shape of the funnel plot seemed to be symmetrical, further suggesting that no publication bias was found in studies of HLA-A1 and HLA-B5 (Fig. 3). There was some indication of publication bias for the studies of DQB1*0301 using Egger's test ($P = 0.048$, Table 2). Removing either

the study by Zhang *et al*. or the one by McManus *et al*. resulted in a loss of the publication bias (P > 0.1), but it had little effect on the pooled ORs and 95%CIs; in contrast, removing either the study by Waine *et al*. or the one by Hirayama *et al*. led to an increased chance of publication bias (*P* = 0.038 and 0.022, respectively). One limitation of our study is that the publication bias could not be assessed for three significant alleles, DQA1*0501, DRB1*0901 and DQB1*0201, because there were fewer than three studies for each variant. Moreover, non-significant association must be interpreted with cautious for DPA1*0103, DPA1*0201, DQA1*0601, DQB1*0601, DRB1*1202, DRB1*1302, A9, B15 and BW40 variants because of high Q and I-squared values. Therefore, more studies are required to validate on these variants.

A limitation of many meta-analyses is that studies that reports of non-significant results are less likely to be accepted for publication, and missing data could cause potential bias. As with other complicated diseases, the development of PSHD is probably due to multiple factors, in which allelic variants in different genes may have either additive or conflicting effects. Moreover, other hepatitis diseases, alcohol intake, smoking, and praziquantel treatment doses can all affect the development of PSHD [2], and therefore may have affected our analysis. However, because meta-analyses use selected studies based on defined criteria, they can assess common and significant genetic factors that were not assessed in a systematic way in individual, primary studies. Similar meta-analyses of the genetic studies of pneumococcal and meningococcal infection [71], autoimmune diseases [72,73], and cancer [74] have been already reported. We found no effect of different schistosomal species on the outcome of PSHD;

however, the number of studies for each allele was small (less than 5), and further studies should focus on this issue.

In conclusion, the association between post-schistosomal hepatic disorder and risk factors including eggs load, lack of treatment, repeated infection and HLA types has been reported in previous studies. However, to the knowledge of the authors this is the first systemic meta-analysis that combines individual studies to improve the strength of the evidence. This meta-analysis identified positive associations between eight HLA types—DQB1*0201, DQB1*0303, DRB1*0901, A1, A2, B5, B8, and B12—and post-schistosomal hepatic disorder and negative associations with two variants, DQA1*0501 and DQB1*0301. Moreover, we identified the possible existence of common antigenic moieties that would be presented to some pathogenic or protective T-cells that could affect the outcome of the disease. Though previous included studies identified a positive association of DRB1*0901 - DQB1*0303 linkage [41] and negative association of DQA1*0501 -DQB1*0301 haplotype [42] with PSHD, we further propose that individuals bearing the DRB1*0901 - DQB1*0201 and A1-B8 haplotypes may be at increased risk for the development of PSHD, but further studies are required to confirm our hypothesis.

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Footnotes

The authors have declared that no competing interests exist. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Figure legends

Fig. 1. Flow diagram of the search and review process.

Fig. 2. Meta-analysis forest plot showing the pooled odd ratio (OR) for PSHD susceptibility with 95% confidence intervals (95% CI) of significant HLA alleles (A), significant HLA serotypes (B) and contrasting effective HLA alleles (C). The size of the plots represents the study size. All HLA variants—DQB1*0201, DQB1*0303, and DRB1*0901 were associated with *S. japonicum*-induced PSHD in all studies (A), while HLA antigens-A1, A2, B5, B8, and B12 were correlated with *S. mansoni*-induced PSHD in all studies except one study by Wang *et al* (B).

Fig. 3. Funnel plots for evaluation of publication bias of HLA-A1 (A) and HLA-B5 (B) where there are at least five studies on the same genetic variation. Each circle represents each study in the meta-analysis.

Fig. 1 Flow diagram of the searching and reviewing process

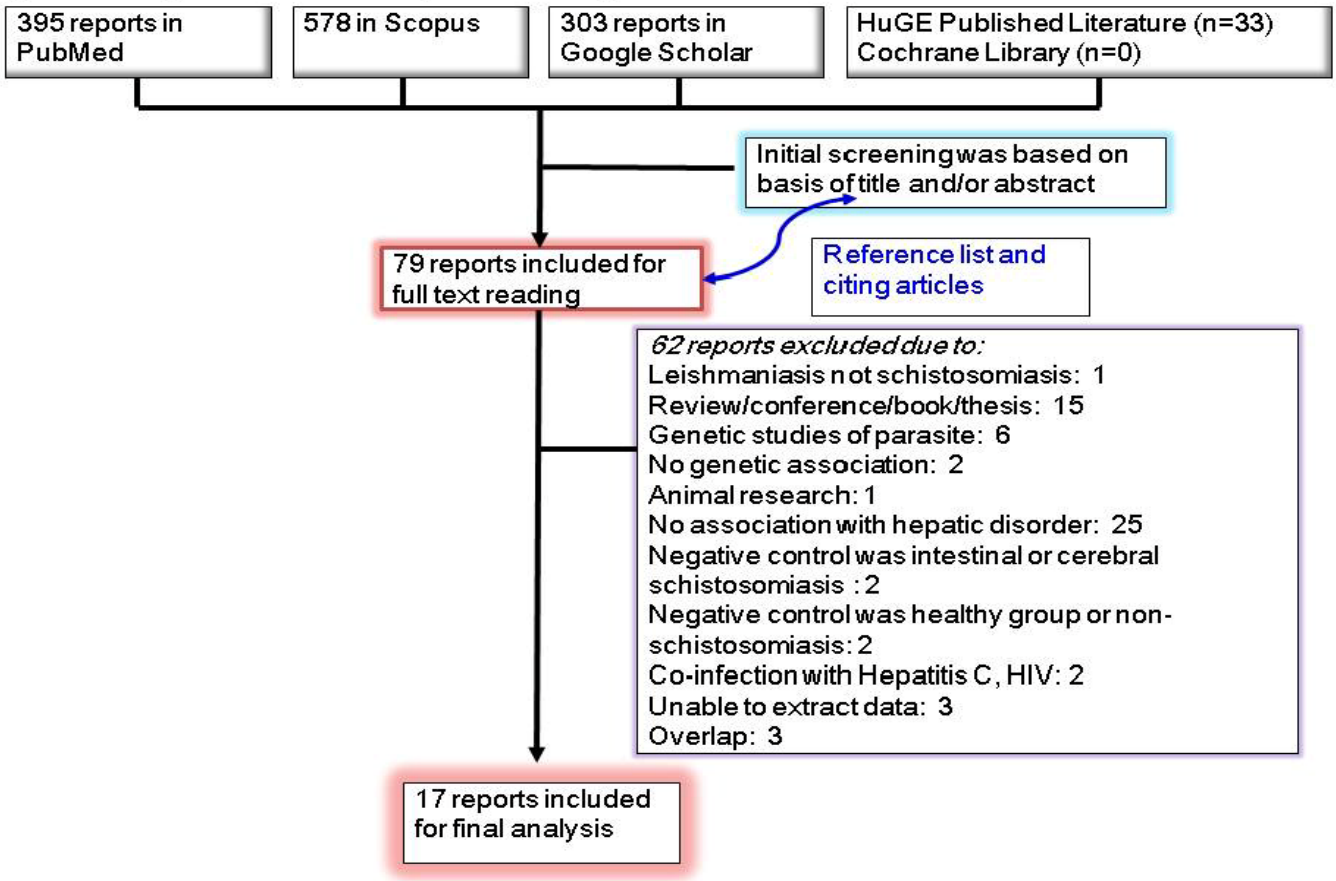

Fig 2 (A)

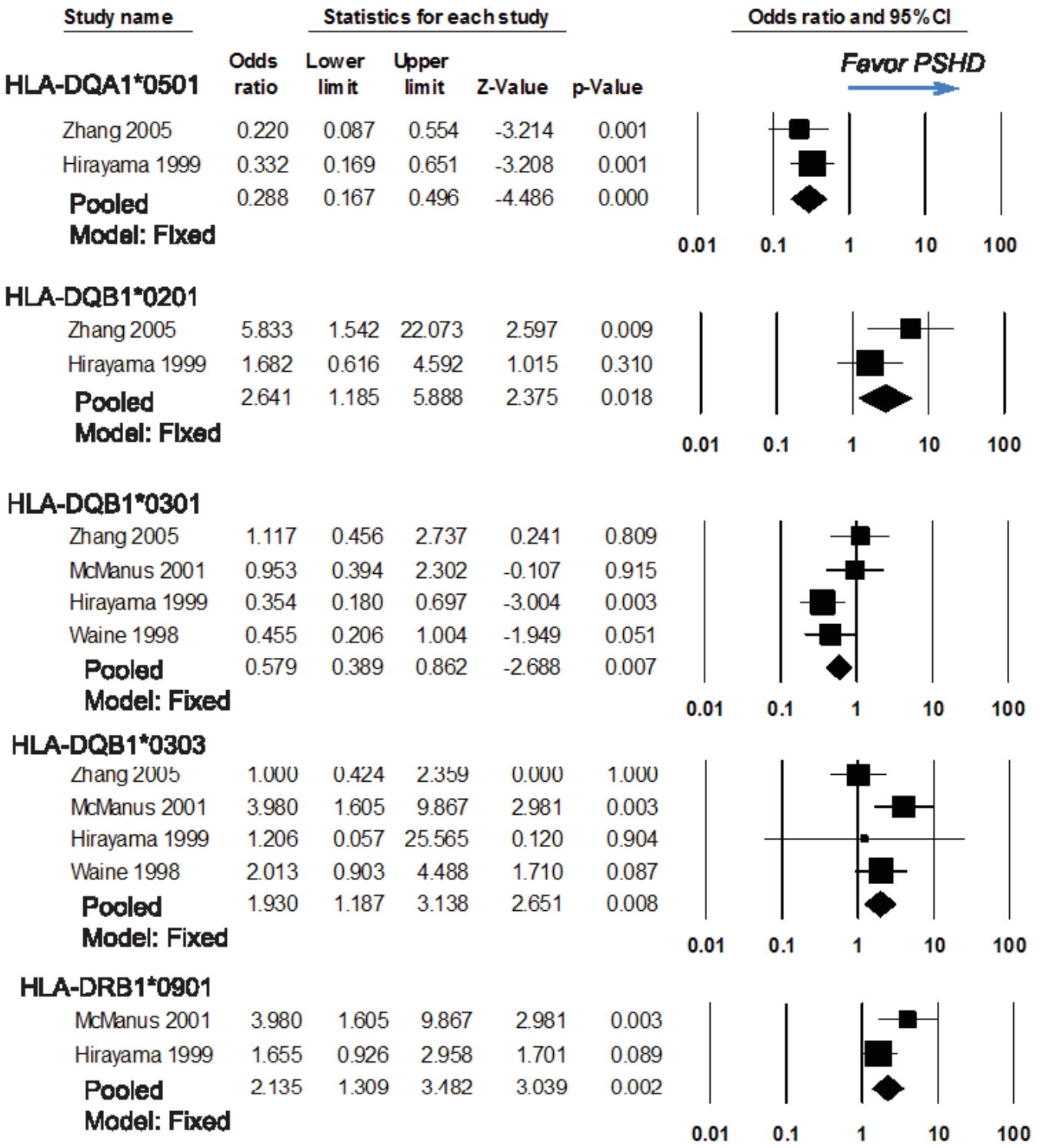
Study name
Statistics for each study
Odds ratio and 95% CI
Odds ratio
Lower limit
Upper limit
Z-Value
p-Value
Favor PSHD
HLA-DQA1*0501
Zhang 2005 0.220 0.087 0.554 -3.214 0.001
Hirayama 1999 0.332 0.169 0.651 -3.208 0.001
Pooled Model: Fixed 0.288 0.167 0.496 -4.486 0.000
HLA-DQB1*0201
Zhang 2005 5.833 1.542 22.073 2.597 0.009
Hirayama 1999 1.682 0.616 4.592 1.015 0.310
Pooled Model: Fixed 2.641 1.185 5.888 2.375 0.018
HLA-DQB1*0301
Zhang 2005 1.117 0.456 2.737 0.241 0.809
McManus 2001 0.953 0.394 2.302 -0.107 0.915
Hirayama 1999 0.354 0.180 0.697 -3.004 0.003
Waine 1998 0.455 0.206 1.004 -1.949 0.051
Pooled Model: Fixed 0.579 0.389 0.862 -2.688 0.007
HLA-DQB1*0303
Zhang 2005 1.000 0.424 2.359 0.000 1.000
McManus 2001 3.980 1.605 9.867 2.981 0.003
Hirayama 1999 1.206 0.057 25.565 0.120 0.904
Waine 1998 2.013 0.903 4.488 1.710 0.087
Pooled Model: Fixed 1.930 1.187 3.138 2.651 0.008
HLA-DRB1*0901
McManus 2001 3.980 1.605 9.867 2.981 0.003
Hirayama 1999 1.655 0.926 2.958 1.701 0.089
Pooled Model: Fixed 2.135 1.309 3.482 3.039 0.002
0.01
0.1
1
10
100

Fig 2(B)

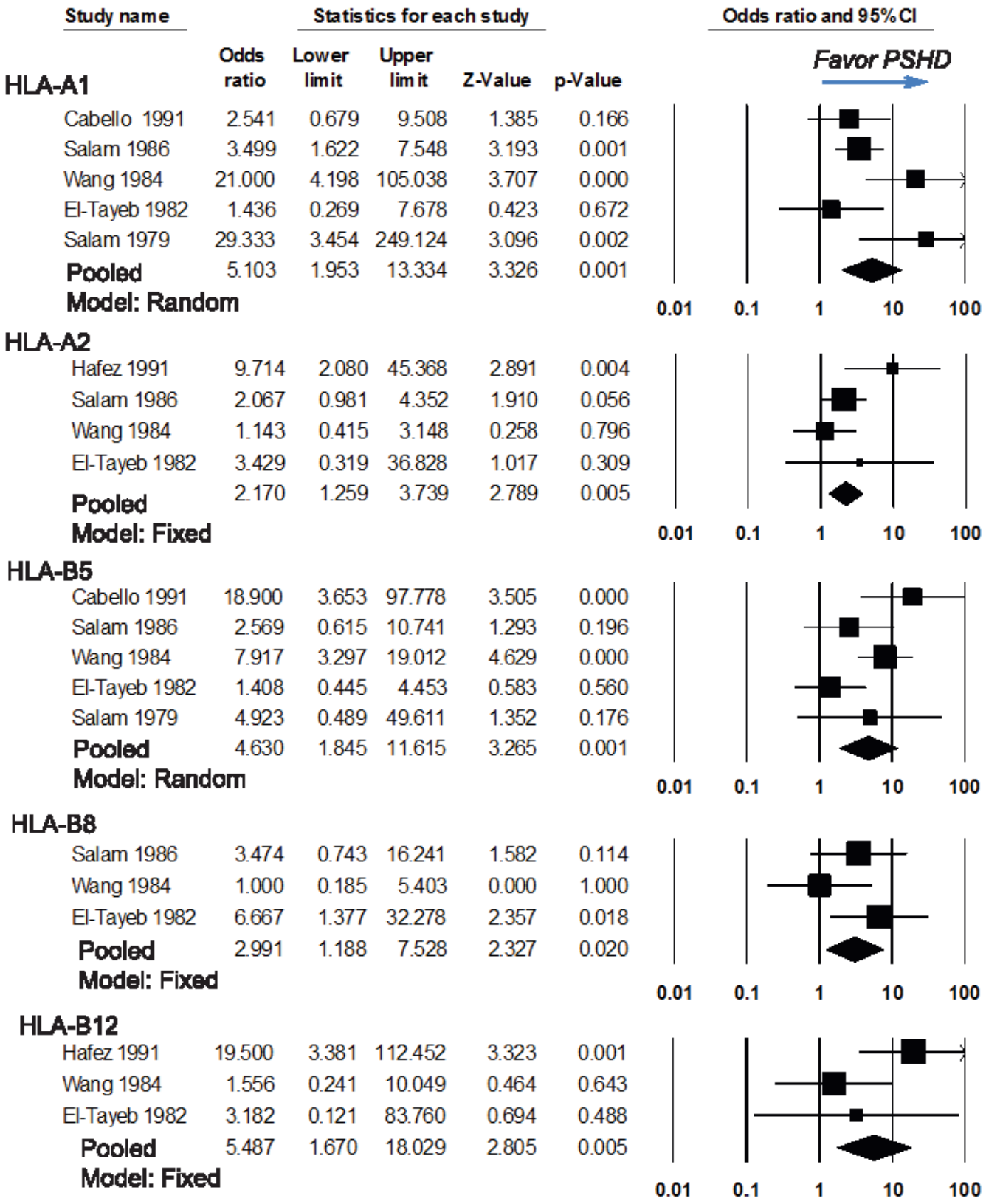
Study name
Statistics for each study
Odds ratio and 95% CI
Odds ratio
Lower limit
Upper limit
Z-Value
p-Value
Favor PSHD
HLA-A1
Cabello 1991 2.541 0.679 9.508 1.385 0.166
Salam 1986 3.499 1.622 7.548 3.193 0.001
Wang 1984 21.000 4.198 105.038 3.707 0.000
El-Tayeb 1982 1.436 0.269 7.678 0.423 0.672
Salam 1979 29.333 3.454 249.124 3.096 0.002
Pooled 5.103 1.953 13.334 3.326 0.001
Model: Random
HLA-A2
Hafez 1991 9.714 2.080 45.368 2.891 0.004
Salam 1986 2.067 0.981 4.352 1.910 0.056
Wang 1984 1.143 0.415 3.148 0.258 0.796
El-Tayeb 1982 3.429 0.319 36.828 1.017 0.309
Pooled 2.170 1.259 3.739 2.789 0.005
Model: Fixed
HLA-B5
Cabello 1991 18.900 3.653 97.778 3.505 0.000
Salam 1986 2.569 0.615 10.741 1.293 0.196
Wang 1984 7.917 3.297 19.012 4.629 0.000
El-Tayeb 1982 1.408 0.445 4.453 0.583 0.560
Salam 1979 4.923 0.489 49.611 1.352 0.176
Pooled 4.630 1.845 11.615 3.265 0.001
Model: Random
HLA-B8
Salam 1986 3.474 0.743 16.241 1.582 0.114
Wang 1984 1.000 0.185 5.403 0.000 1.000
El-Tayeb 1982 6.667 1.377 32.278 2.357 0.018
Pooled 2.991 1.188 7.528 2.327 0.020
Model: Fixed
HLA-B12
Hafez 1991 19.500 3.381 112.452 3.323 0.001
Wang 1984 1.556 0.241 10.049 0.464 0.643
El-Tayeb 1982 3.182 0.121 83.760 0.694 0.488
Pooled 5.487 1.670 18.029 2.805 0.005
Model: Fixed
0.01
0.1
1
10
100

Fig 2(C)

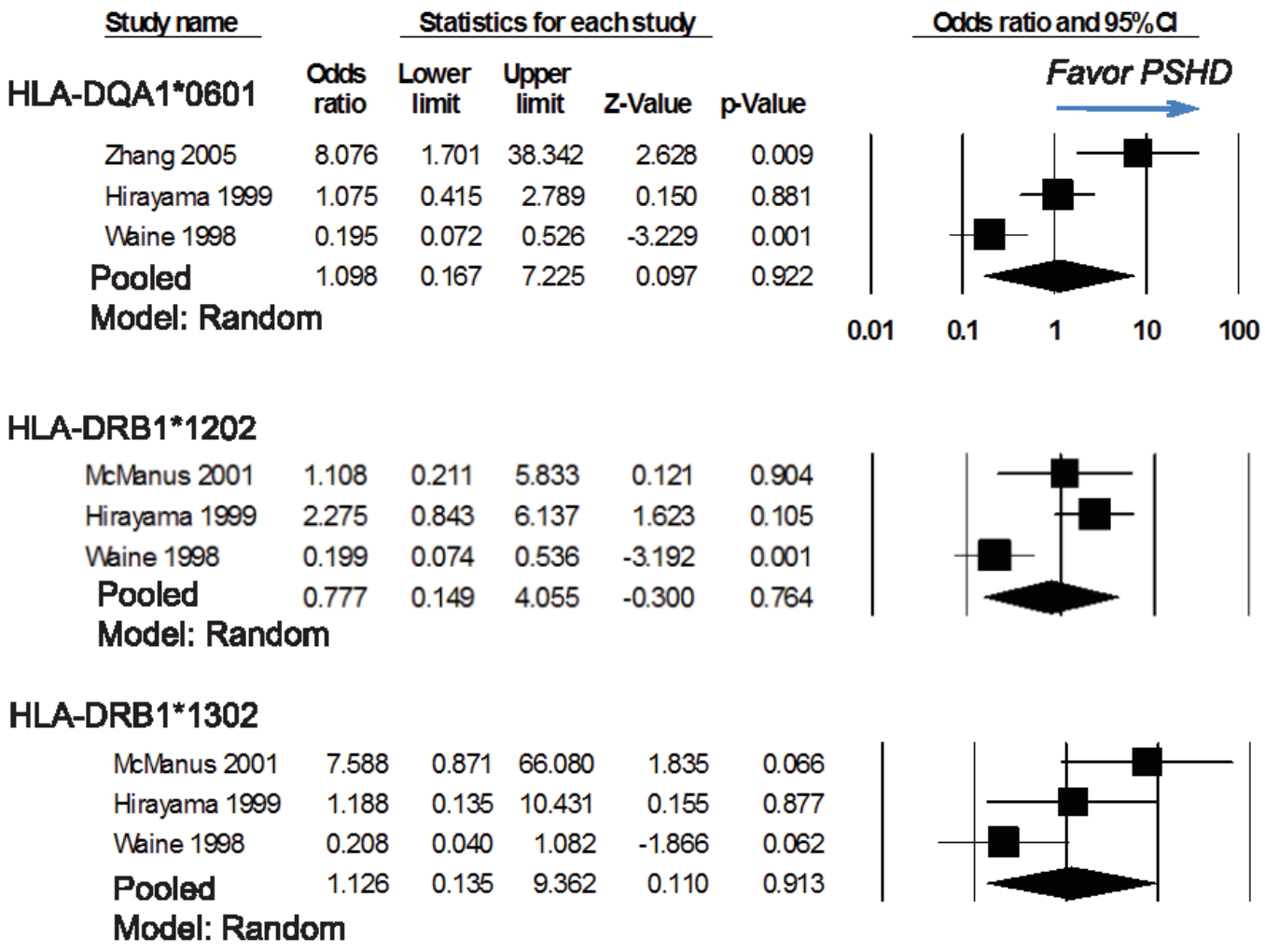
Study name
Statistics for each study
Odds ratio and 95% CI
Favor PSHD
HLA-DQA1*0601
Odds ratio
Lower limit
Upper limit
Z-Value
p-Value
Zhang 2005 8.076 1.701 38.342 2.628 0.009
Hirayama 1999 1.075 0.415 2.789 0.150 0.881
Waine 1998 0.195 0.072 0.526 -3.229 0.001
Pooled 1.098 0.167 7.225 0.097 0.922
Model: Random
0.01
0.1
1
10
100
HLA-DRB1*1202
McManus 2001 1.108 0.211 5.833 0.121 0.904
Hirayama 1999 2.275 0.843 6.137 1.623 0.105
Waine 1998 0.199 0.074 0.536 -3.192 0.001
Pooled 0.777 0.149 4.055 -0.300 0.764
Model: Random
HLA-DRB1*1302
McManus 2001 7.588 0.871 66.080 1.835 0.066
Hirayama 1999 1.188 0.135 10.431 0.155 0.877
Waine 1998 0.208 0.040 1.082 -1.866 0.062
Pooled 1.126 0.135 9.362 0.110 0.913
Model: Random

Fig 3

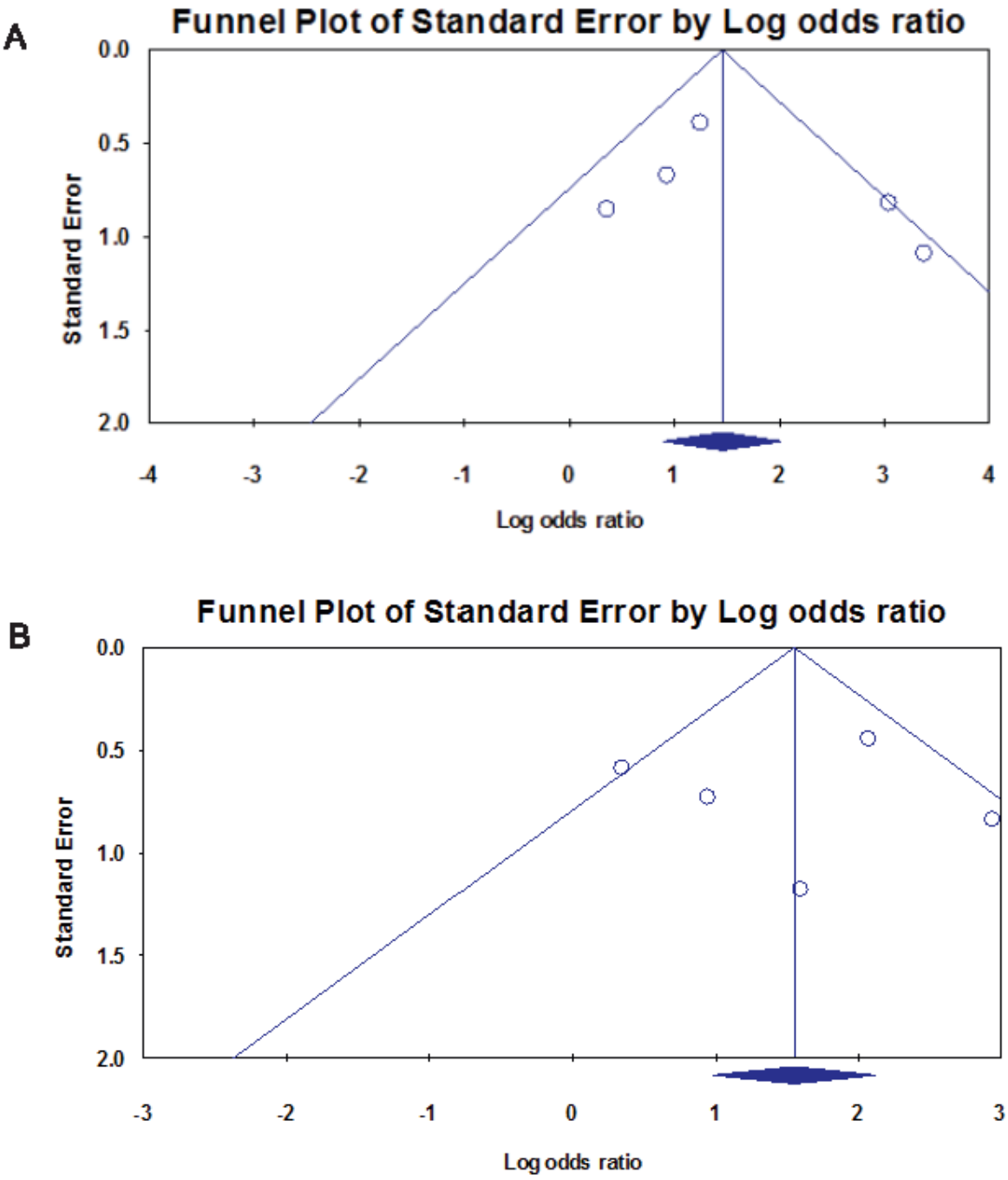
A
Funnel Plot of Standard Error by Log odds ratio
Standard Error
0.0
0.5
1.0
1.5
2.0
-4
-3
-2
-1
0
1
2
3
4
Log odds ratio
B
Funnel Plot of Standard Error by Log odds ratio
Standard Error
0.0
0.5
1.0
1.5
2.0
-3
-2
-1
0
1
2
3
Log odds ratio

Table 1. Characteristic of studies included in this analysis.

Authors	Year	Country	Strain	Study design	PSHD		Control		Diagnostic method of PSHD	Genetic method	Number of alleles studied
					No. (female%)	Mean age (±SD or range)	No. (female%)	Mean age (±SD or range)			
Elsammak	2008	Egypt	*S. mansoni*	Case control	22 (41)	53.7 (± 5.6)	22 (41)	48.5 (± 5.4)	Ultrasound	PCR-RFLP	1 (LA)
Eriksson	2007	Uganda	*S. mansoni*	Case control	22 (ND)	ND	274(ND)	ND	Ultrasound	PCR-SSOP	1 (ECP)
Cheng	2005	China	*S. japonicum*	Case control	45 (31)	54.9 (± 11)	44 (34)	51.6 (± 9.2)	ND	PCR-SSOP	16 (HLA)
Blanton	2005	Egypt	*S. mansoni*	Family based	48 (25)	42.3 (± 15)	188(33)	39.1 (±13.2)	Ultrasound	UTSG	48(11genes)
Zhang	2005	China	*S. japonicum*	Case control	46 (41)	51. 8 (± 10.)	43(30)	55. 3(± 7. 8)	Ultrasound	PCR-SSOP	41 (HLA)
Chevillard	2003	Sudan	*S. mansoni*	Case control	29 (ND)	ND	76 (ND)	ND	Ultrasound	PCR-SSCP	5 (IFN-γ)
Moukoko	2003	Sudan	*S. mansoni*	Case control	27 (ND)	ND	71 (ND)	ND	Ultrasound	PCR-RFLP	4 (TNF-α)
Hirayama	2002	China	*S. japonicum*	Case control	156 (ND)	ND	36 (ND)	ND	Ultrasound	ND	1 (IL-13)
McManus	2001	China	*S. japonicum*	Case control	40 (ND)	ND	44 (ND)	ND	Ultrasound	PCR-SSOP	34 (HLA)
Hirayama	1999	China	*S. japonicum*	Case control	186 (40)	ND	44 (57)	ND	Ultrasound	PCR-SSOP	104 (HLA)
Waine	1998	China	*S. japonicum*	Case control	64 (ND)	59. 4 (± 8.8)	44 (ND)	54. 5(± 7. 1)	Ultrasound	PCR-SSOP	53 (HLA)
Hafez	1991	Egypt	*S. mansoni*	Case control	19 (26)	11.2 (7-14)	20 (40)	9.9 (7-14)	Biopsy	CDM	29 (HLA)
Cabello	1991	Brazil	*S. mansoni*	Case control	23 (ND)	ND	41 (ND)	ND	Clinical	CDM	38 (HLA)
Salam	1986	Egypt	*S. mansoni*	Case control	88 (ND)	11. 8 (± 1.1)	46 (ND)	12. 8 (± 0.5)	Biopsy	CDM	32 (HLA)
Wang	1984	China	*S. japonicum*	Case control	30 (ND)	ND	30 (ND)	ND	Clinical	CDM	24 (HLA)
El-Tayeb	1982	Egypt	*S. mansoni*	Case control	17 (ND)	37.3 (14-60)	17 (ND)	33.9 (14-55)	Clinical	CDM	25 (HLA)
Salam	1979	Egypt	*S. mansoni*	Case control	28 (32)	ND	23 (26)	ND	Clinical	CDM	37 (HLA)

ND, not described

PCR-RFLP, PCR-restriction fragment length polymorphism

PCR-SSOP, PCR-sequence-specific oligonucleotides probes

UTSG, ultra-high throughput SNP genotyping

PCR-SSCP, PCR-single strand conformation polymorphism

CDM, complement-dependent microlymphocytotoxicity

LA, lymphotoxin-α

ECP, Eosinophil Cationic Protein

Table 2. Summary of associations between HLA alleles and hepatic disorders, pooled odds ratios (OR) with corresponding 95% confidence intervals (95%CI) of the published results were calculated where more than one study had investigated the allele.

HLA types	No. of study	Heterogeneity			Model	Association with hepatic disorders			***Egger's* 2-*tailed*** bias
		Q value	*P* value	I-squared		*P* value	OR	95%CI	*P* value
DPA1*0103	2	6.06	0.014	83.5	Random	0.500	1.62	0.40-6.62	ND
DPA1*0201	2	3.80	0.051	73.7	Random	0.893	1.22	0.06-22.99	ND
DPA1*0401	2	0.002	0.97	0	Fixed	0.636	0.77	0.25-2.32	ND
DQA1*0101	2	0.00	0.993	0.0	Fixed	0.280	0.46	0.11-1.88	ND
DQA1*0101/4	2	0.83	0.362	0.0	Fixed	0.087	2.59	0.87-7.72	ND
DQA1*0102	3	1.28	0.527	0.0	Fixed	0.670	0.89	0.51-1.54	0.205
DQA1*0103	3	1.61	0.448	0.0	Fixed	0.073	1.76	0.95-3.25	0.21
DQA1*0201	3	3.48	0.175	42.6	Fixed	0.948	1.03	0.44-2.40	0.584
DQA1*0301	2	0.19	0.666	0.0	Fixed	0.389	1.43	0.64-3.21	ND
DQA1*0401	3	3.21	0.201	37.7	Fixed	0.624	0.75	0.23-2.42	0.052
DQA1*0501	**2**	**0.50**	**0.480**	**0.0**	**Fixed**	**<0.0001**	**0.29**	**0.17-0.50**	**ND**
DQA1*0601	3	16.57	0.000	87.9	Random	0.922	1.10	0.17-7.23	0.601
DQB1*0201	**2**	**2.14**	**0.144**	**53.2**	**Fixed**	**0.018**	**2.64**	**1.19-5.89**	**ND**
DQB1*0301	**4**	**5.67**	**0.129**	**47.0**	**Fixed**	**0.007**	**0.58**	**0.39-0.86**	**0.048**
DQB1*0302	4	4.12	0.249	27.1	Fixed	0.425	0.83	0.52-1.32	073
DQB1*0303	**4**	**4.80**	**0.187**	**37.5**	**Fixed**	**0.008**	**1.93**	**1.19-3.14**	**0.91**
DQB1*0401	3	1.93	0.380	0.0	Fixed	0.80	1.10	0.53-2.30	0.71
DQB1*0402	2	1.30	0.254	23.2	Fixed	0.29	0.32	0.04-2.64	ND
DQB1*0501	4	3.26	0.353	8.0	Fixed	0.40	1.75	0.48-6.43	0.11
DQB1*0502	4	1.54	0.672	0.0	Fixed	0.169	0.63	0.33-1.22	0.61
DQB1*0503.1	3	4.47	0.107	55.2	Fixed	0.68	1.37	0.30-6.23	0.23
DQB1*0601	4	13.20	0.004	77.3	Random	0.301	0.60	0.23-1.58	0.92
DQB1*0602	4	4.37	0.224	31.4	Fixed	0.902	0.96	0.50-1.83	0.62
DQB1*0603	3	2.53	0.283	20.9	Fixed	0.254	2.73	0.49-15.33	0.20
DQB1*0604	3	2.45	0.294	18.3	Fixed	0.501	0.55	0.09-3.19	0.93
DRB1*01	3	1.69	0.429	0.0	Fixed	0.848	0.85	0.16-4.48	0.33
DRB1*0301	3	4.60	0.100	56.5	Random	0.32	2.33	0.44-12.34	0.19
DRB1*0401	2	0.62	0.433	0.0	Fixed	0.95	1.06	0.18-6.21	ND
DRB1*0403	3	2.08	0.353	3.8	Fixed	0.56	1.38	0.47-4.05	0.115
DRB1*0404	3	1.38	0.502	0.0	Fixed	0.174	0.34	0.07-1.62	0.98
DRB1*0405	3	0.97	0.614	0.0	Fixed	0.42	1.35	0.66-2.78	0.49
DRB1*0406	3	1.99	0.370	0.0	Fixed	0.46	1.56	0.48-5.05	0.39
DRB1*0701	3	2.35	0.309	14.8	Fixed	0.661	0.81	0.31-2.11	0.22
DRB1*0901[a]	3	3.88	0.144	48.4	Fixed	0.49	1.18	0.75-1.86	0.85

DRB1*0901[b]	**2**	**2.54**	**0.111**	**60.7**	**Fixed**	**0.002**	**2.39**	**1.31-3.48**	**ND**
DRB1*1001	4	2.52	0.472	0.0	Fixed	0.91	0.92	0.25-3.46	0.09
DRB1*1101	3	4.77	0.092	58.1	Random	0.162	0.46	0.15-1.37	0.004
DRB1*1104	2	1.34	0.247	25.4	Fixed	0.91	0.88	0.09-8.58	ND
DRB1*1201	3	4.09	0.130	51.1	Fixed	0.87	1.08	0.45-2.62	0.31
DRB1*1202	3	11.89	0.003	83.2	Random	0.76	0.78	0.15-4.06	0.90
DRB1*1301	2	1.53	0.215	34.8	Fixed	0.42	2.42	0.28-20.94	ND
DRB1*1302	3	6.82	0.033	70.7	Random	0.91	1.13	0.14-9.36	0.31
DRB1*1401	3	1.06	0.589	0.0	Fixed	0.55	0.61	0.12-3.07	0.79
DRB1*1403	2	0.04	0.843	0.0	Fixed	0.24	0.28	0.03-2.31	ND
DRB1*1404	2	0.09	0.767	0.0	Fixed	0.69	1.43	0.24-8.65	ND
DRB1*1405	3	2.46	0.293	18.6	Fixed	0.69	1.38	0.29-6.43	0.19
DRB1*1407	2	1.69	0.194	40.7	Fixed	0.84	0.81	0.10-6.36	ND
DRB1*1501	3	7.62	0.022	73.7	Random	0.76	0.83	0.24-2.89	0.62
DRB1*1502	2	2.56	0.110	60.9	Fixed	0.80	1.31	0.16-10.58	ND
DRB1*1602	3	1.31	0.518	0.0	Fixed	0.64	0.80	031-2.06	0.71
A1	**5**	**9.35**	**0.053**	**57.2**	**Random**	**0.001**	**5.10**	**1.95-13.33**	**0.44**
A2	**4**	**5.33**	**0.149**	**43.7**	**Fixed**	**0.005**	**2.17**	**1.26-3.74**	**0.48**
A3	2	0.12	0.733	0.0	Fixed	0.45	0.52	0.10-2.75	ND
A9	3	4.65	0.098	57.0	Random	0.72	1.45	0.26-1.38	0.002
A11	2	1.80	0.180	44.5	Fixed	0.76	1.16	0.45-2.97	ND
A28	2	0.26	0.608	0.0	Fixed	0.65	1.64	0.19-13.99	ND
AW30+31	2	0.00	0.991	0.0	Fixed	0.33	3.14	0.31-31.39	ND
B5	**5**	**9.01**	**0.061**	**55.6**	**Random**	**0.001**	**4.63**	**1.85-11.62**	**0.99**
B7	2	0.81	0.369	0.0	Fixed	0.74	1.25	0.34-4.61	ND
B8	**3**	**2.65**	**0.266**	**24.5**	**Fixed**	**0.02**	**2.99**	**1.19-7.53**	**0.39**
B12	**3**	**3.87**	**0.144**	**48.4**	**Fixed**	**0.005**	**5.49**	**1.67-18.03**	**0.82**
B13	2	1.61	0.205	37.7	Fixed	0.24	1.82	0.68-4.90	ND
B15	2	4.21	0.040	76.2	Random	0.95	1.13	0.04-31.86	ND
B17	2	1.33	0.249	24.7	Fixed	0.53	1.57	0.38-6.51	ND
BW22	2	1.69	0.193	41.0	Fixed	0.59	0.68	0.17-2.77	ND
BW40	2	2.71	0.100	63.1	Random	0.57	1.97	0.19-19.97	ND

[a]fibrosis FI-III vs. F0

[b]fibrosis FII-III vs. F0-I

OR, pooled odds ratio

95%CI, 95% confidence interval

ND, not performed when there is less than three studies

Bold line indicates significant association