REVIEW

Association of Transforming Growth Factor-β1 rs1982073 Polymorphism with Susceptibility to Acute Renal Rejection: a Systematic Review and Meta-Analysis

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Purpose: The association of rs1982073 (codon 10) polymorphism at Transforming Growth Factor- β1 (TGF-β1) gene with acute renal rejection (ARR) has been reported by several studies. However, the results were controversial. To derive a more precise estimation of this association, a meta-analysis was performed.

Methods: The eligible literatures were identified through PubMed, Scopus, Web of Science, EMBASE, SciELO, WanFang, and CNKI databases up to July 01, 2019. The pooled odds ratios (ORs) with corresponding 95% confidence intervals (CIs) were used to calculate the strength of the association.

Results: A total of 23 case-control studies with 795 ARR cases and 1,562 non-AR controls were selected. Pooled data revealed that there was no significant association between TGF-β1 codon 10 polymorphism and an increased risk of ARR in the overall population (C vs. T: OR=0.908, 95% CI 0.750-1.099, p = 0.322; CT vs. TT: OR=1.074, 95% CI 0.869-1.328, p = 0.507; CC vs.TT: OR=0.509, 95% CI=0.738-1.253, p = 0.770; CC+CT vs. TT: OR = 0.917, 95% CI 0.756-1.112, p = 0.376, and CC vs. CT+TT: OR=0.995, 95% CI 0.809-1.223, p = 0.959). Moreover, stratified analysis revealed no significant association between the TGF-β1 rs1982073 polymorphism and ARR risk by ethnicity and cases type (recipient and donor).

Conclusion: The current meta-analysis demonstrated that the TGF-β1 rs1982073 polymorphism was not significantly associated with increased risk of ARR. However, studies with a larger number of subjects among different ethnic groups are needed to further validate the results.

Keywords: Acute Renal Rejection; TGF-β1; Polymorphism; Meta-analysis.

INTRODUCTION

Acute renal rejection (ARR) has been identified as the main cause of renal graft dysfunction during the first year after transplantation[1–3]. ARR is associated with chronic structural and functional damage, which causes loss of graft and decrease in patient survival. Moreover, it is associated with other conditions such as cardiovascular disease and overall mortality[4]. The improvement of renal transplantation results in the last two decades is largely due to a progressive decrease in the incidence of acute rejection[5]. Many scientists acknowledge that ARR is a multifactorial disease which mediated by complex immunological mechanisms and a network of interactions between cytokines regulates the immune response to transplanted renal[6,7]. Several risk factors for ARR have been identified including low histocompatibility between donor and recipient, the age of donor and recipient, ethnicity, gender, ischemia time, delayed graft function, graft non-adherence, and reduced immunosuppression[8,9].

In the recent years, there is an increasing body of research highlighting the effects of genetic variants in different cytokines such as IL-2, IL-4, IL-10, TNF-α, and TGF-β1 in development of ARR (10–12). TGF-β1 is a multifunctional cytokine with immunosuppressive and fibrogenic properties. TGF-β1 belongs to a family of multi-functional polypeptides, produced by many cell types, including T lymphocytes, monocytes, vascular endothelium and fibroblasts[13,14]. TGF-β1 has been conventionally recognized as a guardian against different organ acute rejection[15]. The pivotal function of TGF-beta in the immune system is to maintain tolerance via the regulation of lymphocyte proliferation, differentiation, survival and in both suppressive and inflammatory immune responses[16,17]. It has been known that TGF-β is a cytokine required for the induction and maintenance of transplantation tolerance. Central for transplantation tolerance is the role for TGF-β in the induction of Foxp3 and regulatory capacity in CD4(+) T cells[18,19]. Moreover, TGF-β1 has been implicated in many different disorders development of various disorders, including coronary heart disease, human cancers,

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

First Author	Country (Ethnicity)	Subjects	Genotyping Method	Immunosuppressive Protocol	AR/non-AR	AR					non-AR					MAFs	HWE
						Genotype			Allele		Genotype			Allele			
						TT	CT	CC	T	C	TT	CT	CC	T	C		
Marshall 2000a	UK (Caucasian)	R	SSP-PCR	CsA, AZA, Steroids	114/76	46	55	13	147	81	39	48	8	126	64	0.336	0.201
Marshall 2000b	UK (Caucasian)	D			77/68	34	32	11	100	54	30	24	14	84	52	0.382	0.037
Alakulppi 2004	Finland (Caucasian)	R	SSP-PCR	CsA/FK506, MMF/AZA, Steroids	50/241	31	19 (CT+CC)	-	-	-	115	126 (CT+CC)	-	-	-	NA	NA
Ligeiro 2004a	USA (Caucasian)	R	SSP-PCR	CsA, AZA, Steroids	31/35	12	12	7	36	26	14	15	6	43	27	0.385	0.571
Ligeiro 2004b	USA (Caucasian)	D			31/35	5	22	4	32	30	14	14	7	120	60	0.400	0.324
Park 2004	Korea (Asian)	R	SSP-PCR	CsA, AZA, steroids	28/100	3	18	7	24	32	25	50	25	100	100	0.937	1.000
Dmitrienko 2005	Canada (Caucasian)	R	RFLP-PCR	CsA/FK506, MMF/AZA , Steroids	50/50	16	24	10	56	44	16	24	10	56	44	0.440	0.854
Guo 2005a	China (Asian)	R	Microarray	CsA/FK506, MMF, Steroids	39/90	18	15	6	51	27	18	57	15	93	87	0.483	0.011
Guo 2005b	China (Asian)	D			39/90	6	33	0	45	33							
Chow 2005	China (Asian)	R	SSP-PCR	CsA	52/77	8	44 (CT+CC)	-	-	-	13	64 (CT+CC)	-	-	-	NA	NA
Gendzekhadze 2006	Venezuela (Mixed)	R	SSP-PCR	CsA, MMF, Steroids	30/33	12	12	6	36	24	10	14	9	34	32	0.484	0.386
Hueso 2006	Spain (Caucasian)	R	RFLP-PCR	CsA/FK506, steroids, MMF/SRL	14/63	6	5	3	17	11	20	28	15	68	58	0.460	0.402
Canossi 2007a	Italy (Caucasian)	R	SSP-PCR	CsA, MMF/AZA, Steroids	25/61	4	15	6	23	27	14	29	18	57	65	0.532	0.725
Canossi 2007b	Italy (Caucasian)	D			20/50	5	12	3			13	26	11	52	48	0.480	0.768
Brabcova 2007	Czech (Caucasian)	R	SSP-PCR	CsA/FK506, MMF, Steroids	190/246	32	91	67	155	225	34	128	84	196	296	0.601	0.179
Grinyo 2008	Spain (Caucasian)	R	AS-PCR	CsA, MMF, Steroids	63/161	18	34	11	70	56	66	69	26	201	121	0. 272	0.272
Mendoza 2008	Mexico (Mixed)	R	SSP-PCR	CsA/FK506, AZA, Steroids	19/32	11 (TT+CT)		8	-	-	25 (CT+CC)		7	-	-	NA	NA
Manchanda 2008a	India (Asian)	R	ARMS-PCR	CsA, AZA, Steroids	18/82	1	11	6	13	23	19	45	18	83	81	0.493	0.376
Manchanda 2008b	India (Asian)	D			18/82	3	6	9	12	24	13	48	21	74	90	0.591	0.011
Karimi 2012	Iran(Asian)	R	ARMS-PCR	CsA, MMF, Steroids	29/71	5	8	16	18	40	17	24	30	58	84	0.591	0.011
Seyhun 2012	Turkey (Caucasian)	R	SSP-PCR	CsA/FK506, MMF, Steroids	19/71	6	10	3	22	16	16	31	24	63	79	0.556	0.330
Saigo 2014	Japan(Asian)	R	DS	NA	24/111	5	16	3	26	22	22	51	36	95	123	0.564	0.612
Seyhun 2015	Turkey (Caucasian)	R	SSP-PCR	CSA, TAC/ MPA, MMF, AZA	28/62	6	15	7	27	29	16	28	18	60	64	0.516	0.450
28	18	60	64	0.516	0.450												

Abbreviations: ARR: Acute Renal Rejection; R: Recipient; D: Donor; PCR: Polymerase Chain Reaction; SSP: Single Specific Primer; RFLP: Restriction Fragment Length Polymorphism; AS: Allele-specific; ARMS: Amplification Refractory Mutation System; DS: Direct Sequencing; AR: Acute Rejection; non- AR: Non Acute Rejection; NA: Not Applicable; MAFs: Minor Allele Frequencies; HWE: Hardy–Weinberg equilibrium.

rheumatoid arthritis, and asthma[20,21].

The human TGF-β1 gene has previously been mapped to chromosome 19q13.1–13.3, consists of seven exons and spanning a region of 23 kbp[22,23]. Several common single nucleotide polymorphisms (SNPs) such as +869T>C, +915G>C, -509C>T, and codon 25 (+74G>C) have been identified at TGF-β1 gene[23]. Among them, TGF-β1 rs1982073 (codon 10) polymorphism has been extensively studied in organ translation outcomes[15]. TGF-β1 rs1982073 polymorphism is lo-

Table 2. Summary risk estimates for association of TGF-β1 rs1982073 polymorphism with risk of ARR.

			Heterogeneity		Odds Ratio			Publication Bias	
Subgroup	**Genetic Model**	**Type of Model**	**I^2 (%)**	**PH**	**OR**	**95% CI**	**Z_{test}**	**POR**	**PBeggs**	**PEggers**
Overall Population	C vs. T	Random	52.32	0.004	0.908	0.750-1.099	-0.999	0.322	0.944	0.521
	CT vs. TT	Fixed	33.07	0.076	1.074	0.869-1.328	0.664	0.507	0.381	0.249
	CC vs.TT	Fixed	0.00	0.509	0.961	0.738-1.253	-0.293	0.770	0.871	0.880
	CC+CT vs. TT	Fixed	36.87	0.047	0.917	0.756-1.112	-0.885	0.376	0.096	0.056
	CC vs. CT+TT	Fixed	6.70	0.372	0.995	0.809-1.223	-0.051	0.959	0.032	0.163
By Ethnicity										
Caucasians	C vs. T	Random	57.76	0.009	0.852	0.662-1.096	-1.248	0.212	0.212	0.196
	CT vs. TT	Fixed	2.45	0.420	1.137	0.889-1.452	1.024	0.306	9.303	0.290
	CC vs.TT	Fixed	0.00	0.918	0.969	0.712-1.319	-0.199	0.842	0.837	0.902
	CC+CT vs. TT	Fixed	28.16	0.161	0.943	0.719-1.238	-0.423	0.672	0.246	0.253
	CC vs. CT+TT	Fixed	0.00	0.947	0.917	0.715-1.176	-0.681	0.496	0.114	0.074
Asians	C vs. T	Random	53.86	0.043	1.048	0.738-1.487	0.262	0.793	0.133	0.042
	CT vs. TT	Random	62.30	0.014	1.133	0.528-2.432	0.321	0.748	0.548	0.162
	CC vs.TT	Fixed	50.94	0.057	1.031	0.588-1.809	0.107	0.915	1.000	0.921
	CC+CT vs. TT	Random	58.65	0.024	1.159	0.582-2.309	0.421	0.674	0.229	0.037
	CC vs. CT+TT	Random	53.81	0.043	1.060	0.560-2.004	0.178	0.858	0.386	0.122
By Subjects										
Recipient	C vs. T	Fixed	19.61	0.235	0.960	0.837-1.101	-0.588	0.556	0.692	0.950
	CT vs. TT	Fixed	32.29	0.110	0.984	0.776-1.249	-0.130	0.897	0.234	0.348
	CC vs.TT	Fixed	0.00	0.450	0.983	0.734-1.315	-0.118	0.906	1.000	0.797
	CC+CT vs. TT	Random	41.16	0.044	0.889	0.661-1.196	-0.778	0.437	0.095	0.127
	CC vs. CT+TT	Fixed	0.00	0.699	1.019	0.814-1.275	0.162	0.872	0.095	0.468
Donor	C vs. T	Random	84.00	≤0.001	0.687	0.313-1.511	-0.933	0.351	0.806	0.597
	CT vs. TT	Fixed	23.63	0.264	1.495	0.941-2.374	1.703	0.083	1.000	0.867
	CC vs.TT	Fixed	2.04	0.395	0.866	0.460-1.632	-0.444	0.657	0.462	0.639
	CC+CT vs. TT	Fixed	0.00	0.458	1.261	0.815-1.950	1.041	0.298	1.000	0.518
	CC vs. CT+TT	Fixed	57.47	0.052	0.866	0.505-1.486	-0.523	0.601	0.806	0.778
Genotyping Methods										
SSP-PCR	C vs. T	Random	58.99	0.009	0.817	0.621-1.076	-1.437	0.151	0.152	0.291
	CT vs. TT	Fixed	12.18	0.328	1.106	0.845-1.446	0.733	0.463	0.008	0.054
	CC vs.TT	Fixed	0.00	0.822	0.929	0.666-1.296	-0.433	0.665	0.755	0.672
	CC+CT vs. TT	Fixed	28.29	0.167	0.862	0.678-1.097	-1.208	0.227	0.046	0.027
	CC vs. CT+TT	Fixed	0.00	0.619	0.908	0.710-1.162	-0.767	0.443	0.062	0.089
ARMS-PCR	C vs. T	Fixed	0.00	0.947	1.648	1.092-2.486	2.379	0.017	1.000	0.425
	CT vs. TT	Fixed	23.61	0.270	1.128	0.464-2.738	0.265	0.791	1.000	0.527
	CC vs.TT	Fixed	0.00	0.597	2.195	0.941-5.116	1.820	0.069	0.296	0.241
	CC+CT vs. TT	Fixed	0.00	0.412	1.542	0.695-3.419	0.287	1.066	1.000	0.495
	CC vs. CT+TT	Fixed	0.00	0.711	2.010	1.133-3.567	2.386	0.017	1.000	0.694
HWE*	C vs. T	Random	54.61	0.004	0.877	0.712-1.080	-1.233	0.218	0.773	0.435
	CT vs. TT	Random	11.86	0.318	1.151	0.906-1.462	1.152	0.249	0.324	0.251
	CC vs.TT	Fixed	0.00	0.482	0.954	0.718-1.268	-0.322	0.748	0.820	0.789
	CC+CT vs. TT	Random	41.50	0.031	0.949	0.715-1.261	-0.360	0.719	0.107	0.068
	CC vs. CT+TT	Fixed	5.570	0.388	0.989	0.794-1.232	-0.097	0.922	0.014	0.160

Abbreviations: ARR: acute renal rejection; PCR: Polymerase Chain Reaction; SSP: Single Specific Primer; ARMS: Amplification Refractory Mutation System.
*By excluding HWE violating studies.

cated at position 10 (exon 1) in the signal peptide and has a central role in exporting of the newly synthesized protein through endoplasmic reticulum (ER) membrane [24].

In the recent decade, an increasing number of studies are being conducted on the impact of TGF-β1 rs1982073 (codon 10) polymorphism on the clinical outcomes of renal transplantation[15,25]. Nevertheless, the results of these studies were not always consistent and controversial. For example, li et al., reported that TGF-β1 rs1982073 polymorphism might be useful in predicting the risk of ARR. By contrast, Karimi et al., in a case-control study showed that TGF-β1 rs1982073 (codon 10) polymorphism was not significantly associated with risk of ARR in the Iranian patients[26]. To clarify the association between TGF-β1 rs1982073 polymorphism and ARR risk, we performed this meta-analysis of all eligible published studies.

MATERIALS AND METHODS

Literature Search Strategy

A comprehensive literature search in PubMed, Scopus, EMBASE, Cochrane Library, Web of Science, Elsevier, SciELO, SID, WanFang, VIP, Chinese Biomedical Database (CBD) and Chinese National Knowledge Infrastructure (CNKI) to identify all eligible studies on TGF-β1 rs1982073 polymorphism with risk of ARR published up to July 01, 2019. The combination of following keywords and terms were adopted in the electronic searches: (''Acute Renal'' OR ''Renal Graft Rejection'' OR "Acute Renal Rejection" OR "Renal Allograft Rejection") AND ("Transforming growth Factor-β1" OR ''TGF-β1'') AND (''Codon 10'' OR ''+869T>C'' OR ''+10T>C'' OR ''T869C'' OR ''rs1982073'' OR ''Leu10>Pro10'') AND (''Gene'' OR "Single Nucleotide Polymorphism" OR "SNPs" OR ''Genotype'' OR ''Allele'' OR ''Variation'' OR "Variant" OR ''Mutation''). Moreover, a manual search of the reference lists performed to retrieved articles for

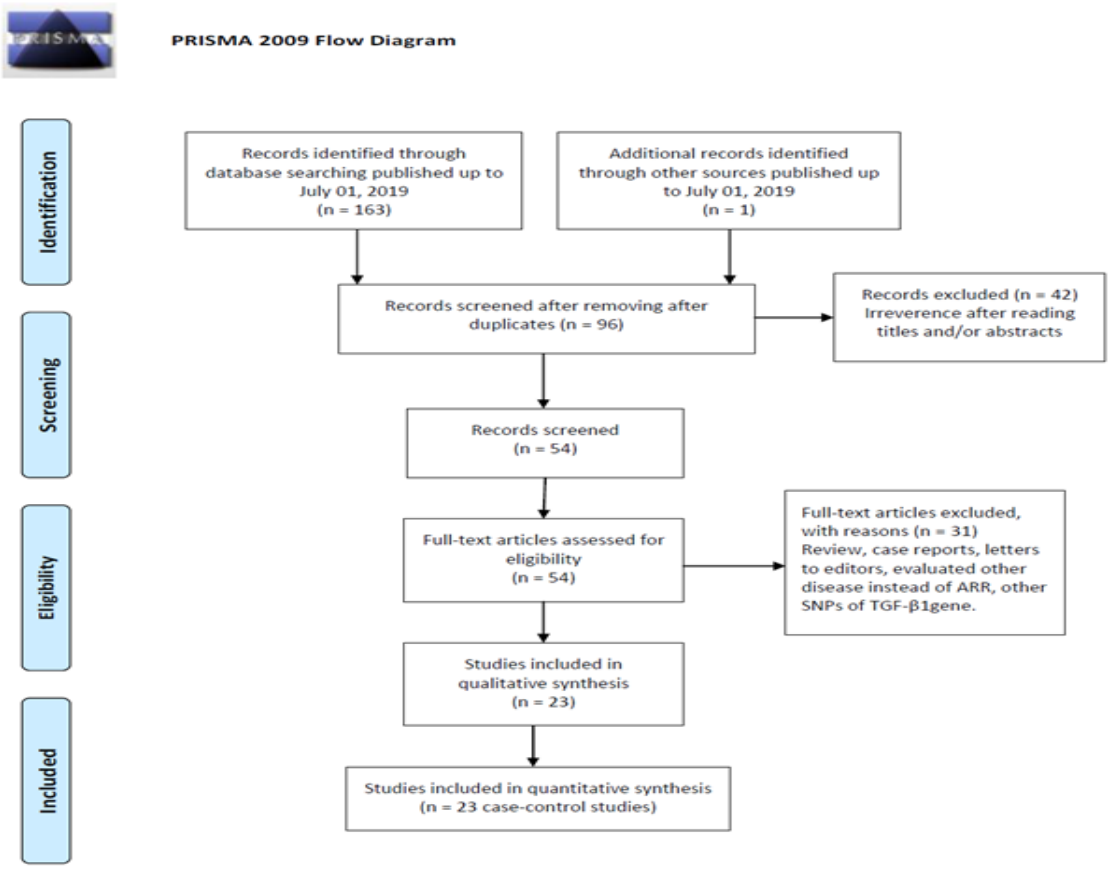

Figure 1. Flow diagram of selecting eligible studies for the meta-analysis.

additional potential studies. Publication language was restricted to English, Chinese, and Farsi. Moreover, non-English publications were translated and included in the meta-analysis.

Inclusion and Exclusion Criteria

The inclusion criteria for the gene association studies in this meta-analysis were as follows: 1) studies with case-control or cohort design; 2) only full-text published studies; 3) studies evaluated the association of TGF-β1 rs1982073 (codon 10) polymorphism with ARR risk; 4) provided the genotype distribution in both cases and controls for estimating an odds ratio (OR) with 95% confidence interval (CI); and 5) at least two comparison groups (ARR group vs. non-AR group). The exclusion criteria were as follows: 1) case only studies (without controls); 2) non-human studies; 3) family-based, sibling, twins and linkage studies; 4) studies without details of genotype frequencies, which were unable to calculate ORs; 5) studies on other polymorphisms of TGF-β1 gene; 6) abstracts, case reports, case series, letters, comments, conference presentations, posters, editorials, reviews, and previous meta-analyses; and 7) duplica¬tion of the previous publication; and 8) duplicates or overlapping studies. If the authors published two or more studies using the same data or overlapping data, the newest publication or the publication with the largest sample size was selected. There was no any limitation by ethnicity, race, placed or geography area.

Data Extraction

Two authors (HN and MJA) carefully extracted data from all eligible studies using a standardized form. Then, they have checked the data extraction results and reached consensus. Any disagreement between the two authors was resolved by discussion with a third author. The following data were collected from each study: first author, year of publication, country of origin, ethnicity (Asians, Caucasians, African, Mixed population), type of cases (recipient and donor), genotyping method, number of cases and controls, genotypes frequencies of cases and controls, minor allele frequencies (MAFs) and Hardy-Weinberg equilibrium test in control subjects (non-ARR). In this meta-analysis the diverse ethnicities were categorized as Caucasian, Asian, Africans, and Mixed population (unknown or more than one racial group).

Statistical Analysis

An ethical approval was not necessary as this study was a meta-analysis based on previous studies. The strength of the association between TGF-β1 rs1982073 (codon 10) polymorphism and ARR risk was measured by odds ratios (ORs) with 95% confidence intervals (CIs). The statistical significance of the pooled OR was determined using the Z-test. Pooled estimates of the OR were obtained by calculating a weighted average of OR from each study. The pooled ORs was calculated under all five genetic models, i.e., allele (C vs. T), homozygote (CC vs. TT), heterozygote (CT vs.

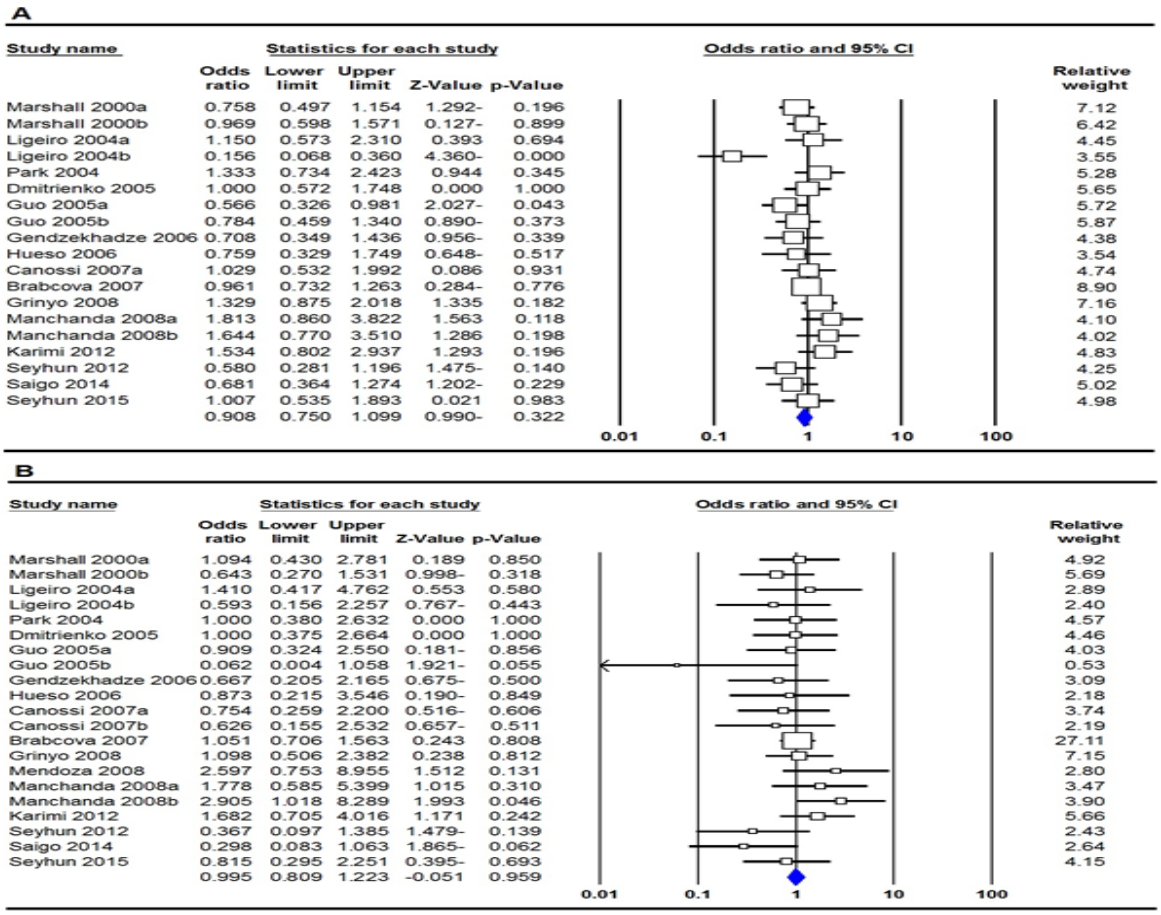

Figure 2. Forest plot for association of TGF-β1 rs1982073 polymorphism with risk of ARR in the overall population. A: allele model (C vs. T); B: recessive model (CC vs. CT+TT).

TT), dominant (CC+CT vs. TT) and recessive (CC vs. CT+TT). Between-study heterogeneity was estimated by Cochran's χ^2 based Q-statistic test, in which it was considered to be statistically significant at $P \leq 0.05$. In addition, I2-value was used to quantify the proportion of heterogeneity, with the range of 0 to 100% (''I^2<25% represents no heterogeneity, I^2 = 25–50% represents moderate heterogeneity, I2 = 50–75% represents large heterogeneity, and I^2>75% represents extreme heterogeneity). Accordingly, when between-study heterogeneity existed ($p < 0.05$, $I^2 > 50\%$) a random-effects model weighted (the DerSimonian-Laird method) was applied to give a more conservative result; otherwise, a fixed-effects model weighted (the Mantel-Haenszel method) method was selected. Fisher's exact test was used to assess the Hardy-Weinberg equilibrium (HWE) in the control group, in which the significance set at P<0.05. A stratification analysis was conducted by ethnicity, type of subjects, genotyping methods and HWE to found out the source of heterogeneity. To check the stability and reliability of the pooled ORs, a sensitivity analysis was performed by omitting a single study each time from the all selected studies and reanalyzing the remainder. Begg's funnel plot a scatter plot of effect against a measure of study size and Egger's test were used to determine the presence of publication bias in the current meta-analysis; which P<0.05 indicated that the result was statistically significant. All statistical analyses were performed using Comprehensive Meta-Analysis (CMA) Software version 2.0 (Biostat, Englewood, NJ). All tests were two-sided, and the P values of < 0.05 were considered statistically significant.

RESULTS

Studies Characteristics

As shown in **Figure 1**, initially, a total of 403 results were identified by electronic and manual searches up to July 01, 2019. After reading the titles and abstracts, 365 were excluded because they were obviously irrelevant papers to TGF-β1 rs1982073 polymorphism or duplicates. Then, 19 articles were excluded because they were case reports, case only studies, reviews, previous meta-analysis, did not report usable data. Finally, a total of 23 case-control studies in 18 publications with 795 ARR cases and 1,562 non-AR controls were selected [10,11,26–41]. The characteristics of each study are summarized in **Table 1**. All eligible studies were published in English between November, 2000 and June, 2015. Among them, 13 studies were based on Caucasian populations (5,410 cases and 6,438 controls), eight studies were based on Asian populations (3,137 cases and 3,700 controls), and two studies were based on mixed populations (331 cases and 405 controls). The included studies were performed in UK, USA, Canada, China, Venezuela, Italy, Czech, India, Iran, Spain and Turkey. The genotypes and allele frequency was not applicable

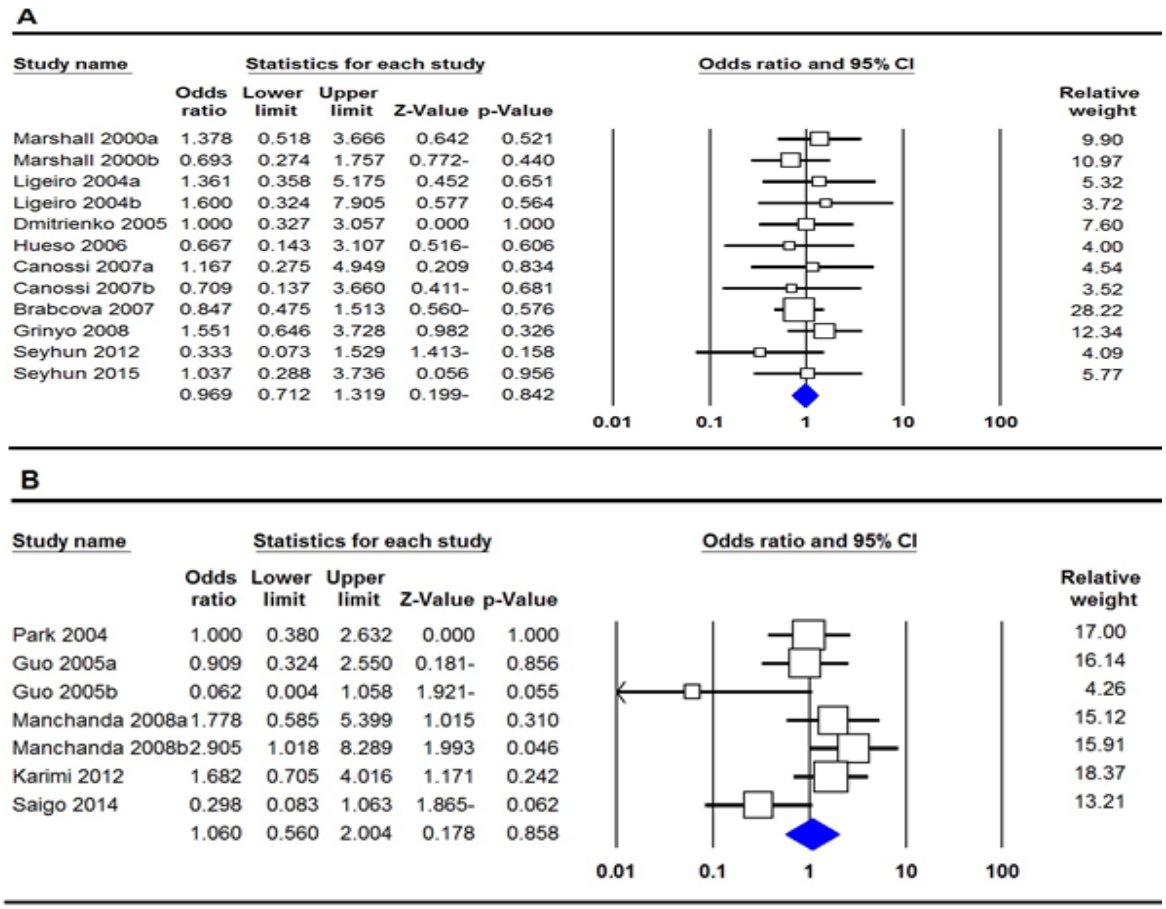

Figure 3. Forest plot for association of TGF-β1 rs1982073 polymorphism with risk of ARR by ethnicity. A: Caucasians (homozygote model: CC vs.TT); B: Asians (recessive model: CC vs. CT+TT).

for three studies. The allele, genotype and minor allele frequency (MAF) distributions in the cases and controls are shown in Table 1. Moreover, the distribution of genotypes in the controls was in agreement with Hardy-Weinberg equilibrium (HWE) for all selected studies, except for four studies (**Table 1**).

Quantitative Data Synthesis

The summary of the meta-analysis of the association of between TGF-β1 rs1982073 polymorphism and risk of ARR are shown in **Table 2**. Pooled data revealed that there was no significant association between TGF-β1 rs1982073 polymorphism and an increased risk of ARR under all five genetic models, i.e., allele (C vs. T: OR = 0.908, 95% CI 0.750-1.099, p = 0.322, **Fig 2A**), heterozygote (CT vs. TT: OR = 1.074, 95% CI 0.869-1.328, p = 0.507), homozygote (CC vs.TT: OR = 0.509, 95% CI 0.738-1.253, p = 0.770), dominant (CC+CT vs. TT: OR = 0.917, 95% CI 0.756-1.112, p = 0.376), and recessive (CC vs. CT+TT: OR = 0.995, 95% CI 0.809-1.223, p = 0.959, **Fig 2B**). Moreover, we performed subgroup analyses by ethnicity, type of cases (recipient and donor) and genotyping methods. When stratified by ethnicity, no significant association was found in Caucasian and Asian populations (**Figure 3A, 3B**). Moreover, subgroup analysis type of cases (recipient and donor) revealed that TGF-β1 rs1982073 polymorphism was not significantly associated with ARR risk in recipient and donor groups (**Table 2**). However, there was a significant association between TGF-β1 rs1982073 polymorphism and an increased risk of ARR in ARMS-PCR group of studies (C vs. T: OR = 1.648, 95% CI 1.092-2.486, p = 0.017 and CC vs. CT+TT: OR=0.2.010, 95% CI 1.133-3.567, p = 0.017), but in SSCP-PCR group of studies.

Between-Study Heterogeneity Test

As shown in **Table 2**, there was a significant between-study heterogeneity only under the allele model (I^2 =52.32; PH=0.004) in the overall population. We conducted subgroup analysis by ethnicity, type of cases, genotyping methods and HWE to found the potential source of heterogeneity in the meta-analysis. Results showed that the heterogeneity was significantly decreased by type of cases and genotyping methods. However, after subgroup analysis by ethnicity and excluding HWE-violating studies a moderate to high heterogeneity was appeared, indicating that ethnicity and HWE might be potential source of between-study heterogeneity in the current met-analysis (**Table 2**).

Sensitivity Analysis

We performed a sensitivity analysis to assess the influence of each individual study on the pooled ORs by sequential omission of individual studies. However, the corresponding pooled ORs were not materially altered by removing any individual study. Moreover, we have

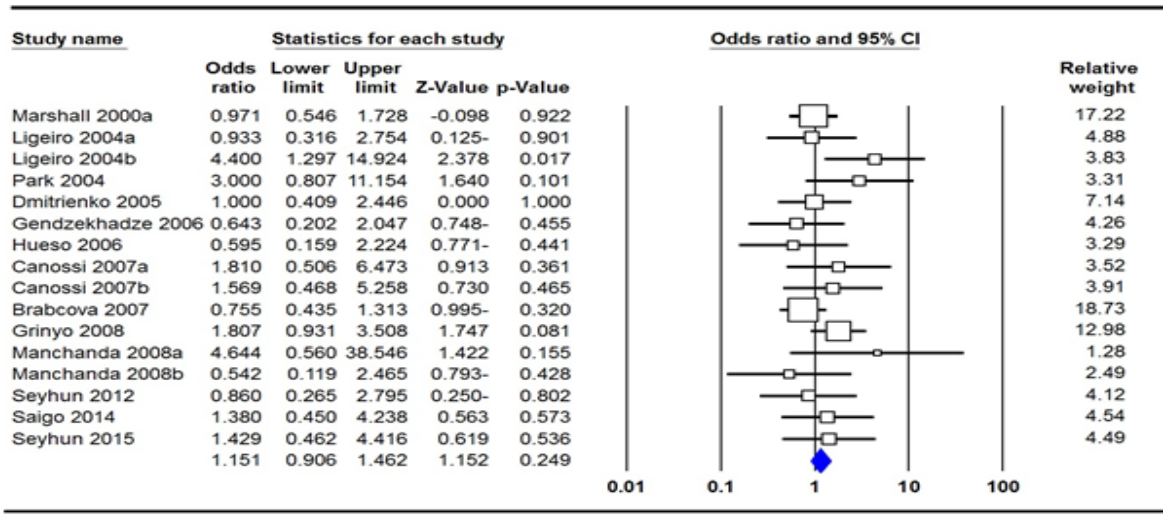

Figure 4. Forest plot for association of TGF-β1 rs1982073 polymorphism with risk of ARR after excluding HWE-violating studies under the heterozygote model (CT vs. TT).

performed sensitivity analysis by excluding HWE-violating studies. As shown in **Table 2** and **Figure 4**, sensitivity analysis showed that the initial results were not considerably adjusted by omitting the HWE-violating studies. Therefore, the sensitivity analysis confirmed that the results of the present meta-analysis were statistically stable.

Publication Bias

Publication bias was assessed by Begg's funnel plot and Egger's test. The shape of the funnel plots did not revealed any asymmetry under all five genetic models in the overall population (**Figure 3**). Then, Egger's test was performed to provide statistical evidence of funnel plot asymmetry. The results indicated a lack of publication bias under all five genetic models, i.e., allele (PBeggs = 0.661; PEggers = 0.856), heterozygote (PBeggs = 0.381; PEggers = 0.508), homozygote (PBeggs = 0.661; PEggers = 0.991, **Figure 5A**), dominant (PBeggs = 0.191; PEggers = 0.199, **Fig 2B**) and recessive (PBeggs = 0.137; PEggers = 0.485).

DISCUSSION

To date, the cause of ARR has not yet been fully clarified. In recent years, numerous studies have revealed an association between TGF-β1 rs1982073 and ARR risk [15]. However, the relationship remains controversial. In the current meta-analysis, a total of 23 case-control studies with 795 ARR cases and 1,562 non-AR controls were selected. After pooling the data from all eligible studies, we have shown that TGF-β1 rs1982073 polymorphism was not significantly associated with an increased risk of ARR in the overall population and by ethnicity. Moreover, our subgroup analysis revealed that ARR was not associated with genotype of TGF-β1 rs1982073 polymorphism in renal recipients or donors (**Table 2**). Thus, our results indicated that TGF-β1 rs1982073 polymorphism might not be useful biomarker to identify patients predisposed to ARR.

The current meta-analysis results are inconsistent with a previous meta-analysis in that revealed that TGF-β1 rs1982073 polymorphism was not significantly associated with risk of ARR. Ge et al., in a meta-analysis have found a positive association between TGF-β1 rs1982073 polymorphism and ARR. In recent years, some studies already studied potential associations TGF-β1 rs1982073 polymorphism with risk of ARR. However, by including recently published studies which have strong reverse association with TGF-β1 rs1982073 polymorphism, our pooled data did not show a significant association between TGF-β1 rs1982073 polymorphism and ARR in the overall population under all five genetic models. Omrani et al., showed that the TGF-β1 rs1982073 polymorphism did not play a major role in kidney allograft survival[42]. In a meta-analysis, Warlé et al., also failed to show a significant association of TGF-β1 rs1982073 (codon 10) and rs1800471 (codon 25) polymorphisms with an increased risk of acute liver graft rejection[43]. Hueso et al., found an independent association between T allele at TGF-β1 rs1982073 polymorphism in recipient and independent predictors of subclinical rejection (SCR)[28]. Moreover, Cho et al., reported that TGF-β1 rs1982073 (codon 10) and rs1800471 (codon 25) polymorphisms were not significantly associated with an increased risk of development of chronic allograft nephropathy in Korean renal transplant recipients[25]. Therefore, our findings are in accordance with the mentioned studies revealed that C allele of TGF-β1 rs1982073 loci was not associated with an increased risk of ARR. However, this result was contradictory to studies performed by Chow et al., Park et al., and Ge et al., which observed an increased risk in renal transplant recipients. In 2005, Chow et al., have demonstrated that the CC genotype of TGF-β1 rs1982073 polymorphism was a potential risk factor for failure of kidney allograft function[41]. In 2004, Park et al., have evaluated the association of TGF-β1 polymorphisms with ARR risk in renal transplant recipients and their donors. They found that the CC genotype in the renal transplant recipients were associated with recurrent acute rejection episodes in Korean population[38]. Ge et al., have found recipient TGF-β1 haplotypes were significantly associated with an increased risk of acute rejection in solid organ transplant recipients, particu-

larly in patients receiving cardiac allograft. In addition, they revealed that the donor TGF-β1 rs1982073 polymorphism was significantly associated with acute rejection of solid organ transplant in recipients under the heterozygote and dominant models, especially among patients in CsA/ FK 506 group compared with those in CsA group[44].

As shown in **Table 2**, there was a global variation for MAFs of TGF-β1 rs1982073 in the healthy subjects, suggesting a potential subgroup analysis in the worldwide population. However, analysis by ethnicity did not show a significant association between TGF-β1 rs1982073 and ARR under all five genetic models. In addition, most of the selected studies were conducted in Caucasian population, which might be caused to reduce the potential effects of subgroup analysis by ethnicity. Between-study heterogeneity is a common issue when interpreting the pooled data of meta-analyses[45–47].

It could be attributable to differences in several factors such as environmental factors, criteria or methodological factors in study design, sample size, source of controls, type of cases, genotyping methods, and so on [48,49]. In the present meta-analysis there was a significant heterogeneity under two genetic models. However, after subgroup analysis a moderate to high heterogeneity appeared in the Asians under four genetic models, indicating that ethnicity might be potential source of between-study heterogeneity in the met-analysis.

All of the studies included in this meta-analysis met our inclusion criteria. In spite of these, several limitations that exist in the current meta-analysis have to be addressed. First, the sample size was relatively small which may lead to a relatively small power. Second, we only selected published studies electronically in English, Chinese, and Farsi, so it is possible that some pertinent studies published in other languages or unpublished studies with negative results may have been missed. Therefore, publication bias may exist; even no statistical evidence suggests publication bias in the current meta-analysis. Third, there were only two studies that evaluated the association in mixed populations and subgroup analysis was performed only in Caucasians and Asians. Therefore, it is unknown whether the results will extend to other populations such as Africans and mixed populations. Fourth, only small numbers of studies were included in some subgroups such as donors and ARMS-PCR group of studies. Therefore, these subgroup analyses may not have enough statistical power with the small sample size and the conclusions may be biased. Fifth, our study was designed to analyze the association of TGF-β1 rs1982073 polymorphism with ARR; however, a haplotype analysis may be more powerful to find a significant association between TGF-β1 polymorphisms (such as rs1982073 and rs1800471 polymorphisms) and ARR risk. Moreover, due to lack of data, we did not evaluate the effects of gene-gene and gene-environment interactions on development of ARR.

CONCLUSIONS

This meta-analysis result revealed that TGF-β1 rs1982073 (codon 10) polymorphism was not significantly associated with an increased risk of ARR. Moreover, there was no significant association by ethnicity and genotypes of recipients or donors. Thus, our results indicated that TGF-β1 rs1982073 polymorphism might not be useful biomarker to identify patients predisposed to ARR. Data from large-scale, multicenter, epidemiological studies are still needed to validate our findings and the molecular mechanism for the association need to be elucidated in future studies.

CONFLICTING INTERESTS

The authors declared no potential conflicts of interest with respect to the research or publication of this article.

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