

REVIEW ARTICLE

Three polymorphisms in the IL-10 gene and the risk of HCV infection: a meta-analysis plus a Chinese Association Study involving 1140 subjects

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SUMMARY

The influence of an immunosuppressive cytokine, interleukin-10 (IL-10), on the outcome of hepatitis C virus (HCV) infection has been increasingly reported recently. A number of polymorphisms appear to control the level of IL-10 production. Among them, –592C/A, –819C/T and –1082G/A in the IL-10 gene are three most studied single nucleotide polymorphisms. To provide a more definitive conclusion about their association with the risk of HCV infection, a meta-analysis was performed by combining and summarizing a total of 17 studies. A biological justification for the choice of genetic model was provided. The results indicated no significant association between these IL-10 polymorphisms and the susceptibility to HCV infection [–592C/A: odds ratio (OR) 0·99, 95% confidence interval (CI) 0·78–1·25; –819C/T: OR 0·90, 95% CI 0·69–1·18; –1082G/A: OR 1·34, 95% CI 0·90–2·00]. However, this analysis did not account for the possible risk modifications by other factors, such as ethnicity and virus persistence. Therefore, the effects of ethnicity and virus persistence were investigated using Bayesian meta-regression and subgroup analysis. Finally, an extended case-control association study was conducted in a Chinese population involving 1140 subjects. Both serum level and genotype data of IL-10 –1082G/A were determined. As a result, a low prevalence of G allele was observed. Significantly higher IL-10 production was observed in HCV patients, especially patients with the GG genotype.

Key words: Association study, HCV, IL-10, meta-analysis, polymorphism.

INTRODUCTION

Hepatitis C virus (HCV) is a leading cause of liver disease worldwide [1]. About 80% of infected patients fail to clear the virus and develop chronic infection [2]. Most of these patients may further progress to liver cirrhosis and eventually hepatocellular carcinoma (HCC). To date, HCV infection has been attributed to

several molecular mechanisms concerning transcription, translation and secretion pathways [3, 4]. Most of these proposed mechanisms have implicated that cytokines may play an important role in the regulation of immune responses against viral infection [5–7].

Interleukin-10 (IL-10) is one of the cytokines involved in HCV infection. It is located on chromosome 1 and encodes five exons (5·1 kb) [8]. The IL-10 level differs widely between individuals probably due to its polymorphisms in the promoter region of the

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IL-10 gene, which includes three frequent point mutations –592C/A (rs1800872), –819C/T (rs1800871) and –1082G/A (rs1800896) [9]. Although –592C, –819C and –1082G alleles have been previously associated with higher IL-10 production [10], the population-based studies [11] investigating the association between IL-10 polymorphisms and risk of HCV infection have yielded conflicting results [12–25]. Some of the studies show a significant association [17, 18, 20], while others do not [6, 14]. In the presence of these inconsistent results, a new meta-analysis, conducted by compiling all available data and organizing them into a coherent summary, may provide a more definitive conclusion about the association between these IL-10 polymorphisms and the susceptibility to HCV.

The fact that some studies did not find the effect of IL-10 polymorphisms on the risk of HCV infection also implies an involvement of other risk factors. These factors might modify the outcome of meta-analysis. For example, ethnic difference in the examined populations could lead to a differential distribution of IL-10 promoter genotypes, resulting in a different response to HCV infection [26]. This type of heterogeneity can be explored by the inclusion of covariates at study level [27], such as meta-regression and subgroup analysis [28]. In subgroup analysis, the studies are stratified into homogeneous subgroups and meta-analysis is then conducted in each subgroup. Compared to subgroup analysis, meta-regression can accommodate study-level factors directly [29]. The study is the unit of analysis and the outcomes are effect size [i.e. odds ratios (ORs)]. Hence the effect size outcomes are correlated with risk factors measured at study level.

This report aims to determine the association of IL-10–592C/A, –819C/T and –1082G/A polymorphisms with the risk of HCV infection. The possible risk modifications by ethnicity and HCV persistence were investigated using both Bayesian meta-regression and subgroup analysis. Finally, an extended case-control study was conducted in a Chinese population involving 1140 subjects for the further assessment of the association evaluated here.

METHODS

Literature search

The systematic survey of eligible studies for meta-analysis was performed using electronic database

engines, including PubMed, the Cochrane Database for Systematic Reviews and EMBASE. The language of the reviewed articles was limited to English. The key words used for search were ‘hepatitis C’ or ‘HCV’, ‘interleukin-10’ or ‘IL-10’, ‘polymorphism’ and combinations thereof. The abstract of each citation was screened in order to select studies that explicitly focused on the association of IL-10 –592C/A, –819C/T and –1082G/A polymorphisms with the risk of HCV infection. Articles satisfying these criteria were further evaluated by two independent reviewers to determine if the inclusion criteria, listed below, were met. If so, the full article text was obtained. In addition, each reference cited in the obtained articles was reviewed in order to identify additional work not indexed by the database engines provided above. Efforts were also made to contact authors for missing data.

Inclusion/exclusion criteria

Inclusion criteria were (1) an original article, (2) a population-based association study of IL-10 polymorphisms at positions –592, –819 and –1082 with HCV infection being the outcome, and (3) a human study. Studies were excluded if they: (1) contained overlapped data, (2) were an animal study, (3) were without healthy controls, or (4) contained incomplete data and failed to respond to a data request. If more than one paper with the same data was found, the one that contained original data was reviewed.

Statistical analysis

Relevant data, including details of genotypes and frequencies of IL-10 at three positions –592, –819 and –1082, were first extracted. The effect size for each study was the OR. As both dominant and recessive modes have been employed in the selected studies, a biological justification for the choice of genetic model was required [30]. The procedure to capture the inheritance mode in association studies has been described previously [12, 28]. Using –592C/A polymorphism as an example here, λ (the ratio of $\log OR_{CA}$ and $\log OR_{CC}$) was determined; the pairwise estimates of the OR of CA vs. AA (OR_{CA}) and the OR of CC vs. AA (OR_{CC}) were performed by conducting two separate meta-analyses [30]. Values of λ equal to 0 and 1 correspond to the recessive and dominant genetic model, respectively. Afterwards, the OR in the full meta-analysis was estimated using the selected inheritance mode.

A χ^2 test was performed to determine if observed frequencies of genotypes conformed to Hardy–Weinberg (HW) expectations [31]. Studies that deviated from the HW equilibrium were excluded from meta-analysis. The heterogeneity of the group of ORs was assessed using H statistics and the I^2 metric, which suggest the presence of heterogeneity when $H \geq 1.2$ and $I^2 \geq 25\%$ [32]. Publication bias was assessed using a funnel plot. Cumulative meta-analysis was also performed to examine whether the magnitude of effect changed markedly with sample size. Then the ORs were pooled, and 95% confidence intervals (CIs) were calculated using meta-analysis [33, 34]. In the meta-analysis, the Mantel–Haenszel and DerSimonian–Laird methods were employed in fixed-effects and random-effects models, respectively. Furthermore, sensitivity analyses were conducted to evaluate the robustness of our results. We removed each study individually to evaluate its effect on the pooled OR [35]. All parameters of the model were estimated by the ‘metan’ package within Stata software v. 10.1 [36]. The significance of the pooled OR was determined by Z test, and a 95% CI was calculated.

Bayesian meta-regression was performed to determine the impact of ethnicity on the association of IL-10 –592C/A, –819C/T, –1082G/A polymorphisms with the risk of HCV infection [37]. The procedure has been described previously [28]. Briefly, log ORs of selected studies were regressed on an intercept and covariates (i.e. ethnicity). The significance of effect was identified by the value and CI of the corresponding regression coefficient ($\beta_{\text{ethnicity}}$). The regression process was performed using Markov Chain Monte Carlo (MCMC) and Gibbs sampling algorithms through Bayesian computation software WinBUGS. The prior distribution for standard deviation σ was given as a uniform distribution with a mean of 0 and a variance of 100 to reflect the non-informative situation of effect size. Ten thousand iterations were dismissed as burn-in, and the subsequent 100 000 iterations were used for parameter estimation. Satisfactory convergence of the simulated Markov chains to the target posterior distributions was assessed using visual inspection of posterior traces and running chains with different initial values of the model parameters. Next, subgroup meta-analysis was performed by stratifying the existing studies into subgroups according to ethnicity and virus persistence, and conducting quantitative pooling separately for each subgroup.

An association study in a Chinese population involving 1140 subjects

New subjects

This study was approved by the institutional review board of Nanjing Medical University, Nanjing, China. Chronic HCV patients ($n=586$) were consecutively recruited between January 2007 and March 2011 at the First Affiliated Hospital of Nanjing Medical University, Nanjing, China [328 men, 258 women; mean age (range) 47.9 (15–75) years]. They were biologically unrelated ethnic Han Chinese from Nanjing City and surrounding regions in Jiangsu Province, China. Diagnosis in all patients was confirmed by both biochemical and molecular assays, including detection of anti-HCV antibody using a third-generation commercial enzyme immunoassay (Chiron Corporation, USA) and detection of HCV RNA in serum using an Amplicor HCV assay (Roche Diagnostics, Germany). Patients presenting with other causes of chronic hepatitis (i.e. alcoholic or autoimmune hepatitis), testing positive for other hepatotropic viral antigens, or treated with antiviral drugs before liver biopsy or echography were excluded. A total of 554 healthy control subjects tested negative for HCV infection were selected randomly from blood donors from the same region during the same time period as the cases were recruited [302 men, 252 women; mean age (range) 51.7 (16–79) years]. Informed consent was obtained from case and control subjects.

DNA extraction and genotyping

Peripheral blood (5 ml) was collected in EDTA tubes (BD Biosciences, Germany). Genomic DNA was extracted and purified from whole-blood lymphocytes using a blood DNA kit (Qiagen, USA) according to the manufacturer’s instructions. Genotyping of IL-10 polymorphism at position –1082 was performed by an allele-specific PCR (AS-PCR) assay. The PCR primer sequences were as follows: (i) primer A (sense): 5'-ACTACTAAGGCTTCTTTGGGAA-3'; (ii) primer G (sense): 5'-CTACTAAGGCTTCTTTGGGAG-3' and (iii) generic primer (antisense): 5'-CAGTGC-CAACTGAGAATTTGG-3' [38]. The PCR was performed in a 20- μ l volume of reaction mixture containing 10 \times PCR buffer (500 mM KCl, 100 mM Tris–HCl, 0.8% Nonidet P40; Fermentas, USA), 2 mM MgCl₂, 0.8 mM dNTPs, 0.5 μ M specific primer, 50 ng template DNA and 0.5 U *Taq* polymerase (Fermentas). The PCR cycle conditions were 94 °C

for 5 min, followed by 35 cycles at 94 °C for 30 s, 68 °C for 60 s with a final extension step at 72 °C for 10 min. The amplified AS-PCR products were identified by gel electrophoresis on 2% agarose gels stained with ethidium bromide and visualized with ultraviolet light.

Measurement of serum IL-10

Serum samples were subjected to an enzyme-linked immunosorbent assay (ELISA). The assay was performed in the First Affiliated Hospital of Nanjing Medical University using an IL-10 ELISA kit (R&D systems, USA) on a model 680 Microplate Reader (Bio-Rad, USA).

Statistical analysis

The association between IL-10 $-1082G/A$ polymorphism and susceptibility to HCV in this Chinese population was analysed using χ^2 test. ORs and the corresponding 95% CIs were calculated. Student's *t* test was used to test differences in the means of IL-10 serum levels between groups.

RESULTS

Characteristics of studies

Seventeen case-control studies met the inclusion criteria, of which 14 studies/subgroups reported data for polymorphism meta-analysis. Three studies were excluded because they lacked either a control group or genotype data [24, 26, 39]. All the selected studies presented original data on independent samples. Of these, 10, 9 and 14 studies reported data concerning IL-10 polymorphisms of $-592C/A$, $-819C/T$ and $-1082G/A$, respectively. The numbers of patients and controls are listed in Table 1.

Six studies were excluded from the meta-analysis assessing the association between IL-10 $-1082G/A$ polymorphism and the susceptibility to HCV because the observed distribution of genotypes in controls was inconsistent with HW equilibrium [12, 15, 18, 21, 23, 25]. All control samples of $-592C/A$ and $-819C/T$ polymorphisms were in HW proportions. The results of homogeneity analysis suggested no statistically significant heterogeneity across the studies of IL-10 polymorphisms at positions -592 ($H=1.00$, $I^2=0\%$, $P=0.758$) and -819 ($H=1.00$, $I^2=0\%$, $P=0.932$), whereas a high degree of heterogeneity was found in $-1082G/A$ polymorphism ($H=1.56$, $I^2=53.3\%$, $P=0.029$). In addition, no clear evidence for

publication bias was observed in the inversed funnel plots of the polymorphisms evaluated here. The resulting *P* values were 0.19, 0.53 and 0.70, respectively. The cumulative meta-analyses did not show that sample size had a significant impact on the pooled ORs (see Supplementary Fig. S1). Sensitivity analyses excluding one study at a time for studies on the association of IL-10 polymorphisms with the risk of HCV infection confirmed the direction and magnitude of statistical significance of the overall effect, even after the exclusion of the largest study (see Supplementary Fig. S2).

The choice of genetic model

The plots of $\log OR_{CA}$ vs. $\log OR_{CC}$ ($-592C/A$), $\log OR_{CT}$ vs. $\log OR_{CC}$ ($-819C/T$) and $\log OR_{GA}$ vs. $\log OR_{GG}$ ($-1082G/A$) are shown in Figure 1, in which the slope represents λ . The resulting values of λ for $-592C/A$ and $-819C/T$ polymorphisms were 0.708 ± 0.014 and 0.715 ± 0.017 , respectively, suggesting dominant models. In addition, a recessive model was pronounced in the $-1082G/A$ polymorphism, $\lambda = -0.004 \pm 0.005$. Therefore, full meta-analyses were performed using dominant inheritance mode for polymorphisms at positions -592 ($CC+CA$ vs. AA) and -819 ($CC+CT$ vs. TT), and recessive mode for -1082 (GG vs. $GA+AA$). Notably, the plots in Figure 1 can help to check the consistency of λ across studies and identify outlier studies. Ideally, all studies would be expected to lie along a straight line with slope λ in the absence of heterogeneity in the genetic model. Here no significant outliers or influential studies were visually identified. To further elucidate the advantages of biological justification, study-specific estimates of λ and bootstrapped 95% CIs are depicted in the Supplementary material (Supplementary Fig. S3), which can help assess whether any departures from linearity in Figure 1 can be explained by sampling error [30]. The findings revealed that the selected genetic models were consistent across studies. Otherwise, it may be better to perform joint pairwise comparisons using a general bivariate meta-analysis model [40].

Meta-analysis of the association between IL-10 polymorphisms and the risk of HCV infection

Fixed-effects models were employed for the full meta-analysis of the relationship between IL-10 $-592C/A$ and $-819C/T$ polymorphisms and the susceptibility

Table 1. Characteristics of case-control studies included in the full meta-analysis

Study (first-named author)	Country	- 592				- 819				- 1082			
		Genotype for cases		Genotype for controls		Genotype for cases		Genotype for controls		Genotype for cases		Genotype for controls	
		CC+CA	AA	CC+CA	AA	CC+CT	TT	CC+CT	TT	GG	GG+AA	GG	GA+AA
Afzal [23]	Pakistan	-	-	-	-	73	16	84	15	15	74	3*	96
Dogra [25]	India	-	-	-	-	-	-	-	-	38	32	42*	28
Gao [21]	China	35	20	40	35	-	-	-	-	2	53	1*	73
Chuang [12]	Taiwan	70	73	69	65	71	72	69	65	0	143	0*	133
Bouzgarrou [13]	Tunisia	-	-	-	-	-	-	-	-	19	81	12	91
Abbas [14]	Pakistan	87	12	57	5	87	12	57	5	17	82	9	53
Pereira [6]	Brazil	110	18	84	10	110	18	84	10	17	111	13	81
Chen [15]	Taiwan	36	36	87	93	36	36	86	94	0	72	0*	180
Falleti [22]	Italy	21	29	35	61	-	-	-	-	8	42	25	71
Persico [16]	Ireland	218	10	104	6	218	10	104	6	74	154	18	92
Mangia [20]	Italy	246	24	136	9	246	24	136	9	40	230	23	122
Zein [19]	USA	29	1	33	3	29	1	33	3	12	18	6	30
	Egypt	20	2	41	3	20	2	41	3	5	17	14	30
Lio [18]	Italy	-	-	-	-	-	-	-	-	27	33	34*	101
Vidigal [17]	Ireland	69	9	33	3	69	9	33	3	27	51	6	30

* The distribution of genotypes in controls was not consistent with Hardy-Weinberg equilibrium law ($P < 0.0001$).

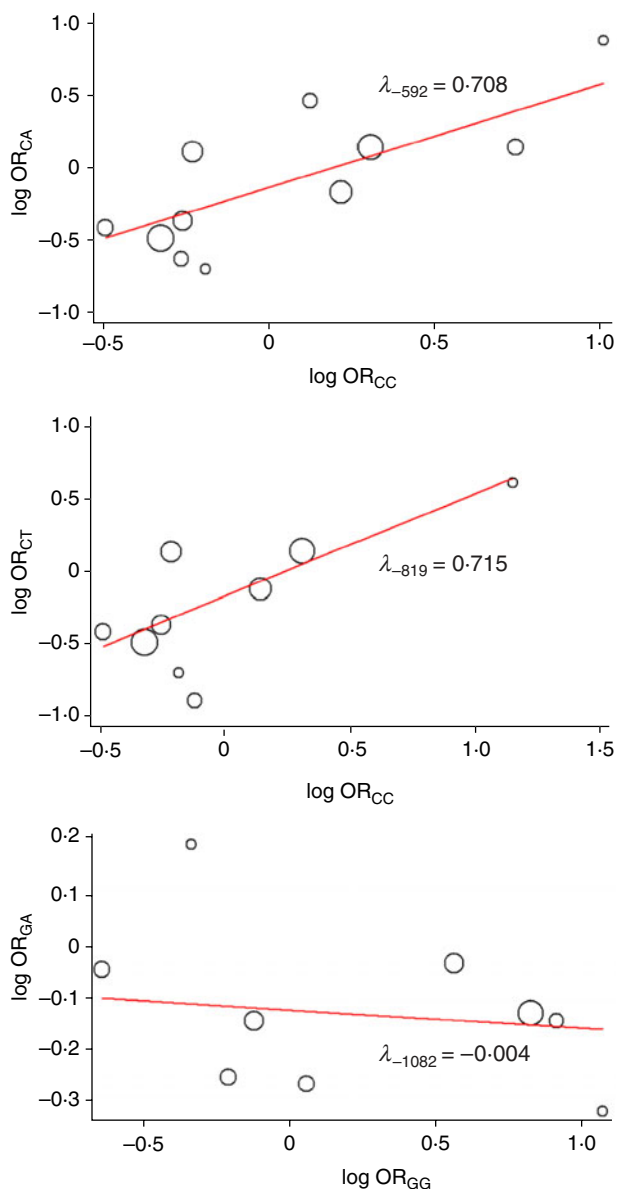


Fig. 1 [colour online]. Plots of log odds ratio (OR)_{CA} against log OR_{CC} (–592C/A), log OR_{CT} against log OR_{CC} (–819C/T) and log OR_{GA} against log OR_{GG} (–1082G/A) for IL-10 polymorphisms and risk of HCV infection. The slope of the solid line represents λ and size of circular symbols denotes the weight of study.

to HCV. The pooled ORs were 0.99 (95% CI 0.78–1.25) and 0.90 (95% CI 0.69–1.18), respectively (Fig. 2). In the case of –1082 G/A polymorphism, a random-effects model was used due to the presence of heterogeneity. The pooled OR and 95% CI were 1.34 and 0.90–2.00, respectively (Fig. 2). Overall, no significant association was found between the risk of HCV infection and the IL-10 polymorphisms investigated here.

It should be noted that the above conclusion cannot prevent an assumption of possible risk modifications by other factors, and also that the selected studies contained data on several ethnic populations, including primarily Caucasians and Asians. Thus, subgroup meta-analysis was performed to evaluate the effect of polymorphisms in these two ethnic populations, by conducting quantitative pooling individually. A forest plot assessing the ethnicity effect is shown in Figure 3. All the 95% CIs included the null value 1. For studies on the association of –1082G/A polymorphism, the interaction appeared a little stronger in Caucasians ($P=0.193$). Further, considering the high heterogeneity described previously, a binomial logistic regression analysis using a Bayesian model [41] with ethnic status (Caucasians=1, Asians=0) as the dependent variable and the distribution of genotypes as an independent variable was performed to illustrate whether ethnicity was a risk modification factor. As a result, no clear evidence for the modification effect of ethnicity was observed ($\beta_{-1082} = -0.274 \pm 0.636$) when HCV patients were compared to healthy controls.

Moreover, HCV infection can become chronic in most infected individuals. Only a few infected people spontaneously clear the virus. To date, HCV persistence has also been related to the production of inappropriate levels of IL-10 [17]. Therefore, the studies on patients with chronic HCV were grouped and another meta-analysis was performed in comparison with healthy controls. Under the premise of chronic HCV, no significant associations were further observed for these polymorphisms (data not shown).

Chinese association study

The demographic data of the study is presented in Table 2. Representative genotyping data are shown in Supplementary Fig. S4. The size of the AS-PCR product was 258 bp. The genotype and allele frequencies of IL-10 –1082G/A were 79.2% for AA genotype, 16.9% for GA genotype, 3.9% for GG genotype, 87.5% for A allele and 12.5% for G allele in controls and 71.9% for AA genotype, 15.6% for GA genotype, 12.5% for GG genotype, 79.7% for A allele and 20.3% for G allele in cases. No statistically significant difference was observed between chronic HCV patients and healthy controls for IL-10–1082G/A polymorphism (OR 2.64, 95% CI 0.690–10.1, $P=0.156$). Notably, the G allele was found less frequently in this Chinese population

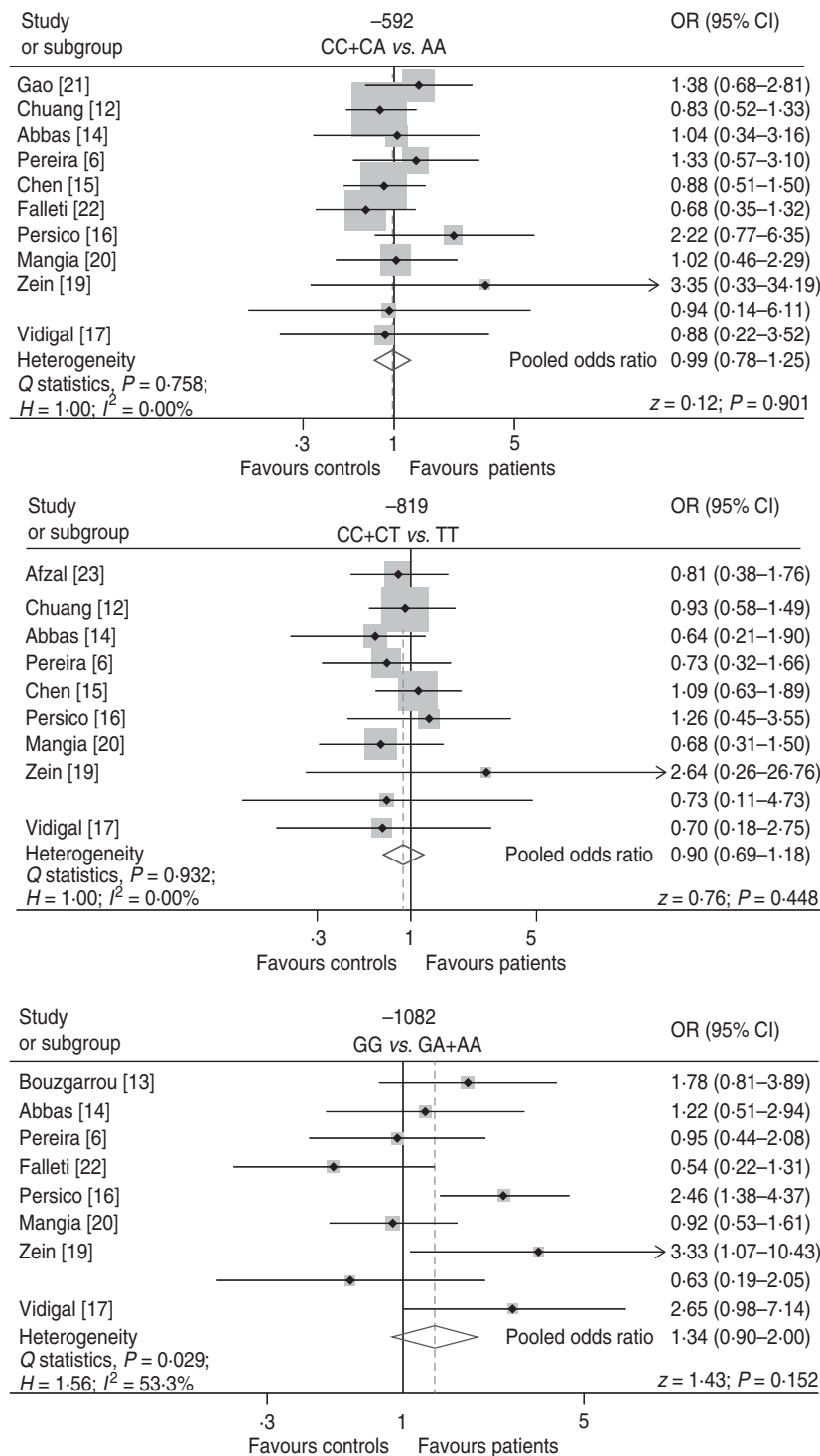


Fig. 2. Association between IL-10 polymorphisms and risk of HCV in full meta-analysis. The pooled odds ratio (OR) and 95% confidence interval (CI) were generated using either a fixed-effects model (Mantel–Haenszel method) or a random-effects model (DerSimonian–Laird method). Studies are ordered by publication year.

(i.e. 12.5%) than in Caucasians (36.1–57.0%), while approaching its prevalence in Taiwanese (3.4% and 7.8%). Furthermore, the ELISA results indicated that

the serum level of IL-10 was significantly higher in HCV patients than in controls ($P < 0.0001$, Fig. 4a). In particular, a significantly higher serum IL-10 was

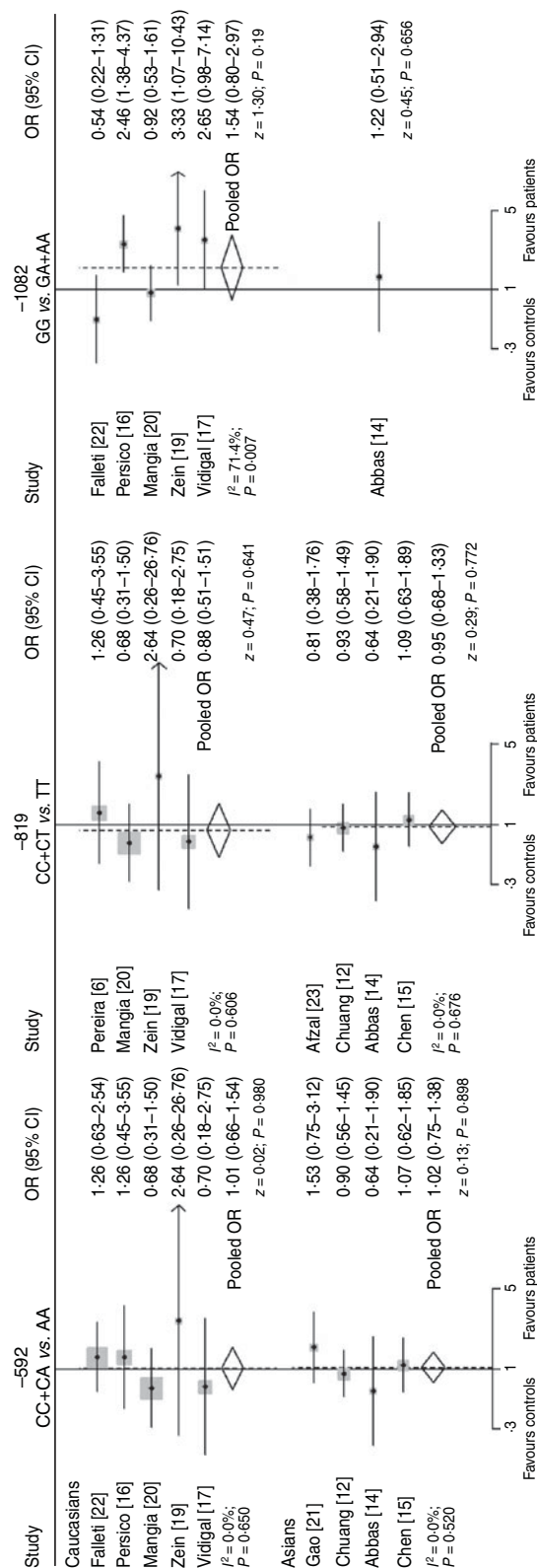


Fig. 3. IL-10 polymorphisms and risk of HCV in (a) Caucasian and (b) Asian populations. Studies are ordered by publication year. OR, Odds ratio; CI, confidence interval.

Table 2. Demographic data of HCV patients and controls

Data	HCV patients (n = 586)	Controls (n = 554)
Age (yr)	47.9 ± 15.3	51.7 ± 16.1
Body mass index	23.1 ± 4.2	23.7 ± 4.1
Anti-HCV/HCV RNA	Positive	Negative
Gender		
Male	328	302
Female	258	252
IL-10 -1082		
GG	73	22
GA	92	94
AA	421	438

observed in patients with the GG genotype ($P < 0.0001$, Fig. 4b).

DISCUSSION

Genetic association studies commonly have three genotype groups for a bi-allelic polymorphism, one of which is thought to be associated with a disease. In practice, three groups are often reduced to two by assuming a specific genetic model, such as a dominant or recessive model. However, a biological justification for the choice of generic model is rarely available [30, 31]. Lack of coherence or transparency is a common issue in choosing a model of meta-analysis. In this report, different genetic models were found in the selected epidemiological studies. As mentioned previously, inappropriate specification of inheritance mode could increase the risk of false negatives. For example, the association between IL-10-1082G/A polymorphism and the risk of HCV infection was more significant using a recessive model ($P = 0.0018$) than a dominant one ($P = 0.0742$) in the study by Persico *et al.* [16]. Therefore, we used a linear regression of pairwise estimates (two log ORs), weighted by study to determine the most appropriate genetic mode [40]. The results indicated dominant inheritance in IL-10-592C/A and -819C/T polymorphisms and recessive inheritance in -1082G/A. Furthermore, the presence of both dominant and recessive inheritances in the IL-10 gene suggested a complex interaction between genotype and protein expression. Therefore, the genetic contribution of single genes towards a complex disease is unlikely to act in a simple Mendelian fashion. To confirm dominantly and recessively inherited polymorphisms,

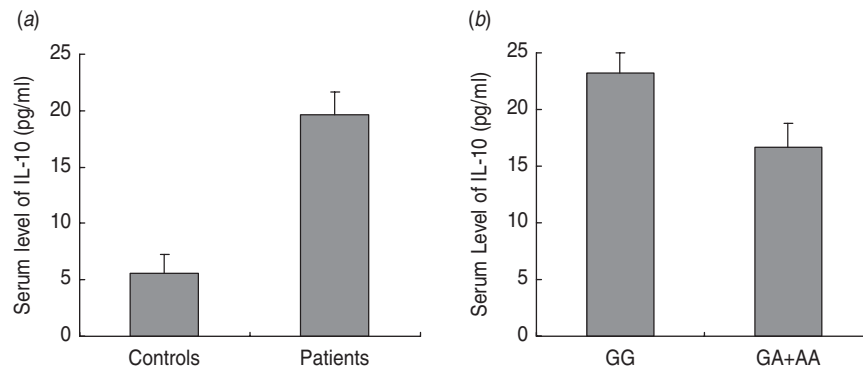


Fig. 4. Serum levels of IL-10 in (a) HCV patients and controls, and (b) HCV patients with GG and GA + AA genotypes.

additional phenotype-genotype studies need to be performed.

No overall significant association was observed between the IL-10 polymorphisms and the risk of HCV infection using meta-analysis. However, the effect of polymorphisms on the susceptibility to HCV may be modified by other risk factors such as ethnicity. This hypothesis has been recently suggested by the fact that African patients composed 53% of all HCV-positive patients while constituting only slightly less than one third of HCV-negative patients [42]. In addition, significantly higher IL-10 production was generated *in vitro* by peripheral blood mononuclear cells (PBMCs) isolated from healthy Caucasian subjects compared to first nation subjects [43]. One possible explanation can be the different amounts of IL-10 produced in populations, which was probably results from differential distribution of the cytokine polymorphisms [44]. It has been reported that PBMCs obtained from individuals presenting IL-10 -1082G/G genotype produced twofold greater quantities of IL-10 compared to individuals exhibiting GA or AA genotypes at the same position [45]. Moreover, Asians had a significantly higher proportion of genotypes that resulted in low IL-10 production [13]. Nevertheless, the significance of either subgroup analysis or Bayesian meta-regression in this study was not enough to accept or reject the hypothesis suggested above. Therefore, to further examine whether IL-10 -1082G/A is associated with the risk of HCV infection, an extended case-control study was conducted in a Chinese population. Despite no significant association being found, a low prevalence of G allele was observed. Furthermore, significantly higher IL-10 production was observed in HCV patients and especially patients with the GG genotype, which were also observed previously [13, 17].

Surprisingly, no essential role of IL-10 polymorphisms was found in the persistence of HCV. On the contrary, the incidence of chronicity has been associated with the ability of proteins to modulate immune responses upon various occasions. For example, enhanced production of IL-10 from antigen-presenting cells in the early stages of infection can alter the development of protective T-cell responses [46], contributing to impaired IFN- γ production and proliferation of HCV-specific T cells [47, 48]. However, it should be noted that some of those patients with chronic HCV infection may progress to liver fibrosis, cirrhosis and eventually HCC [49]. Thus, the association between the genetic polymorphisms and chronic HCV may depend on the severity/progress of HCV. Case-control studies have shown that women carrying IL-10 -1082G/G genotype were associated with a higher risk of HCV persistence but a lower risk of progress to cirrhosis [50]. This finding was also supported by the protective role of IL-10 against progressive fibrosis in chronic infection [51]. The variability of outcomes in the progress of HCV precludes making a more definitive statement on the association evaluated here. The effort in grading the severity of HCV could help elucidate the role of IL-10 polymorphisms in HCV progress. Notably, other suspected risk factors for the susceptibility to HCV (e.g. age, sex and body mass index) also deserve extensive investigation.

It should be noted that the risk of bias of individual studies such as selection bias could be a potential issue in meta-analysis [52]. In this meta-analysis, sampling bias as one of the types of selection biases might cause some members of the population to be less likely to be included than others, resulting in that all participants are not equally balanced or objectively represented. Since we cannot obtain more information about the

individual studies in meta-analysis, we are uncertain whether there are other selection biases existing in those individual studies, e.g. if the selection process does not aim to include a patient spectrum similar to the population in which the test will be used in practice, if the populations are systematically different between comparison groups within a study (e.g. important baseline imbalances), if the studies apply inclusion/exclusion criteria uniformly to all comparison groups, etc. Furthermore, selection bias may be present in the process of meta-analysis due to the inability to identify and include all conducted and relevant studies [53]. Such selection bias can cause exaggerated or even false-positive gene-disease associations. Failure to include all relevant studies is largely caused by selective publication of studies with certain results, and the inability to identify studies published in languages other than English. All these biases could lead to meta-analysis an incomplete set of the evidence and produce summary results potentially biased.

As mentioned previously, a number of cytokines are involved in HCV infection. Among the cytokines identified, T-cell cytokines have been highly associated with the host immune response to HCV infection. IL-10 is an anti-inflammatory T-helper cytokine (Th2), while it can also be produced by regulatory T (T_{reg}) cells [54]. The balance between the T cell-mediated immunities has been proven to be a determinant in many viral or non-viral infections [15]. On the other hand, the imbalance in immune-mediated diseases is commonly mediated by a variety of genetic polymorphisms in the genes. As HCV infection is an interaction between multiple genetic events, effects due to linkage disequilibrium (LD) between genotype variants cannot be excluded. In this study, some of these were considered in meta-analysis using a Bayesian hierarchical model. As a result, in the presence of strong LD between IL-10 -592C/A, -819C/T and -1082G/A polymorphisms, no more evidence for their clear association with the risk of HCV infection was found (data not shown). It is noteworthy that the interactions of these single nucleotide polymorphisms (SNPs) with other genetic variants were not taken into account here. To further model the effects of more SNPs in a region as well as gene \times gene interactions, highly computationally efficient methods will be needed, in addition to the amount of previous information available on haplotype frequencies. Therefore, large whole-genome association studies (GWAS) are eagerly awaited to resolve this issue in the near future.

CONCLUSIONS

The present analysis provides increased support for clarifying the overall association of IL-10 -592C/A, -819C/T and -1082G/A polymorphisms with the risk of HCV infection. Moreover, Bayesian meta-regression and subgroup analysis have demonstrated their attractiveness for exploration of the possible impacts of ethnicity and HCV persistence on the association evaluated here. Finally, an extended case-control study conducted in a Chinese population showed a low prevalence of G allele, and significantly higher IL-10 levels in HCV patients, especially patients with GG genotype.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit <http://dx.doi.org/10.1017/S0950268812002154>.

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DECLARATION OF INTEREST

None.

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