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BRIEF ARTICLE

Interleukin-10-1082G/A polymorphism and acute liver graft rejection: A meta-analysis

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Abstract

AIM: To investigate the association between interleukin (IL)-10-1082 (G/A) promoter polymorphism and acute rejection (AR) in liver transplant (LT) recipients.

METHODS: Two investigators independently searched the Medline, Embase, China National Knowledge Infrastructure, and Chinese Biomedicine Databases. Summary odds ratios (ORs) and 95% CIs for IL-10-1082 G/A polymorphism and AR were calculated in a fixed- and a random-effects model as appropriate.

RESULTS: This meta-analysis included seven case-control studies, which comprised 652 cases of LT recipients in which 241 cases developed AR and 411 cases did not develop AR. Overall, the variant A allele was not associated with AR risk when compared with the wild-type G allele (OR = 0.94, 95% CI: 0.64-1.39). Moreover, similar results were observed when the AA genotype was compared with the AG/GG genotype (OR = 1.05, 95% CI: 0.55-2.02). When stratifying for ethnicity, no significant association was observed among either Caucasians or Asians. Because only one study was performed in Asian patients, the result of subgroup analysis by ethnicity would not be reliable for Asians. Limiting the analysis to the studies with controls in the Hardy-Weinberg equilibrium, the results were persistent and robust. No publication bias was found in the present study.

CONCLUSION: This meta-analysis suggests that IL-10-1082 G/A polymorphism may be not associated with AR risk in LT recipients among Caucasians.

Key words: Liver transplantation; Acute rejection; Interleukin-10; Gene polymorphism; Meta-analysis

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INTRODUCTION

Liver transplantation is regarded as an effective therapeutic option for end-stage liver disease as survival after liver transplantation has dramatically improved during

the last two decades. Despite this success, graft dysfunction occurs in up to 13% of the patients during the first year after transplantation and rises to 35% in 5 years[1,2]. Acute rejection (AR) and recurrence of disease are two major immunological complications, which may lead to graft dysfunction. The inflammatory microenvironment within the graft may play a role in the precipitation of rejection[3], although the underlying mechanisms involved in such events remain unclear. A network of short-acting cytokines and growth factors in turn determines this environment. Cytokines have a central role in the immunologic events that occur after transplantation and are intimately implicated in graft rejection.

Interleukin-10 (IL-10), whose encoding gene is located on chromosome 1 (1q31-1q32), is an immunoregulatory cytokine produced by Th2 cells, monocytes/macrophages, and regulatory T cells, and is capable of downregulating T-cell activation and major histocompatibility complex expression on antigen-presenting cells *in vitro*[4]. Previous studies have suggested that IL-10 mRNA levels are increased just before a rejection episode[5]. The production of cytokines (including IL-10) is under genetic control and varies among individuals as a function of polymorphisms within the regulatory regions of the various genes that determine the transcriptional activation[6-9]. The promoter of the *IL-10* gene contains three biallelic polymorphisms at positions -1082 (base G to A, dbSNP no. rs1800896), -819 (base C to T, dbSNP no. rs1800871), and -592 (base C to A, dbSNP no. rs1800872) from the transcription start site, and these influence the capacity of cells to produce IL-10[10]. For example, the G-to-A polymorphism at position -1082 of the IL-10 promoter reduces IL-10 production[7]. Alloimmune responses and variations in susceptibility to rejection may be influenced by individual variations in cytokine genes. Associations between cytokine gene polymorphisms and rejection of kidney[11,12], heart[13], and lung[14] have been reported.

Over the last two decades, a number of studies have assessed the association between the IL-10-1082 (G/A) promoter polymorphism and AR in liver transplant (LT) recipients in different populations; however, the results are inconsistent and inconclusive[15-22]. In 2005, Warlé *et al*[23] published findings from a meta-analysis of the IL-10-1082 (G/A) polymorphism and AR risk in LT recipients (based on five studies). The pooled results by Warlé *et al*[23] suggested that the IL-10 polymorphism at position -1082 was a genetic risk factor for acute liver graft rejection, and that LT recipients carrying the IL-10-1082. A allele displayed a lower rejection rate. However, this manuscript had some limitations mainly due to the small sample size and data retrieval. In order to derive a more comprehensive estimation of the association between IL-10-1082 polymorphism and AR risk in LT recipients, we conducted a meta-analysis to re-evaluate the association.

MATERIALS AND METHODS

Literature search strategy

We searched the PubMed, Embase, CNKI (China National Knowledge Infrastructure) and Chinese Biomedicine databases for all articles on the association between IL-10 polymorphisms and AR risk in LT recipients (last search update 20th March 2011). The following key words were used: "interleukin-10" or "IL-10"; "acute rejection" or "early graft rejection"; "liver transplantation". The search was performed without restriction on language, but conducted on human subjects. The reference lists of reviews and retrieved articles were hand searched at the same time. We did not consider abstracts or unpublished reports. If more than one article was published by the same author using the same case series, we selected the study where the most individuals were investigated.

Inclusion and exclusion criteria

We reviewed abstracts of all citations and retrieved studies. The following criteria were used to include published studies: (1) evaluation of the association between IL-10-1082 G/A polymorphism and AR in LT recipients; (2) a case-control or cohort design; and (3) sufficient genotype data presented to calculate the odds ratio (OR) with 95% confidence interval (CI). Major reasons for exclusion of studies were: (1) duplicate data; (2) an abstract, comment, review or editorial; and (3) no sufficient data were reported.

Data extraction

Two investigators (Liu F and Li B) extracted information from all eligible publications independently according to the inclusion criteria listed above. Disagreements were resolved by discussion between the two investigators. The following information was collected from each study: first author, year of publication, transplant period, indication for transplantation, patient characteristics (age, gender, *etc*), definition of AR, immunosuppressive regimen, country of the first or corresponding author, ethnicity, number of AR cases and controls (non-AR), genotyping methods and evidence of Hardy-Weinberg equilibrium (HWE). Ethnicities were categorized as Asian or Caucasian.

Statistical analysis

We first assessed HWE in the controls for each study using the goodness-of-fit test (χ^2 or Fisher's exact test) and a $P < 0.05$ was considered as significant disequilibrium. The strength of the association between AR and the IL-10-1082 G/A polymorphism was estimated using the OR and corresponding 95% CI. For the -1082G/A polymorphism, we estimated the risk of the variant A allele compared with the wild-type G allele, and then evaluated the risk of AA *vs* (AG + GG) which assumed a recessive effect of the variant A allele. We also carried out the stratified analyses by ethnicity (Caucasians/Asians) and HWE in controls (yes/no).

Both the Cochran Q statistic[24] to test for heterogeneity and the I^2 statistic to quantify the proportion of the total variation due to heterogeneity[25] were calculated. A P value of more than the nominal level of 0.10 for the Q

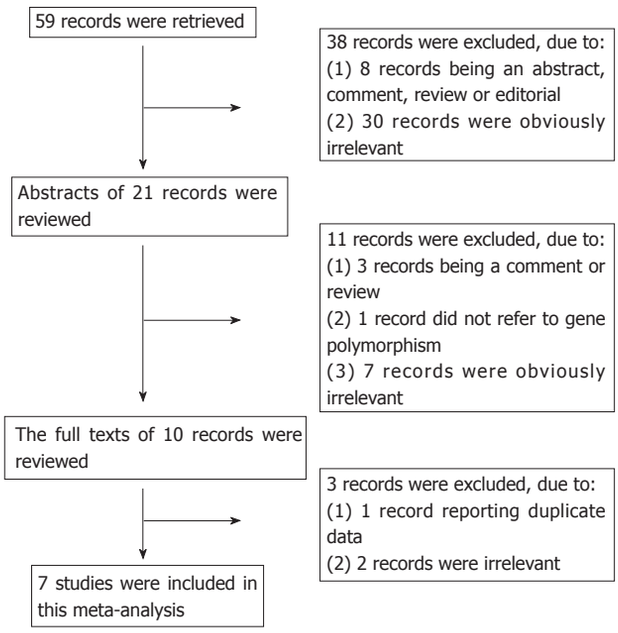

Figure 1 Flow chart of selection of studies and specific reasons for exclusion from the meta-analysis.

statistic indicated a lack of heterogeneity across studies, allowing for the use of a fixed-effects model (the Mantel-Haenszel method)[26]; otherwise, the random-effects model (the DerSimonian and Laird method) was used[27]. Sensitivity analysis was performed to assess the reliability of the results.

Several methods were used to assess potential publication bias. Visual inspection of funnel plot asymmetry was conducted. The Begg's rank correlation method[28] and the Egger's weighted regression method[29] were used to statistically assess publication bias ($P < 0.05$ was considered statistically significant). All analyses were done using STATA software, version 11.0 (STATA Corp., College Station, TX, United States). All the *P* values were two-sided.

RESULTS

Characteristics of studies

There were 59 papers relevant to the search words. *Via* steps of screening the title and reading the abstract, 10 studies were identified[15-22,30,31]. Of these, three studies were excluded (two did not report the association between IL-10-1082 G/A polymorphism and AR in LT recipients[22,30]; two articles[20,31] were published by a different first author using the same case series, and we selected the latest study[20]); thus, seven studies[15-21] which included 241 AR cases and 411 non-AR cases were found to match our inclusion criteria. The flow chart of selection of studies and reasons for exclusion is presented in Figure 1. Characteristics of studies included in the meta-analysis are presented in Tables 1 and 2.

There were six studies of Caucasian descendents, one study of Asian descendents. Studies had been carried out in China, Turkey, the United States, Netherlands, Israel and the United Kingdom. All studies defined rejection as biopsy-proven episodes of AR during the early post-transplant period (AR within first 4-8 wk), treated with high-dose steroids, except for the study of Karasu *et al*[16]. Immunosuppressive regimens in all studies consisted of a calcineurin inhibitor (cyclosporin or tacrolimus) and prednisone with or without azathioprine. Mycophenolate mofetil was only used in a subgroup of patients studied by Xie *et al*[15] and Mas *et al*[17]. Most studies extracted DNA from peripheral blood, and only two studies[15,17] from surgically explant liver tissue from recipients. Several genotyping methods were used, including PCR-RFLP, PCR-SSP, direct sequencing, ARMS-PCR and AS-PCR. The genotype distributions among the controls of all studies were consistent with HWE except for Tambur's study[20].

Quantitative synthesis

Overall, the variant A allele was not associated with AR risk when compared with the wild-type G allele (OR_{random} = 0.94, 95% CI: 0.64-1.39, $P_{heterogeneity}$ = 0.07) (Figure 2). When the AA genotype was compared with AG/GG genotype (recessive model), no significant association was observed (OR_{random} = 1.05, 95% CI: 0.55-2.02, $P_{heterogeneity}$ = 0.01) (Figure 3). When stratified for ethnicity, no significant association was observed among either Caucasians or Asians (for Caucasians: A allele *vs* G allele, OR_{random} = 0.95, 95% CI: 0.61-1.47, $P_{heterogeneity}$ = 0.04; AA *vs* AG/GG, OR_{random} = 1.07, 95% CI: 0.49-2.32, $P_{heterogeneity}$ = 0.01; for Asians: A allele *vs* G allele, OR_{random} = 0.96, 95% CI: 0.34-2.68; AA *vs* AG/GG, OR_{random} = 0.96, 95% CI: 0.33-2.77). Because only one study was performed in Asian patients, the result of subgroup analysis by ethnicity could not be reliable for Asians.

In Tambur's study, the distribution of *IL-10-1082* genotypes among controls was not in HWE. Limiting the analysis to the studies within HWE, the estimated association remained unchanged (A allele *vs* G allele, OR_{fixed} = 0.81, 95% CI: 0.61-1.07, $P_{heterogeneity}$ = 0.13; AA *vs* AG/GG, OR_{random} = 0.98, 95% CI: 0.46-2.11, $P_{heterogeneity}$ = 0.009).

Publication bias

Begg's funnel plot and Egger's test were performed to evaluate the publication bias of studies of AR in LT recipients. Figures 4 and 5 display funnel plots that examined the IL-10-1082 polymorphism and overall AR risk included in the meta-analysis. The shape of funnel plots did not reveal any evidence of funnel plot asymmetry. The statistical results did not show publication bias (A allele *vs* G allele: Begg's test P = 0.55, Egger's test P = 0.26; AA *vs* AG/GG: Begg's test P = 0.76, Egger's test P = 0.67).

DISCUSSION

In spite of major advances in the field of immunosuppressive therapy, acute hepatic allograft rejection remains an important problem after liver transplantation. Almost 30%-50% of patients experience at least one episode of rejection within the first year[32]. Cytokines, a group

Table 1 Baseline characteristics of studies included in the meta-analysis

Ref.	Transplant period	Indications for transplantation	Patients characteristics (age, gender)	Definition of acute rejection	Immunosuppression regimens
Bathgate *et al*[21]	1992-1998	ALD, PBC, PSC, chronic viral hepatitis, acute liver failure, autoimmune hepatitis, other	Not described	Liver biopsy and treatment with high-dose steroids	CsA/tacrolimus + prednisone + azathioprine
Tambur *et al*[20]	Not described	Hepatitis B and/or hepatitis C, PBC, PSC, cryptogenic, other	20-69 yr, M/F: 32/36	Liver biopsy (AR within first 6 wk)	CsA/tacrolimus + prednisone with or without azathioprine.
Warlé *et al*[18]	1992-1999	Hepatitis B, hepatitis C, PBC, PSC, ALD, other	AR group: 47 ± 11 yr, M/F: 22/19 Non-AR group: 49 ± 12 yr, M/F: 20/28	Liver biopsy and treatment with high-dose steroids (AR within first 4 wk)	CsA/tacrolimus + prednisone Maintain target therapeutic blood levels of 100-200 ng/mL for CsA or 5-10 ng/mL for tacrolimus
Fernandes *et al*[19]	Not described	Not described	19-73 yr, M/F: 26/27	Liver biopsy and treatment with high-dose steroids	Tacrolimus + prednisolone
Mas *et al*[17]	1999-2000	Hepatitis B, Hepatitis C, PSC, HCC, ALD, Cryptogenic, other	24-60 yr, M/F: 44/33	Liver biopsy (AR within first 8 wk)	CsA/tacrolimus + steroids + MMF
Karasu *et al*[16]	2002-2003	Viral, nonviral	AR group: 44.4 ± 12.7 yr, M/F: 17/9 Non-AR group: 37.4 ± 11.8 yr, M/F:11/6	Treatment with high-dose steroids (AR within first 8 wk)	CsA/tacrolimus+steroids Maintain target therapeutic blood levels of 5-10 ng/mL for tacrolimus
Xie *et al*[15]	2003-2005	HBV-related cirrhosis, HBV-related HCC, fulminant hepatitis B	AR group: 43.6 ± 9.0 yr, M/F: 35/6 Non-AR group: 46.5 ± 9.0 yr, M/F:130/15	Liver biopsy (AR within first 4 wk)	CsA/tacrolimus + prednisolone + MMF

ALD: Alcoholic liver disease; PBC: Primary biliary cirrhosis; PSC: Primary sclerosing cholangitis; HCC: Hepatocellular carcinoma; HBV: Hepatitis B viral; CsA: Cyclosporine A; MMF: Mycophenolate mofetil; AR: Acute rejection.

Table 2 Characteristics of studies included in the meta-analysis

Ref.	Country	Ethnicity	No. of	Case		Control		Genotyping methods	HWE in controls
			case/control	AA	AG/GG	AA	AG/GG		
Bathgate *et al*[21]	United Kingdom	Caucasian	68/76	16	52	22	54	PCR-SSP	Yes
Tambur *et al*[20]	Israel	Caucasian	33/30	19	14	14	16	PCR-SSP	No
Warlé *et al*[18]	Netherlands	Caucasian	41/48	6	35	17	31	ARMS-PCR	Yes
Fernandes *et al*[19]	United States	Caucasian	13/40	4	9	15	25	AS-PCR	Yes
Mas *et al*[17]	United States	Caucasian	19/55	12	7	12	43	DNA-sequencing	Yes
Karasu *et al*[16]	Turkey	Caucasian	26/17	12	14	8	9	PCR-SSP	Yes
Xie *et al*[15]	China	Asian	41/145	36	5	128	17	PCR-RFLP	Yes

PCR-SSP: Polymerase chain reaction and sequence-specific primer typing; ARMS-PCR: Amplification refractory mutation system-polymerase chain reaction; AS-PCR: Allele-specific polymerase chain reaction; PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism; HWE: Hardy-Weinberg equilibrium.

of small, soluble, or cell membrane-bound protein or glycoprotein molecules, play an essential role in the regulation of inflammatory and immune responses. Despite the many variables that influence acute rejection, previous reports indicate that cytokine genotypes that result from polymorphisms can sometimes correlate with acute allograft rejection[33,34]. Alloimmune responses and variations in susceptibility to rejection may be influenced by individual variations in cytokine genes. An association between susceptibility to graft rejection and polymorphism in cytokine gene promoters in kidney, heart, lung, and bone marrow recipients has been reported by some centers[11-14,35], although others have not confirmed this[36-38].

IL-10 is an antiinflammatory cytokine, which can inhibit the production of tumor necrosis factor-α, IL-1, IL-6, IL-8, and IL-12 in monocytes/macrophages and interferon-γ in T cells[4]. Therefore, in the context of allograft rejection, local IL-10 release may have inhibitory properties on macrophages, T cells, and cytokines. However, the role of IL-10 in LT patients remains controversial. For example, some studies have suggested that IL-10 *mRNA* levels are increased just before a rejection episode[5], while others have indicated that IL-10 levels are unchanged during rejection of the LT[39]. In animal models, overexpression of IL-10 by gene transfer prolonged graft survival of orthotopic LTs[40]. Since some studies[33,34] reported that cytokine genotypes that result from polymorphisms can sometimes correlate with acute

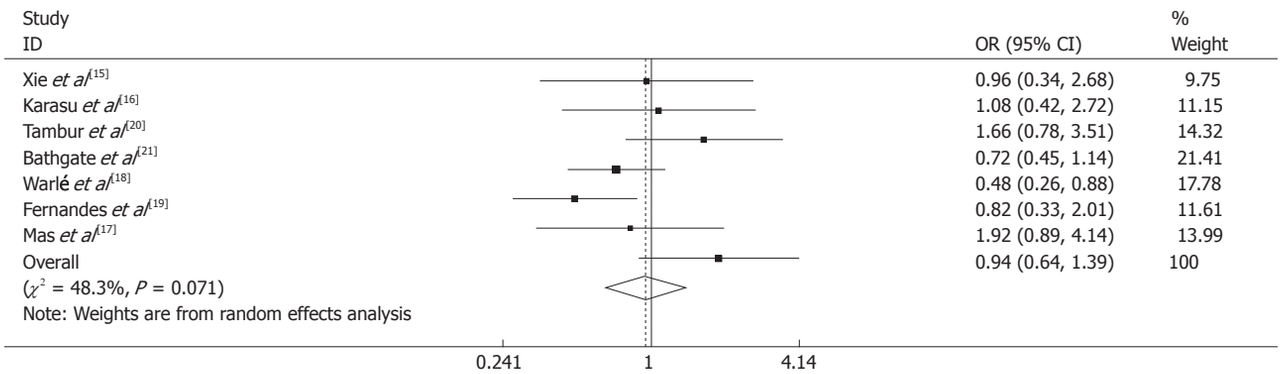

Figure 2 Odds ratios and 95% CI of individual studies and pooled data for the association of the interleukin-10-1082 G/A polymorphism and acute rejection comparing A allele with G allele. OR: Odds ratios.

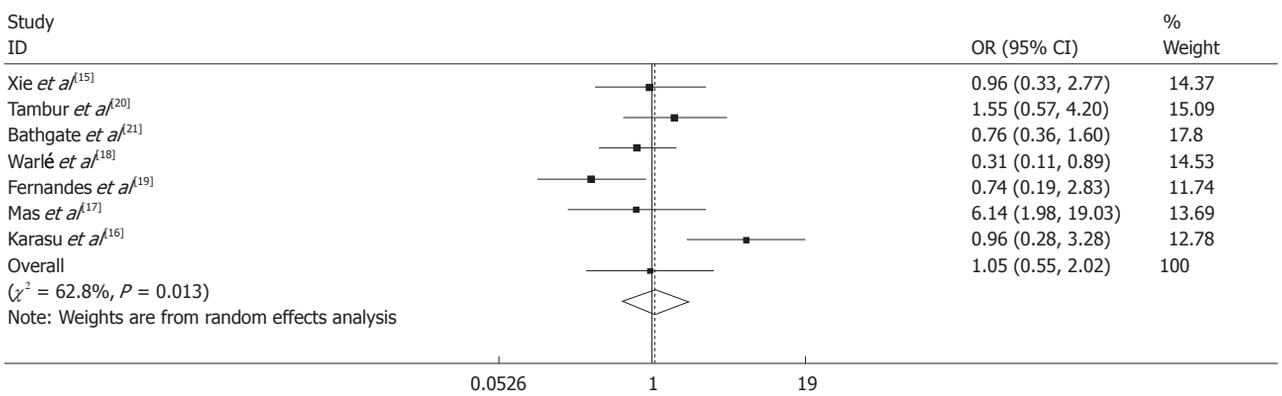

Figure 3 Odds ratios and 95% CI of individual studies and pooled data for the association of the interleukin-10-1082 G/A polymorphism and acute rejection comparing AA genotype with AG/GG Genotype. OR: Odds ratios.

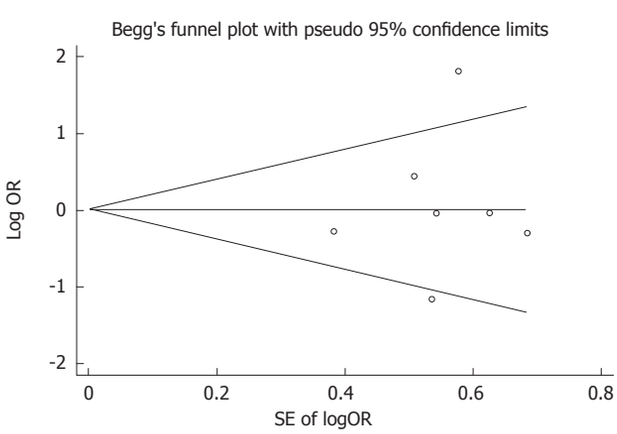

Figure 4 Begg's funnel plot of interleukin-10-1082 G/A polymorphism and acute rejection risk in liver transplant recipients (AA *vs* AG/GG). OR: Odds ratios.

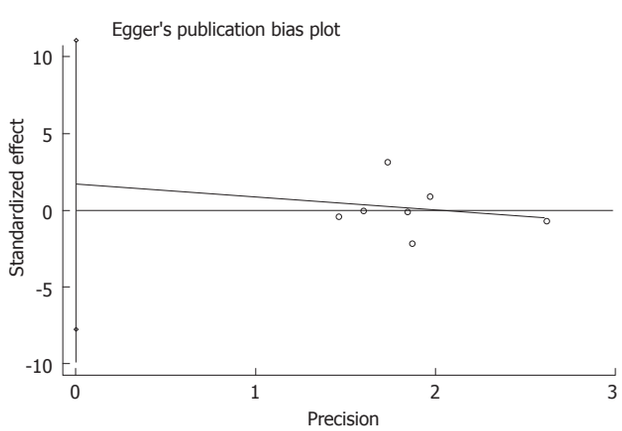

Figure 5 Egger's publication bias plot of interleukin-10-1082 G/A polymorphism and acute rejection risk in liver transplant recipients (AA *vs* AG/GG).

allograft rejection, a number of studies have assessed the association between the IL-10-1082 promoter polymorphism and AR in LT recipients in different populations. However, some of the results were conflicting, even in the same population, and thus a systematic review and meta-analysis of the association between *IL-10-1082* G/A polymorphism and AR risk was of great value.

A meta-analysis can overcome some problems caused by a single study, such as small sample size, low test power and selection bias; however, some concerns have to be addressed before aggregating data. First, the definition of AR, as the main outcome measure for this analysis, should be consistent among included studies. In six studies, AR was defined as "early" biopsy-proven AR within the first 4-8 wk after liver transplantation, treated with high-dose steroids. However, Karasu *et al*[16] defined AR as an increase in liver enzymes in the absence of vascular or biliary problems, associated with an improvement

after treatment by increasing the dose of immunosuppressive drugs or pulse steroid therapy within the first 8 wk. In the overall meta-analysis performed in this study, the number of patients from the Karasu *et al*[16] study is small, suggesting that this factor probably had little effect on the overall estimates. Moreover, the immunosuppressive regimen among different studies included is also an important factor which should be addressed. All LT patients included in this meta-analysis received more or less the same type of immunosuppression: a calcineurin inhibitor and prednisone, with or without azathioprine. However, there were some differences in the type of induction therapy, dosages and maintenance of target levels in blood, which can provide a possible explanation for significant heterogeneity in a recessive model.

This meta-analysis was based on seven case-control studies and showed that *IL-10-1082* G/A polymorphism was not associated with the risk of AR in LT recipients. Our result is not consistent with a previous systemic review[23]. This is most probably because the previous meta-analysis had a relatively small sample size (the Warlé *et al*[23] meta-analysis included only five studies for *IL-10-1082* G/A polymorphism and AR risk in LT recipients) and may have generated a very rough risk estimate. The G-to-A polymorphism at position -1082 of the IL-10 promoter reduces IL-10 production[7], and individuals with the *IL-10-1082*-GG genotype showed the greatest IL-10 production after *in vitro* stimulation, whereas *IL-10-1082*-GA and -AA showed intermediate and low production, respectively[7,41]. Moreover, previous studies[42,43] showed that Th2 cytokines, such as IL-10, are associated with graft tolerance. Therefore, it can be deduced that patients with an IL-10 genotype corresponding to low IL-10 production are more susceptible to rejection, whereas the IL-10 genotype corresponding to high production is found mainly among nonrejectors. However, our result is inconsistent with the above hypotheses. This is probably because the notion, derived mainly from animal studies, that IL-10 has a role in human allograft tolerance needs re-evaluation. In addition, since the effect of the *IL-10-1082* promoter polymorphism on *in vitro* and thus *in vivo* cytokine production is still inconclusive[7,44,45], its biological effect on acute liver graft rejection remains speculative.

As previously described, ethnicity can strongly influence the distribution of cytokine gene polymorphisms[46]. In Caucasian patients[17], the IL-10 AA genotype at position -1082 occurred in 32.5%, while among Asian patients[15] it occurred in 88.2%. Therefore, there may be different associations between IL-10-1082 promoter polymorphism and AR in LT recipients among different ethnicities. Nevertheless, our results were inconsistent with our hypothesis. When stratifying for ethnicity, no significant association was observed among either Caucasians or Asians. The null result may be due to the limited number of studies with only one study (based on Asian patients) available in this meta-analysis, and there is a very high risk of reporting bias for the relationship of the *IL-10-1082* G/A polymorphism and AR risk in the Asian population. In future, additional studies based on Asian patients should be performed to re-evaluate the association between IL-10-1082 G/A polymorphism and AR risk in this population.

It seemed that selection bias could have played a role because the genotype distribution of -1082 G/A polymorphism among control subjects disobeyed the HWE in one study[20]. It is widely believed that deviation from HWE may be due to genetic reasons including non-random mating, or the alleles reflect recent mutations that have not reached equilibrium, as well as methodological reasons including biased selection of subjects from the population or genotyping errors[47,48]. Apart from the reasons for disequilibrium, the results of genetic association studies might be spurious if the distribution of genotypes in the control groups were not in HWE[49,50]. Thus we carried out subgroup analysis by HWE in controls. When excluding the study that was not within HWE, the estimated pooled OR did not change at all, suggesting that this factor probably had little effect on the overall estimates.

However, there some limitations remain in this meta-analysis: (1) our meta-analysis was based on unadjusted OR estimates because not all published studies presented adjusted ORs or when they did, the ORs were not adjusted by the same potential confounders, such as age, sex, ethnicity and exposures. Lack of the information for the data analysis may cause serious confounding bias; (2) there was significant between-study heterogeneity from studies of the *IL-10-1082* G/A polymorphism, and the genotype distribution also showed deviation from HWE in one study; (3) the number of studies and the number of subjects in the studies included in the meta-analysis were small; (4) we must emphasize the fact that this meta-analysis adds more studies and increases the sample size but that it is an update that is not important because it adds patients (small importance); it is important because the statistical analysis reflects that IL-10 polymorphisms are not relevant in AR in liver transplantation; and (5) meta-analysis is retrospective research that is subject to methodological limitations. In order to minimize bias, we developed a detailed protocol before initiating the study, and performed a meticulous search for published studies by using explicit methods for study selection, data extraction and data analysis. Nevertheless, our results should be interpreted with caution.

In conclusion, this meta-analysis suggests that IL-10 -1082 G/A polymorphism may be not associated with AR risk in LT recipients among Caucasians. Since only one study was from an Asian population, it is critical that larger and well-designed multicenter studies based on Asian patients should be performed to re-evaluate the association.

COMMENTS

Background

Interleukin (IL)-10 is an antiinflammatory cytokine, which can inhibit the production of tumor necrosis factor-alpha, IL-1, IL-6, IL-8 and IL-12 in monocytes/

macrophages and interferon-γ in T cells. Therefore, in the context of allograft rejection, local IL-10 release may have inhibitory properties on macrophages, T cells, and cytokines. The production of cytokines (including IL-10) is under genetic control and varies among individuals as a function of polymorphisms within the regulatory regions of the various genes that determine the transcriptional activation. The G-to-A polymorphism at position -1082 of the IL-10 promoter reduces IL-10 production. Alloimmune responses and variations in susceptibility to rejection may be influenced by individual variations in cytokine genes.

Research frontiers

To date, a number of studies (including meta-analysis) have assessed the association between the IL-10 1082 G/A polymorphism and acute rejection (AR) risk in liver transplant (LT) recipients among different populations; however, the results are inconsistent and inconclusive.

Innovations and breakthroughs

Contrary to the finding of the previous meta-analysis, this study suggests that IL-10-1082 G/A polymorphism may be not associated with AR risk in LT recipients among Caucasians.

Applications

It can be seen from this paper that IL-10-1082 G/A polymorphism could not alter susceptibility to AR risk in LT recipients. It suggests that a common variant in the functional region of a meaningful gene had little effect on human disease.

Peer review

This appears to be a well-done meta-analysis study.

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