Association between the *AGTR1* A1166C polymorphism and risk of IgA nephropathy: a meta-analysis

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ABSTRACT. Numerous studies have evaluated the association between the A1166C polymorphism in the angiotensin II type 1 receptor (*AGTR1*) gene and immunoglobulin A nephropathy (IgAN) risk. However, this relationship remains controversial. Our aim was to evaluate the relationship between this polymorphism and IgAN susceptibility by performing a meta-analysis. Articles were identified in the PubMed, Google Scholar, and China National Knowledge Infrastructure databases, and after selection, five eligible studies were included. Statistical analyses were carried out using Stata 12.0, combining data from all the relevant studies. The pooled odds ratios (ORs) regarding the association between the *AGTR1* A1166C polymorphism and IgAN risk were not statistically significant [A *vs* C: OR = 0.64, 95% confidence interval (CI) = 0.24-1.68; AA *vs* AC+CC: OR = 1.02, 95%CI = 0.74-1.39; CC *vs* AC+AA: OR = 1.20, 95%CI = 0.48-2.98;

AC *vs* AA+CC: OR = 0.96, 95%CI = 0.70-1.31]. In conclusion, the *AGTR1* gene A1166C polymorphism may not be correlated with IgAN susceptibility. However, further studies should be performed to confirm this finding.

Key words: A1166C; Angiotensin II type 1 receptor; IgA nephropathy; Gene polymorphism; Meta-analysis

INTRODUCTION

Immunoglobulin A (IgA) nephropathy (IgAN) is an immune complex-mediated glomerulonephritis characterized pathologically by deposition of IgA-IgG immune complexes in the mesangium of the kidney (Donadio and Grande, 2002). IgAN has become the most common primary glomerulonephritis worldwide, being particularly prevalent in Southeast Asia (D'Amico, 1987), and is a major factor in end-stage renal failure. Despite much investigation, the pathogenesis of IgAN is not yet fully understood. A family history of chronic nephritis, susceptibility to the common cold, preference for salty foods, frequent consumption of raw eggs, and a high intake of carbohydrates, including rice, are significantly associated with increased IgAN risk (Wakai et al., 1999). In addition, many reports have indicated that certain genetic polymorphisms are associated with susceptibility to this disease (Suh et al., 2013; Zhou et al., 2013).

Renin-angiotensin system (RAS) activity is an important modulator of blood pressure and plays a key role in renal diseases (Mochel et al., 2013). In the kidney, the RAS regulates renal cell growth and contributes to elevated transforming growth factor β production, leading to fibrosis (Ruiz-Ortega and Egido, 1997). Angiotensinogen interacts with renin to produce angiotensin I, which is then catalyzed to form angiotensin II (Oudart, 2005). This latter is the main mediator of RAS action, and exerts its effects via two distinct angiotensin II receptor subtypes: type 1 (angiotensin II type 1 receptor, AGTR1) and type 2 (Miura et al., 2011). The AGTR1 is a G protein-coupled receptor that mediates most of the biological actions of the RAS.

The human *AGTR1* gene consists of five exons, and spans over 55 kb of chromosome 3q21-25 (Guo et al., 1994). The A1166C polymorphism (rs5186) is located in the 3' untranslated region, and comprises an A to C transversion at nucleotide 1166. Previous studies have demonstrated that this sequence variation is associated with susceptibility to coronary heart disease, breast cancer, and diabetic nephropathy (Xi et al., 2011; Ding et al., 2012; Zhang et al., 2013). However, little is known regarding the association between the *AGTR1* gene A1166C polymorphism and susceptibility to IgAN.

Many prior investigations have assessed the influence of this polymorphism on IgAN risk. However, their results are controversial. Meta-analysis can be a useful tool to detect an association that might otherwise remain masked in studies of small sample size, especially in those evaluating low-frequency polymorphisms involving rare alleles (Attia et al., 2003). Thus, we performed the present meta-analysis of all eligible case-control studies to clarify the effect of the A1166C polymorphism on risk of IgAN, and to shed some light on the contradictory findings previously reported.

MATERIAL AND METHODS

Selection of studies

Relevant papers published before May 2015 were identified through searches of PubMed, Google Scholar, and China National Knowledge Infrastructure databases using the following terms:

1) IgA nephropathy, IgAN, immunoglobulin A nephropathy; 2) angiotensin II, gene polymorphism, AT1R, A1166C. References given in eligible articles were also reviewed to locate other potentially relevant reports by manual searching.

Inclusion and exclusion criteria

To be included in our meta-analysis, studies had to: 1) consist of a case-control investigation; 2) assess the association between the *AGTR1* gene A1166C polymorphism and IgAN risk; 3) provide sufficient information to enable estimation of odds ratios (ORs) and their 95% confidence intervals (95%CIs); and 4) contain adequate data for the retrieval of *AGTR1* gene A1166C polymorphism genotype frequencies. Studies were excluded based on the following major criteria: 1) they lacked a control population; 2) they lacked available data regarding genotype frequencies; and 3) they were duplicate studies.

Data extraction

The following data were extracted from each study: first author's last name, year of publication, ethnicity of the population studied, source and number of cases and controls, genotype frequencies, and evidence of Hardy-Weinberg equilibrium (HWE) in the control group. Two authors independently performed the data extraction, with any disagreements being resolved by discussion.

Statistical analysis

The *AGTR1* gene A1166C polymorphism distribution in the control group was tested for HWE using the Pearson chi-square test (Chootrakool et al., 2011). The strength of the association between this polymorphism and susceptibility to IgAN was estimated by ORs and 95%CIs under allele comparison (A *vs* C), and genotype comparisons including AA *vs* AC+CC, CC *vs* AC+AA, and AC *vs* AA+CC (Mao and Huang, 2013). We quantified the effect of heterogeneity with the I^2 test. I^2 ranges between 0 and 100% and represents the proportion of inter-study variability that can be attributed to heterogeneity rather than chance. When $I^2 > 50\%$ indicated heterogeneity across studies, the random-effect model was used for the meta-analysis, otherwise the fixed-effect model was employed. Subgroup analyses were performed to explain the diversity among the results of different studies attributable to ethnicity-specific effects. However, it should be noted that as only one study involved a Caucasian population, the ethnicity-based analysis may not be reliable in regard to the Caucasian subgroup. Sensitivity analysis was performed by omitting each study in turn. A study was suspected of excessive influence if the point estimate of its omitted analysis was outside the 95%CI of the combined analysis. Publication bias was investigated using Begg's funnel plots, with $P < 0.05$ representing statistically significant bias. Statistical analyses were performed with the Stata software (version 12.0; StataCorp., College Station, TX, USA).

RESULTS

Study characteristics

The literature search identified 239 potentially relevant papers. Of these, 234 were excluded according to the inclusion and exclusion criteria above. Thus, five studies published

between 1997 and 2013 were included in our meta-analysis (Pei et al., 1997; Maruyama et al., 2001; Lau et al., 2004; Huang et al., 2010; You et al., 2013), incorporating 579 IgAN cases and 483 controls (Figure 1). One involved Caucasian subjects (Pei et al., 1997), while four focused on Asian populations (Maruyama et al., 2001; Lau et al., 2004; Huang et al., 2010; You et al., 2013). The countries in which these investigations were carried out included Canada, Japan, Singapore, and China. All five articles specified the number of participants carrying the A and C alleles of the *AGTR1* A1166C polymorphism in both IgAN patient and control groups. The average frequency of the A allele in the IgAN group was 87.21%, and that in the control group was 88.41%. Taking into account only Asian subjects, the average frequency of the A allele was 83.84% in the case group and 86.31% among the controls. Genotype frequencies in all control groups were consistent with HWE. The study characteristics are presented in Table 1.
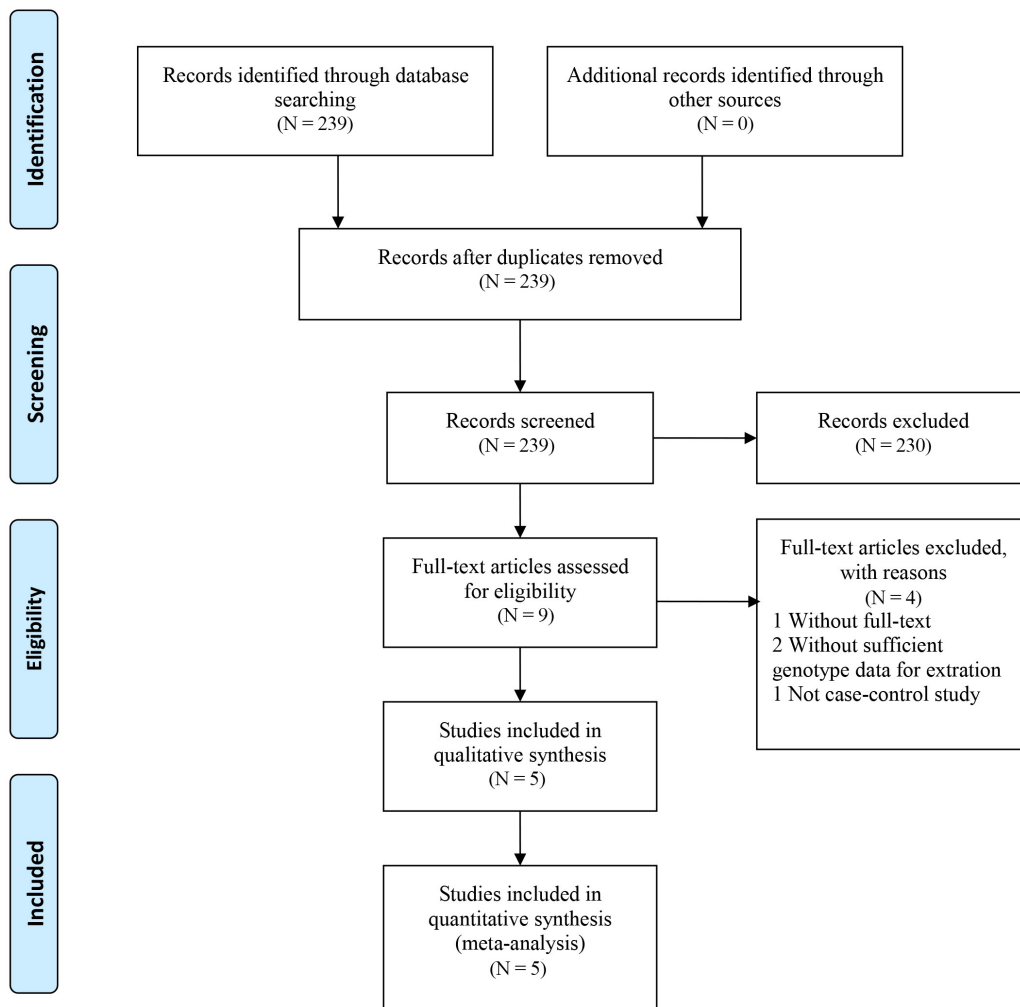

Figure 1. Flow chart describing the selection of articles for our meta-analysis.

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

Reference	Ethnicity	IgAN cases				Controls				A allele (%)		HWE
		AA	AC	CC	Total	AA	AC	CC	Total	Cases	Controls	P value
Pei et al.	Caucasian	82	73	13	168	56	37	7	100	72.0	74.0	0.79
Maruyama et al.	Asian	75	20	0	95	80	19	0	99	89.0	90.0	0.29
Lau et al.	Asian	110	7	1	118	84	10	0	94	96.0	95.0	0.59
Huang et al.	Asian	113	17	0	130	100	20	0	120	93.5	91.7	0.31
You et al.	Asian	62	6	0	68	60	10	0	70	91.2	85.7	0.52

IgAN = immunoglobulin A nephropathy, HWE = Hardy-Weinberg equilibrium.

Quantitative synthesis

Data from five investigations were pooled into our meta-analysis of the association between the *AGTR1* A1166C polymorphism and IgAN susceptibility, a summary of the results of which is presented in Figure 2 and Table 2. In the overall analysis including all studies, no significant association was found (A *vs* C: OR = 0.64, 95%CI = 0.24-1.68; AA *vs* AC+CC: OR = 1.02, 95%CI = 0.74-1.39; CC *vs* AC+AA: OR = 1.20, 95%CI = 0.48-2.98; AC *vs* AA+CC: OR = 0.96, 95%CI = 0.70-1.31). In order to identify potential differences based on ethnicity, we performed a subgroup analysis. Since only one study was performed in a Caucasian population, however, results concerning this subgroup may not be reliable. No significant association was observed among Asian subjects.
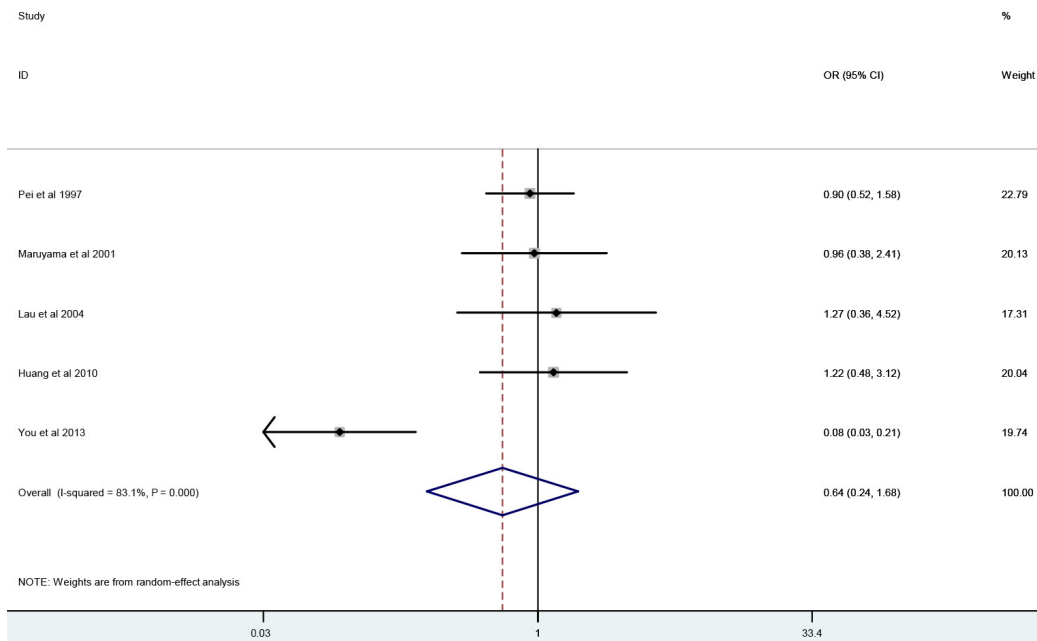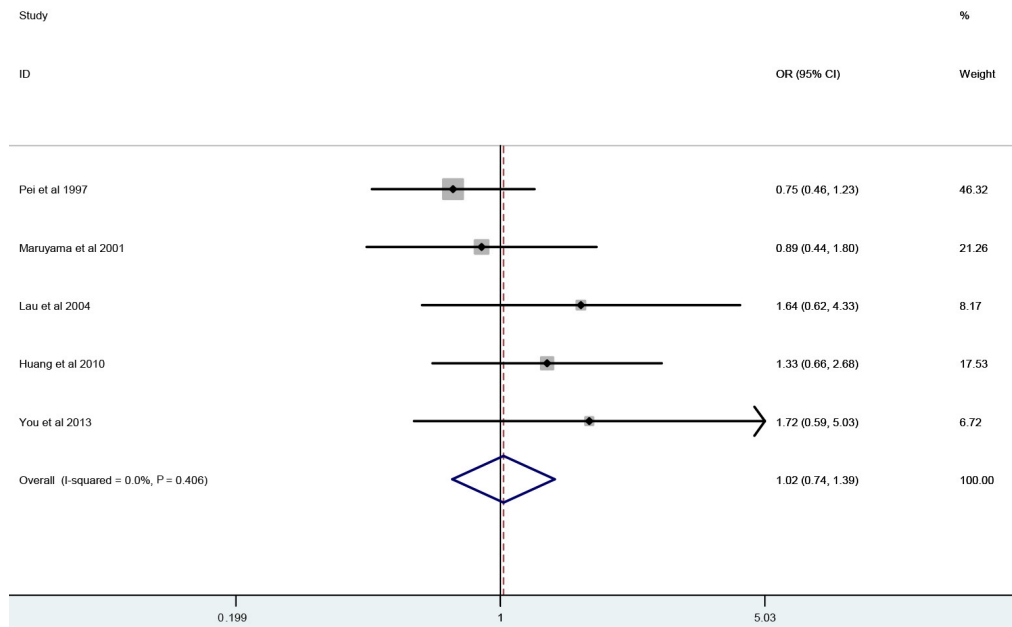
A. A *vs* C

Figure 2. Association of the *AGTR1* A1166C gene polymorphism with IgAN susceptibility in the overall study population. **A.** A *vs* C. **B.** AA *vs* AC+CC. **C.** CC vs AC+AA. **D.** AC *vs* AA+CC. OR = odds ratio, CI = confidence interval.
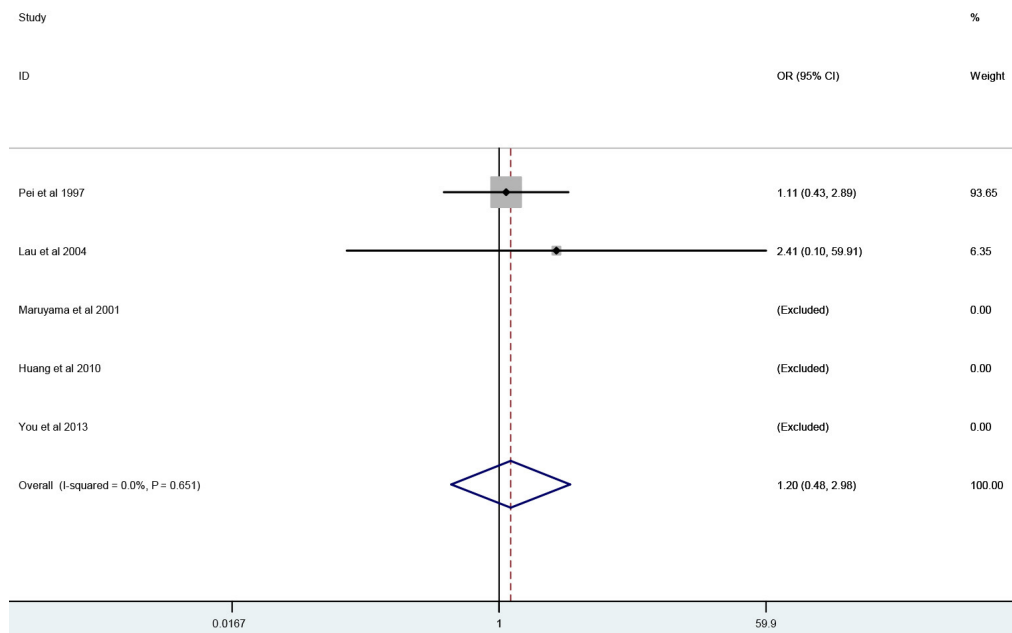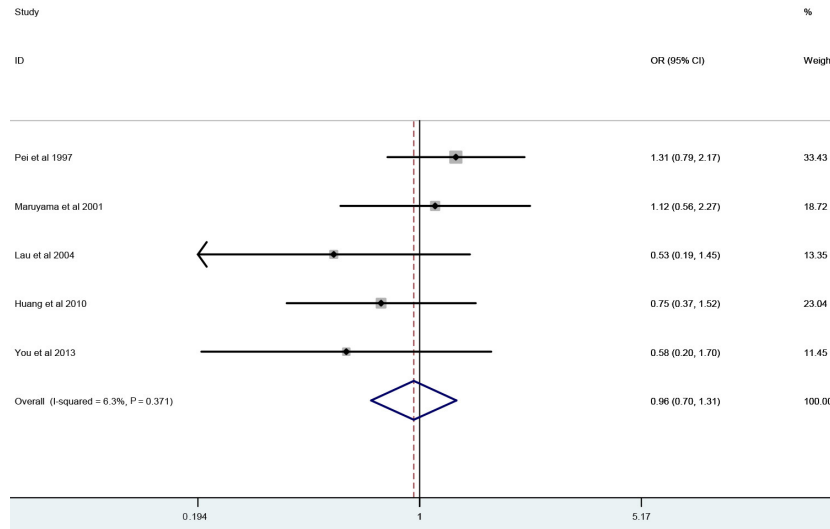
Figure 2. Continued.

B. AA *vs* AC+CC

Study ID	OR (95% CI)	% Weight
Pei et al 1997	0.75 (0.46, 1.23)	46.32
Maruyama et al 2001	0.89 (0.44, 1.80)	21.26
Lau et al 2004	1.64 (0.62, 4.33)	8.17
Huang et al 2010	1.33 (0.66, 2.68)	17.53
You et al 2013	1.72 (0.59, 5.03)	6.72
Overall (I-squared = 0.0%, P = 0.406)	1.02 (0.74, 1.39)	100.00

C. CC *vs* AC+AA

Study ID	OR (95% CI)	% Weight
Pei et al 1997	1.11 (0.43, 2.89)	93.65
Lau et al 2004	2.41 (0.10, 59.91)	6.35
Maruyama et al 2001	(Excluded)	0.00
Huang et al 2010	(Excluded)	0.00
You et al 2013	(Excluded)	0.00
Overall (I-squared = 0.0%, P = 0.651)	1.20 (0.48, 2.98)	100.00

Figure 2. Continued.

D. AC *vs* AA+CC

Sensitivity analysis

Sensitivity analysis was performed by omitting each study in turn to evaluate the influence of single studies on the overall estimation (Figure 3). The corresponding pooled OR and principal results did not change appreciably, indicating that our findings were statistically robust.

Publication bias

Begg's funnel plots were generated to test for possible publication bias in the literature used (Figure 4). No evidence of such bias was observed (P > 0.05, Table 2).
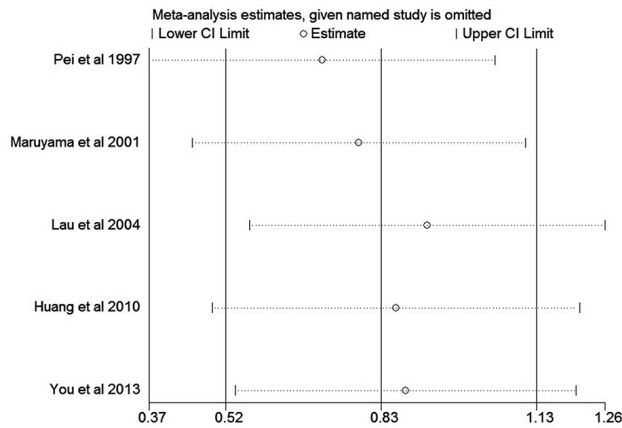

Figure 3. Results of sensitivity analysis using fixed-effect estimates. CI = confidence interval.
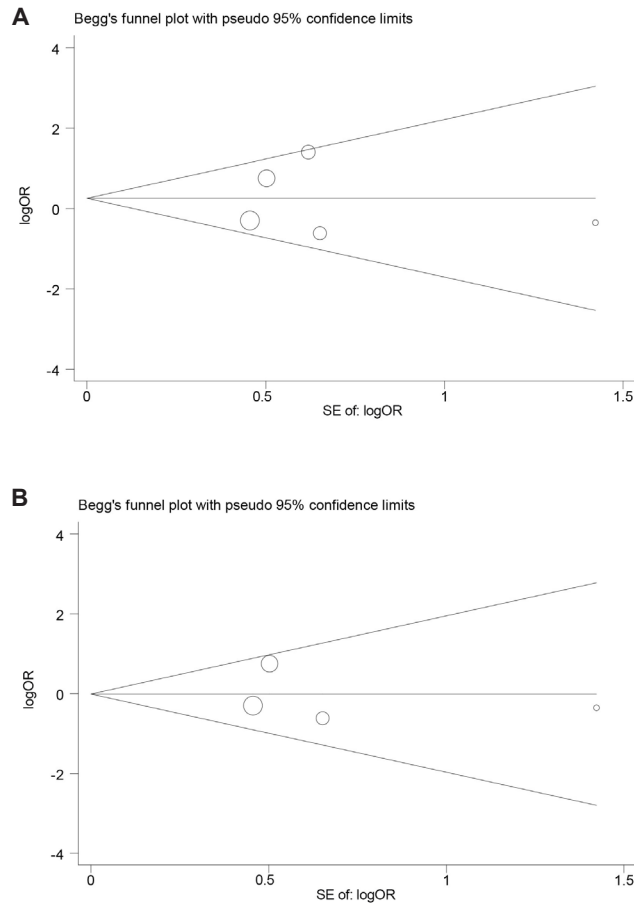
Figure 4. Assessment of publication bias in the analysis of the association between the *AGTR1* A1166C gene polymorphism and IgAN susceptibility. **A.** Publication bias in the overall dataset. **B.** Publication bias in the data concerning Asian subjects. OR = odds ratio.

Table 2. Summary of odds ratios and 95% confidence intervals regarding the relationship between the *AGTR1* gene A1166C polymorphism and immunoglobulin A nephropathy risk.

Subgroup	Genetic model	Sample size		Type of model	Test of heterogeneity		Test of association		Test of publication bias	
		Cases	Controls		I^2 (%)	P	OR	95%CI	Z	P
Overall	A *vs* C	579	483	Random	83.1	0.00	0.64	0.24-1.68	0.24	0.81
	AA *vs* AC+CC			Fixed	0.0	0.41	1.02	0.74-1.39	0.24	0.81
	CC *vs* AC+AA			Fixed	0.0	0.65	1.20	0.48-2.98	0.24	0.81
	AC *vs* AA+CC			Fixed	6.3	0.37	0.96	0.70-1.31	0.24	0.81
Asian	A *vs* C	411	383	Random	86.3	0.00	0.58	0.15-2.28	0.34	0.73
	AA *vs* AC+CC			Fixed	0.0	0.67	1.25	0.83-1.88	0.34	0.73
	CC *vs* AC+AA			Fixed	-	-	2.41	0.15-59.91	-	-
	AC *vs* AA+CC			Fixed	0.0	0.59	0.78	0.52-1.18	0.34	0.73

OR = odds ratio; CI = confidence interval.

DISCUSSION

IgAN, the most prevalent glomerular disease worldwide, is predominantly characterized by IgA deposition in the mesangium and requires a renal biopsy for diagnosis (Hastings et al., 2013). Although in most cases its cause is well known, this disease has proven difficult to diagnose early and treat successfully, reflecting limited advances in our understanding of the molecular mechanisms responsible. Generally, IgAN is considered a multifactorial disease initiated by the interaction between genetic factors and environmental conditions. Previous studies have demonstrated that the RAS is activated at an early stage in IgAN model mice (Ruiz-Ortega and Egido, 1997). The kidney's local RAS has several pathophysiologic functions, not only regulating blood pressure and renal cell growth, but also being implicated in glomerulosclerosis, a process involved in the development of renal fibrosis (Zhang et al., 2014). The modifications to AGTR1 is the primary pathogenic effector of angiotensin II. Recently, a variety of studies have focused on the association between the *AGTR1* gene A1166C polymorphism and IgAN. However, the results obtained from such investigations have been inconclusive. To help resolve these conflicting findings, we conducted this meta-analysis, combining data from similar studies to increase sample size and statistical power and achieve a more robust result.

To the best of our knowledge, this is the first meta-analysis to assess the relationship between the *AGTR1* A1166C polymorphism and risk of IgAN. Our study quantitatively assessed this association, revealing that this *AGTR1* sequence variation is associated with neither increased nor decreased risk of IgAN. Moreover, in the stratified analysis relating to ethnicity, our results indicated that the A1166C polymorphism was not associated with IgAN risk in Asians. However, as only one paper involving a Caucasian population was identified (Pei et al., 1997), further studies focusing on Caucasian patients should be carried out in future. No publication bias was evident in this meta-analysis.

Associations between the *AGTR1* A1166C polymorphism and various diseases have been investigated previously. For example, Zhang et al. (2014) found that this variant is significantly associated with risk of hypertensive disorders of pregnancy. Moreover, in a meta-analysis, Mao et al. (2013) found no correlation between this polymorphism and risk of end-stage renal disease in the overall study population, nor in Caucasian and Asian subgroups. Zhang et al. (2013) observed that it may be involved in the development of coronary heart disease, while Zhang et al. (2011) reported a lack of association with susceptibility to ischemic stroke. In addition, Xi et al. (2011) demonstrated that it might be implicated in the pathogenesis of breast cancer, and Ding et al. (2012) established its possible contribution to the development of diabetic nephropathy.

In our meta-analysis, we found that the *AGTR1* gene A1166C polymorphism was not associated with risk of IgAN. There are several potential explanations for such a negative result. First, most of the included studies comprised few cases and controls, leading to a lack of power to detect common small effects in genetic associations involving multifactorial characteristics (Colhoun et al., 2003). Second, it may be partly attributable to the considerable heterogeneity observed among the investigations used in our meta-analysis. Heterogeneity may result from variations in genetic backgrounds and environmental factors, differences in sample selection (e.g. age, sex, or diagnostic criteria), or dissimilarities in study design (Bai et al., 2012). Third, diagnosis of IgAN depends on renal biopsy, meaning that those patients who did not agree to this procedure were excluded from the analysis (Mao and Huang, 2014). Finally, any potential influence of the A1166C polymorphism may be effected via gene-gene and gene-environment interactions (Qin et

al., 2011). In future, assessment of polymorphism haplotypes combined with environmental factors, rather than individual polymorphisms in isolation, could be used as a risk stratification tool for IgAN.

In conclusion, our meta-analysis indicates that the A1166C polymorphism of the *AGTR1* gene is not associated with IgAN risk. Large-scale case-control and population-based association studies are warranted to validate the findings of the current meta-analysis and investigate the effect of any gene-gene and gene-environment interactions on IgAN susceptibility.

Conflicts of interest

The authors declare no conflict of interest.

REFERENCES

Attia J, Thakkinstian A and D'Este C (2003). Meta-analyses of molecular association studies: methodologic lessons for genetic epidemiology. *J. Clin. Epidemiol.* 56: 297-303.

Bai Y, Wang L, Hu S and Wei Y. (2012). Association of angiotensin-converting enzyme I/D polymorphism with heart failure: a meta-analysis. *Mol. Cell. Biochem.* 361: 297-304.

Chootrakool H, Shi JQ and Yue R (2011). Meta-analysis and sensitivity analysis for multi-arm trials with selection bias. *Stat. Med.* 30: 1183-1198.

Colhoun HM, McKeigue PM and Davey Smith G (2003). Problems of reporting genetic associations with complex outcomes. *Lancet* 361: 865-872.

D'Amico G (1987). The commonest glomerulonephritis in the world: IgA nephropathy. *Q. J. Med.* 64: 709-727.

Ding W, Wang F, Fang Q, Zhang M, et al. (2012). Association between two genetic polymorphisms of the renin-angiotensin-aldosterone system and diabetic nephropathy: a meta-analysis. *Mol. Biol. Rep.* 39: 1293-1303.

Donadio JV and Grande JP (2002). IgA nephropathy. *N. Engl. J. Med.* 347: 738-748.

Guo DF, Furuta H, Mizukoshi M and Inagami T (1994). The genomic organization of human angiotensin II type 1 receptor. *Biochem. Biophys. Res. Commun.* 200: 313-319.

Hastings MC, Moldoveanu Z, Suzuki H, Berthoux F, et al. (2013). Biomarkers in IgA nephropathy: relationship to pathogenetic hits. *Expert Opin. Med. Diagn.* 7: 615-627.

Huang HD, Lin FJ, Li XJ, Wang LR, et al. (2010). Genetic polymorphisms of the renin-angiotensin-aldosterone system in Chinese patients with end-stage renal disease secondary to IgA nephropathy. *Chin. Med. J.* 123: 3238-3242.

Lau YK, Woo KT, Choong HL, Zhao Y, et al. (2004). Renin-angiotensin system gene polymorphisms: its impact on IgAN and its progression to end-stage renal failure among Chinese in Singapore. *Nephron Physiol.* 97: 1-8.

Mao S and Huang S (2013). Lack of association of angiotensin II type 1 receptor A1166C gene polymorphism with the risk of end-stage renal disease. *Ren. Fail.* 35: 1295-1301.

Mao S and Huang S (2014). Association of angiotensinogen gene M235T polymorphism with the risk of IgA nephropathy: a meta-analysis. *Ren. Fail.* 36: 466-472.

Maruyama K, Yoshida M, Nishio H, Shirakawa T, et al. (2001). Polymorphisms of renin-angiotensin system genes in childhood IgA nephropathy. *Pediatr. Nephrol.* 16: 350-355.

Miura S, Karnik SS and Saku K (2011). Review: angiotensin II type 1 receptor blockers: class effects versus molecular effects. *J. Renin Angiotensin Aldosterone Syst.* 12: 1-7.

Mochel JP, Fink M, Peyrou M, Desevaux C, et al. (2013). Chronobiology of the renin-angiotensin-aldosterone system in dogs: relation to blood pressure and renal physiology. *Chronobiol. Int.* 30: 1144-1159.

Oudart N (2005). The renin-angiotensin system: current data. *Ann. Pharm. Fr.* 63: 144-153.

Pei Y, Scholey J, Thai K, Suzuki M, et al. (1997). Association of angiotensinogen gene T235 variant with progression of immunoglobin A nephropathy in Caucasian patients. *J. Clin. Invest.* 100: 814-820.

Qin YH, Zhou TB, Su LN, Lei FY, et al. (2011). Association between ACE polymorphism and risk of IgA nephropathy: a meta-analysis. *J. Renin Angiotensin Aldosterone Syst.* 12: 215-223.

Ruiz-Ortega M and Egido J (1997). Angiotensin II modulates cell growth-related events and synthesis of matrix proteins in renal interstitial fibroblasts. *Kidney Int.* 52: 1497-1510.

Suh JS, Cho SH, Chung JH, Moon A, et al. (2013). A polymorphism of interleukin-22 receptor alpha-1 is associated with the development of childhood IgA nephropathy. *J. Interferon Cytokine Res.* 33: 571-577.

Wakai K, Kawamura T, Matsuo S, Hotta N, et al. (1999). Risk factors for IgA nephropathy: a case-control study in Japan. *Am. J. Kidney Dis.* 33: 738-745.

Xi B, Zeng T, Liu L, Liang Y, et al. (2011). Association between polymorphisms of the renin-angiotensin system genes and breast cancer risk: a meta-analysis. *Breast Cancer Res. Treat.* 130: 561-568.

You YW, Yang FF, Lin Z and Wang J (2013). Gene polymorphism of renin angiotensin system in IgA nephropathy patients with renal damage. *Chin. Gen. Pract.* 16: 2365-2368.

Zhang H, Sun M, Sun T, Zhang C, et al. (2011). Association between angiotensin II type 1 receptor gene polymorphisms and ischemic stroke: a meta-analysis. *Cerebrovasc. Dis.* 32: 431-438.

Zhang K, Zhou B and Zhang L (2013). Association study of angiotensin II type 1 receptor: A1166C (rs5186) polymorphism with coronary heart disease using systematic meta-analysis. *J. Renin Angiotensin Aldosterone Syst.* 14: 181-188.

Zhang L, Yang H, Qin H and Zhang K (2014). Angiotensin II type I receptor A1166C polymorphism increases the risk of pregnancy hypertensive disorders: Evidence from a meta-analysis. *J. Renin Angiotensin Aldosterone Syst.* 15: 131-138.

Zhou XJ, Cheng FJ, Qi YY, Zhao YF, et al. (2013). *FCGR2B* and *FCRLB* gene polymorphisms associated with IgA nephropathy. *PLoS One* 8: e61208.