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Lack of an association between *TSC* gene Arg904Gln polymorphisms and essential hypertension risk based on a meta-analysis

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ABSTRACT. Although there have been several studies investigating a possible association between essential hypertension and *TSC* gene Arg904Gln polymorphisms, the results have been inconsistent. We conducted a meta-analysis of four case-control studies (one study in Europe and three studies in Asia), including 1811 essential hypertension cases and 1381 controls. The pooled results showed no significant associations between any of these polymorphisms and essential hypertension (allele Arg *vs* allele Gln: odds ratio (OR) = 0.94, 95% confidence interval (95%CI) = 0.70-1.27), additive genetic model (Arg/Arg *vs* Gln/Gln: OR = 0.98, 95%CI = 0.43-2.23), dominant genetic model (Arg/Arg + Arg/Gln *vs* Gln/Gln: OR = 0.97, 95%CI = 0.43-2.21), and recessive genetic model (Arg/Arg *vs* Arg/Gln + Gln/Gln: OR = 1.03, 95%CI = 0.45-2.35). Based on the results of our meta-analysis, we conclude that the *TSC* gene Arg904Gln polymorphism is not associated with essential hypertension risk.

Key words: *TSC*; Arg904Gln; Polymorphisms; Essential hypertension; Meta-analysis

INTRODUCTION

Essential hypertension is a complex, multi-factorial, and polygenic disease; it is one of the most important risk factors for cardiovascular disease, leading to over 450,000 deaths in the United States of America in 2004 (Capewell et al., 2010). Essential hypertension is caused by genetic and environmental factors. Many risk factors have been associated with essential hypertension, including age, gender, obesity, and excess salt intake (Stanto et al., 1982). Recently, genetic investigations have revealed several genetic variants that are candidates in the pathogenesis of hypertension (Johnson et al., 2011; Hasi et al., 2011; Fu et al., 2011). Current studies suggest that the thiazide-sensitive Na-Cl cotransporter (*TSC*) gene plays an important role on the risk of essential hypertension in diverse ethnic groups (Melander et al., 2000; Song et al., 2001; Glorioso et al., 2001; Matsuo et al., 2004; Zhan et al., 2007; Wang et al., 2008). The *TSC* gene is located on chromosome 16q13, consisting of 26 exons that encode 1021-amino acid residues (Plotkin et al., 1996). Among the SNPs of the *TSC* gene, Arg904Gln is at amino acid position 904 of the thiazide-sensitive NaCl-cotransporter, the G→A mutation in exon 23 of the *TSC* gene (G2736A), which leads to a replacement of Arg by Gln (Melander et al., 2000). Compared with other SNPs of the *TSC* gene, such as Gly264Ala and C1420T (Melander et al., 2000), it is reported more frequently as associated with essential hypertension risk.

Several studies have evaluated the association between *TSC* gene Arg904Gln polymorphisms and essential hypertension risk. However, the results remain somewhat contradictory and inconclusive. To overcome the limitations of individual studies and to understand the real situation, we made a meta-analysis of four case-control studies.

MATERIAL AND METHODS

Search strategy

We searched the PubMed and Embase database for studies published up to June of 2011, by using items: "thiazide-sensitive Na-Cl cotransporter gene", "*TSC* gene", "Arg904Gln", "G2736A", "hypertension", and "blood pressure". We also sought additional studies by reviewing the reference lists of included articles, conference abstracts, and relevant bibliographies.

Selection criteria

Two reviewers (Zhang F and Yang Y) reviewed all citations and retrieved literature by titles/abstracts and whole texts. Those studies were selected by meeting the following criteria: 1) the publication was a case-control study referring to association between the *TSC* gene and the risk for essential hypertension; 2) the papers must offer sample size, distribution of alleles, genotypes, or other information that can help us infer the results; 3) when multiple publications reported on the same or overlapping data, we used the most recent or largest population as recommended by Little et al. (2002), and 4) we limited the publication languages to English and Chinese.

Data extraction

Data were extracted from each study by two reviewers (Zhang F and Yang Y), independently according to the pre-specified selection criteria. Extraction forms of two reviewers

were compared. Disagreements were discussed. Decisions were made by consensus or by involving a third reviewer. The following information was extracted from the studies: first author, publishing year, studied polymorphisms, ethnicity of subjects, source of controls, and distribution of alleles and genotypes in case and control groups.

Statistical analysis

Crude odds ratios (ORs) with their 95% confidence intervals (95%CIs) for alleles and genotypes were used to assess the strength of association between *TSC* gene Arg904Gln polymorphisms and essential hypertension risk. The pooled ORs were performed for the allele contrasts, additive genetic model, dominant genetic model, and recessive genetic model. Heterogeneity assumption was assessed by the chi-square-based Q-test and the I-squared test. The heterogeneity was not considered significant when $I^2 < 50\%$. With lack of heterogeneity among studies, the pooled OR estimate of each study was calculated by the fixed-effect model (Wang et al., 2008). Otherwise, the random-effect model was used (Matsuo et al., 2004; Zhan et al., 2007). Departure of frequencies of *TSC* gene Arg904Gln polymorphisms from expectation according to Hardy-Weinberg equilibrium (HWE) was assessed by the chi-square test in controls. Funnel plots were used to assess the possibility of publication bias.

The Cochrane Collaboration meta-analysis software, Review Manager 5.0, was used for analysis. A P value of 0.05 for any test or model was considered to be statistically significant.

RESULTS

Eligible studies

After careful examination according to the inclusion criteria, our final pool of eligible studies included four case-control studies with 1811 cases and 1381 controls. As shown in Table 1, there was one study involving European subjects and three studies involving Asian subjects (two in Chinese including 3 ethnic groups and one in Japanese). Almost all of the cases were histologically confirmed. Controls were mainly healthy individuals of the same population, which were matched for age and ethnicity. The genotype distribution in the controls of all studies was in agreement with HWE except for one study (Zhan et al., 2007).

Table 1. Characteristics of case-control studies included in the *TSC* gene Arg904Gln polymorphism and essential hypertension risk analysis.

Author	Ethnicity	Source of control	Case			Control		
			AA	AG	GG	AA	AG	GG
Wang et al., 2008	Kazaks	Population-based	472	117	4	250	53	1
	Uyghurs	Population-based	278	61	0	262	68	7
Zhan et al., 2007	Han	Population-based	167	22	1	83	10	1
Matsuo et al., 2004	Japanese	Hospital-based	332	52	2	338	33	0
Melander et al., 2000	Europe	Population-based	239	48	5	210	54	0

Quantitative synthesis

The main results of this meta-analysis are shown in Table 2 and the heterogeneity tests

are shown in Figures 1-4. Overall, no significant associations were found for the allele contrast (allele Arg *vs* allele Gln: OR = 0.94, 95%CI = 0.70-1.27), additive genetic model (Arg/Arg *vs* Gln/Gln: OR = 0.98, 95%CI = 0.43-2.23), dominant genetic model (Arg/Arg + Arg/Gln *vs* Gln/Gln: OR = 0.97, 95%CI = 0.43-2.21), or recessive genetic model (Arg/Arg *vs* Arg/Gln + Gln/Gln: OR = 1.03, 95%CI = 0.45-2.35).

Table 2. Summary of odds ratio (OR) and 95% confidence interval (95%CI) of the *TSC* gene Arg904Gln polymorphism and essential hypertension risk.

Polymorphism	OR	95%CI	P
A *vs* G*	0.94	0.70-1.27	0.7
AA *vs* GG	0.98	0.43-2.23	0.96
AA+AG *vs* GG	0.97	0.43-2.21	0.94
AA *vs* AG+GG	1.03	0.45-2.35	0.94

*Estimates for a random-effect model.

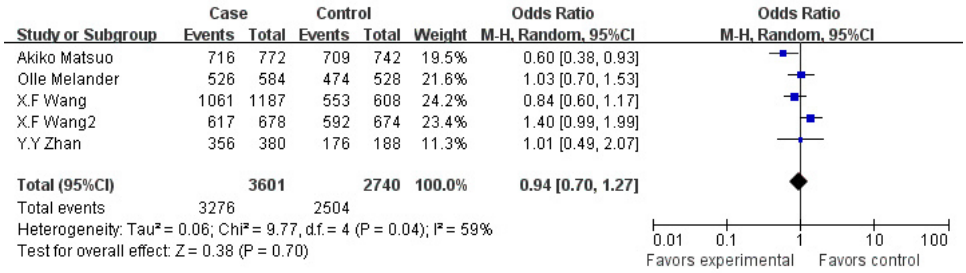

Figure 1. Forest plots of the allele contrast. M-H = Mantel-Haenszel estimator; 95%CI = confidence interval at 95%; d.f. = degrees of freedom.

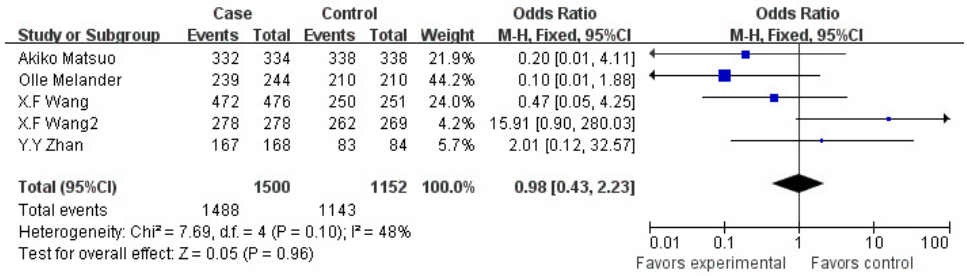

Figure 2. Forest plots of additive genetic model. For abbreviations, see legend to Table 1.

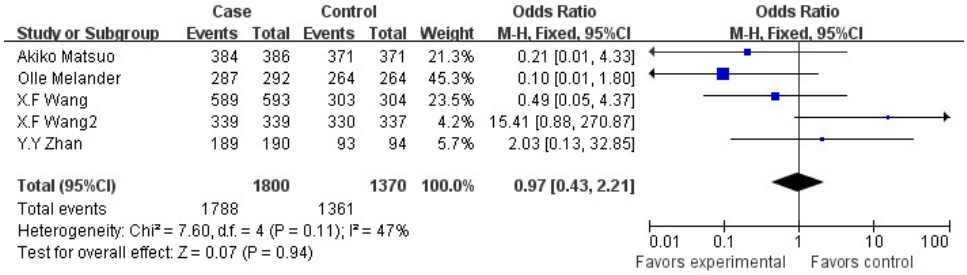

Figure 3. Forest plots of dominant genetic model. For abbreviations, see legend to Table 1.

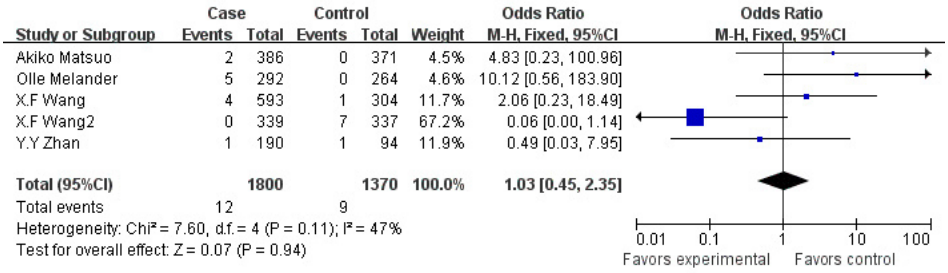

Figure 4. Forest plots of recessive genetic model. For abbreviations, see legend to Table 1.

Sensitivity analysis

Sensitivity analyses were conducted to determine whether modification of the inclusion criteria of the meta-analysis affected the final results. These were carried out by limiting the meta-analysis to studies conforming to HWE and altering corresponding statistic variables and analysis models. Additional sensitivity analyses indicated that the point estimate and 95%CI for the OR were not significantly altered with the exclusion of any individual study. We concluded that our results were statistically robust.

Bias diagnosis

Funnel plots were made to access the publication bias of literatures. As shown in Figure 5, the shape of the funnel plot revealed relatively symmetry.

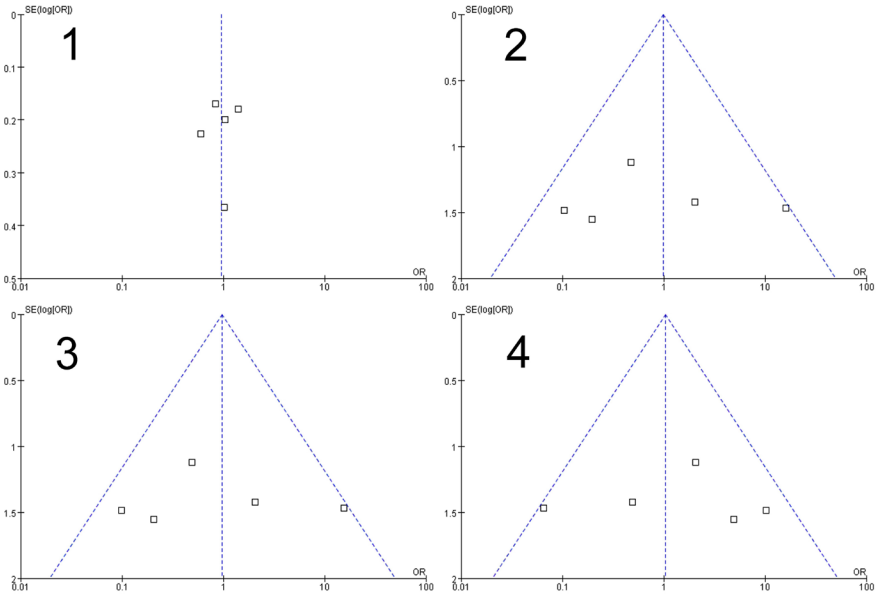

Figure 5. Funnel plots: Funnel plot 1 for allele comparison; Funnel plot 2 for additive genetic model; Funnel plot 3 for dominant genetic model; Funnel plot 4 for recessive genetic model.

DISCUSSION

Hypertension is a common disease caused by polygenic factors combined with environment. Many candidate genes have been reported to be involved in essential hypertension susceptibility, including *TSC*, NEDD4L (Luo et al., 2009), CYP4F2 (Ward et al., 2008), EMILIN 1 (Shimodaira et al., 2010), ATP2B1 (Tabara et al., 2010), apelin-AGTRL1 system (Niu et al., 2010), and so on. Several studies suggest that *TSC* plays a major role in sodium and chloride re-absorption in the distal convoluted tubule, accounting for a significant proportion of the total renal sodium re-absorption (Plotkin et al., 1996; Matsuo et al., 2004). *TSC* is a target of the diuretic effect of thiazides and is an established antihypertensive drug in the management of uncomplicated hypertension. Mutations in the human *TSC* (h*TSC*) gene affect blood pressure regulation, as seen in Gitelman's syndrome (Melander et al., 2000), which is a disease characterized by sodium wasting and low blood pressure. These characteristics validated the *TSC* gene as a logical candidate gene for hypertension susceptibility. In order to obtain persuasive results, we did this meta-analysis to evaluate the association of *TSC* gene Are904Gln polymorphisms with essential hypertension susceptibility.

Melander et al. (2000) found that the Arg904Gln polymorphism is significantly associated with hypertension in the Swedish population. Gln904 homo-zygotes were overrepresented in hypertensive patients compared with normotensive subjects (5 of 292 *vs* 0 of 264; $P = 0.03$). Matsuo et al. (2004) showed that the Arg904Gln (G2736A) polymorphism is significantly associated with prevalence of hypertension ($P < 0.04$), and the estimated OR was 1.8 (95%CI = 1.1-3.0). However, the other two studies were performed on the Chinese Han ethnic group, and other two minorities - Kazaks and Uyghurs (Wang et al., 2008). In Han and Uyghurs, there was no association between the Arg904Gln polymorphism and hypertension risk, but in the minority Kazaks, the results were different from those of the Swedish and Japanese populations. A stronger trend of 904Gln allele in controls than hypertensives ($P = 0.058$) was observed, and the greater prevalence of the 904Gln carrier genotype reached significance ($P = 0.015$).

Combining all of the studies, our meta-analysis found no significant associations between Arg904Gln polymorphisms and essential hypertension for the allele contrast (allele Arg *vs* allele Gln: OR = 0.94, 95%CI = 0.70-1.27), additive genetic model (Arg/Arg *vs* Gln/Gln: OR = 0.98, 95%CI = 0.43-2.23), dominant genetic model (Arg/Arg + Arg/Gln *vs* Gln/Gln: OR = 0.97, 95%CI = 0.43-2.21), or recessive genetic model (Arg/Arg *vs* Arg/Gln + Gln/Gln: OR = 1.03, 95%CI = 0.45-2.35).

A recent study on association of *TSC* gene variants and hypertension in Mongolian and Han populations (Chang et al., 2011) included 385 unrelated Mongolian herdsmen and 523 Han farmers, and found significant differences between the genotype and allele frequencies of rs13306673 between the essential hypertension group and the control group in the Han population and significant associations between the rs7204044 variant and essential hypertension in both the Mongolian and Han ethnic groups. However, the mechanisms by which rs13306673 and rs7204044 might contribute to essential hypertension are currently unknown. So we do not know whether there is a relationship between it and the Arg904Gln or not. More research should be done, and larger data sets should be analyzed.

There are some limitations in our review. First, the bias between the ethnic groups was obvious and the combined studies were few, only four studies referring to five ethnic groups. Second, the controls of one study were hospital-based normal individuals or patients

of other diseases, which did not possess adequate representation (Matsuo et al., 2004). Third, the languages were limited to English and Chinese for the systematic review. Furthermore, the *TSC* gene contains many more polymorphisms than those mentioned in our review. Given the limited evidence available on other *TSC* gene polymorphisms, this article was restricted to the *TSC* gene Arg904Gln polymorphism.

Conclusively, our review suggests that *TSC* gene Arg904Gln polymorphisms are not associated with essential hypertension risk. Large well-designed and multi-center epidemiological studies will be necessary to check genetic factors in diverse ethnicities.

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