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ORIGINAL ARTICLE

α-*adducin* Gly460Trp polymorphism and essential hypertension risk in Chinese: a meta-analysis

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No clear consensus has been reached on the α-*adducin* polymorphism (Gly460Trp) and essential hypertension (EH) risk in Chinese. We conducted a meta-analysis in an effort to systematically explore the possible association. Case-control studies in Chinese and English performed with human subjects were identified by searching the MEDLINE, EMBASE, China Biological Medicine Database, China National Knowledge Infrastructure platform, Wanfang and VIP databases. The fixed-effects model and the random-effects model for dichotomous outcomes were applied to combine the results of the individual studies. We selected 20 studies that met the inclusion criteria, including a total of 5562 patients with hypertension and 4289 controls. Overall, our findings supported the hypothesis that the *ADD1* Gly460Trp polymorphism is associated with EH in the Chinese population. A borderline association was found between the tryptophan (Trp) allele of the Gly460Trp variant and hypertension (P=0.05, Odds ratio (OR)=1.08, 95% confidence interval (CI)=1.00–1.17 and $P_{heterogeneity}$=0.02). Significantly increased risk was observed in the recessive genetic model (P=0.0009, OR=1.24, 95% CI=1.09–1.41 and $P_{heterogeneity}$=0.04) as well as in the homozygote comparison (P=0.006, OR=1.25, 95% CI=1.07–1.46 and $P_{heterogeneity}$=0.03). Furthermore, in the subgroup analysis, our results support a positive association among Chinese Han individuals (P=0.001, OR=1.25, 95% CI=1.09–1.42, $P_{heterogeneity}$=0.08, recessive genetic model; P=0.009, OR=1.26, 95% CI=1.06–1.50, $P_{heterogeneity}$=0.03, homozygote comparison). No apparent association was identified in Kazakhs. Our meta-analysis suggests that the Gly460Trp polymorphism might increase the risk of hypertension in Chinese populations, especially in Han Chinese. Further studies investigating gene–gene, gene–environment and mutual interactions are needed to better understand the role of *ADD1* in hypertension.
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INTRODUCTION

Essential hypertension (EH) is a major public health problem that affects many members of developed countries as well as developing countries. According to the Nutrition and Health Survey of the People's Republic of China, the prevalence of hypertension was estimated to be ~18.8% in the Chinese adult population, and 0.17 billion people were affected by it in 2002 (see ref. 1). This complex disorder is regarded as a multifactorial condition in which various hereditary and environmental factors have a role in determining the occurrence and development of the disease. Some research has suggested that interindividual variation in the risk of hypertension has a genetic component.[2] Approximately 20–60% of the population variability in blood pressure appears to be genetically determined.[3] As a result, candidate genes for hypertension have been extensively studied.

Adducin is a ubiquitously expressed heterodimeric cytoskeleton protein composed of two subunits (an α-subunit and either a β- or γ-subunit depending on the tissue) that are encoded by three genes (*ADD1*, *ADD2* and *ADD3*, respectively) located on different chromosomes.[4] The human α-*adducin* gene was mapped to chromosome 4p16.3 and comprises 16 exons. Animal and clinical studies have suggested that an alteration in *ADD1* might be a contributing factor in the pathogenesis of hypertension. Previous studies using the Milan hypertensive rat strain model of hypertension showed that the α-*adducin* gene point mutation could affect renal sodium transport

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and thus cause hypertension.[5] The role of the α-*adducin* gene in hypertension and other cardiovascular diseases has been extensively evaluated at the molecular level, with particular attention to the rs4961 (Gly460Trp, G460W or G460T) single nucleotide polymorphism at exon 10, (see ref. 6) where a guanine-to-thymine transversion at nucleotide 614 leads to a glycine (Gly) to tryptophan (Trp) substitution at amino acid position 460. In the last decade, a large number of studies seeking to link the Gly460Trp polymorphism to hypertension have been conducted in different races and ethnic groups, but the results have often been unreproducible.[7–9] A similar situation also occurred in China. We therefore performed a formal meta-analysis to further evaluate the relationship of this polymorphism with EH in the Chinese population.

METHODS

Identification and eligibility of relevant studies

Genetic association studies evaluating the α-*adducin* gene Gly460Trp polymorphism and EH in Chinese populations published before 1 May 2010 were included in our meta-analysis. We conducted a systematic computerized literature search of the PubMed, EMBASE, China Biological Medicine Database (http://cbm.imicams.ac.cn), China National Knowledge Infrastructure platform (http://www.cnki.net), Wanfang (http://www.wanfangdata.com.cn) and VIP (http://www.cqvip.com.cn) databases using the MeSH term 'Chinese' or 'China' in combination with the following keywords and MeSH terms: 'hypertension', '*α-adducin*' or '*ADD1*', and 'polymorphism'. Search results were limited to studies on human subjects and articles in Chinese or English. The full text of the retrieved studies was scrutinized, and all of the references cited in the articles were searched to identify potentially relevant studies. To avoid data duplication when potentially overlapping populations were described in multiple reports, we selected the study that provided the fullest amount of information. Thus, individuals enrolled in more than one article were counted only once. If detailed genotyping information was not reported, we contacted the original authors to obtain the relevant data. All retrieved articles were screened to meet all the following criteria: (a) studies investigating the relationship between the *ADD1* Gly460Trp polymorphism and hypertension in Chinese individuals; (b) use of an unrelated case-control design (family-based study designs with linkage considerations were excluded); (c) availability of genotype frequencies in both cases and controls; and (d) a genotype distribution of the control population consistent with Hardy–Weinberg equilibrium (HWE). Hypertension was defined as systolic blood pressure $\geqslant$140 mm Hg and/or diastolic blood pressure $\geqslant$90 mm Hg and/or treatment with anti-hypertensive medication.[10,11]

Data extraction

Two investigators (KL and YL) independently reviewed and extracted the data. Inconsistencies were discussed between the two investigators until a consensus was obtained on all of the items. If they could not reach an agreement, a third investigator (JL) adjudicated the disagreements. For each study, information was collected concerning the following characteristics: first author, year of publication, racial background and location of the study population, source of subjects, methodology used for genotype detection, diagnostic standard for hypertension, matching, clinical features, quality score, sample sizes, and distribution of genotypes and alleles in both case and control groups.

Quality score assessment

The quality assessment score we used was adapted from Niu *et al.*[12] and Zhang *et al.*,[13] which were based on both traditional epidemiologic considerations and genetic issues raised by Thakkinstian *et al.*[14,15] Total scores ranged from 0 (lowest) to 13 (highest). The criteria used for the quality assessment of the genetic association between the *ADD1* Gly460Trp polymorphism and hypertension risk are summarized in the Appendix.

Statistical analysis

Odds ratios (ORs) with 95% confidence intervals (CIs) were used as the metric of choice. We calculated the OR and respective 95% CIs for each study to assess the strength of the association between the α-*adducin* Gly460Trp polymorphism and the risk of EH, according to the method described by Woolf.[16] On the basis of the individual OR, the pooled ORs were assessed for allele comparison (Trp *vs.* Gly), dominant genetic model (GlyTrp+TrpTrp *vs.* GlyGly), recessive genetic model (TrpTrp *vs.* GlyTrp+GlyGly) and homozygote comparison (TrpTrp *vs.* GlyGly). The statistical significance was determined by the Z-test, and a P value of < 0.05 was considered statistically significant. In our study, two models of meta-analysis were used for dichotomous outcomes in the Review-Manager 4.2 software (Oxford, England, UK): the fixed-effects model and the random-effects model. The fixed-effects model used the Peto Mantel–Haenszel's method, which assumes that studies are sampled from populations with the same effect size, and adjusts the study weights according to the in-study variance. The random-effects model used the Der Simonian and Laird's method, which assumes that the studies are taken from populations with varying effect sizes, and calculates the study weights both from in-study and between-study variances, considering the extent of variation or heterogeneity. The chi-squared-based Q-test was performed to check the heterogeneity.[17] A P value >0.10 for the Q-test indicates a lack of heterogeneity among studies, and the pooled OR estimate of each study was calculated with the fixed-effects model.[18] Otherwise, the random-effects model was used.[18] The inconsistency index I^2 was also calculated to quantify the degree of heterogeneity between studies, with I^2 $<$25%, 25–50%, 50–75% and $>$75% to represent low, moderate, large and extreme heterogeneity, respectively.[19] For each genetic contrast, subgroup analysis according to racial descent was only performed for Han Chinese and non-Han Chinese minorities to estimate ethnic-specific ORs when there were at least three independent studies. We also classified all the Han populations as either Northern Han Chinese (N-Han) or Southern Han Chinese (S-Han), with the Yangtze River serving as a geographical boundary.[20–22] Additionally, the source of heterogeneity was further investigated with stratified meta-analyses based on sample sources (population-based or hospital-based).

Sensitivity analyzes were conducted by sequentially deleting each individual study in an attempt to identify the potential influence of each individual data set to the pooled ORs. In addition, an estimate of potential publication bias was carried out by the funnel plot, in which the standard error of log (OR) of each study was plotted against its OR. The symmetry of the plot distribution indicates the absence of publication bias. Funnel-plot asymmetry was assessed with Egger's linear regression test.[23] The significance of the intercept was determined by the *t*-test suggested by Egger, and $P<0.05$ was considered representative of statistically significant publication bias. HWE was tested with a chi-squared test for goodness of fit based on a Web program (http://www.ihg.gsf.de/cgi-bin/hw/hwa1.pl).

All statistical analyses were performed using the software Review-Manager 4.2 and Stata version 10.0 (Stata Corporation, College Station, TX, USA). All statistical tests were two-sided.

RESULTS

Description of studies identified in meta-analysis

Through a comprehensive search, 29 potentially relevant articles concerning the α-*adducin* gene Gly460Trp polymorphism and hypertension in the Chinese population were identified on the basis of our inclusion criteria. A total of 8 of the 29 eligible articles were excluded because they shared the same or overlapping data. We also deleted one study[24] in which the genotype distributions in control individuals departed from HWE (P_{HWE} =0.00466). Finally, a total of 20 studies containing 5562 hypertensive patients and 4289 controls were used in this meta-analysis.[25–44] The study by Zhan *et al.*[35] was an unpublished thesis obtained from a medical doctorate dissertation database that was a sub-database of the CNKI and Wanfang databases. Three studies (Jiang *et al.*,[26] Zhao *et al.*,[31] and Huang *et al.*[36]) included in our meta-analysis only listed the percentages of genotype frequencies in both cases and controls. We therefore calculated the numbers of the three different genotypes (GlyGly, GlyTrp and TrpTrp). To avoid possible bias by calculating, we contacted the original or corresponding authors by e-mail to verify the data. All of them replied and offered the

Table 1 Detailed characteristics of eligible studies considered in the meta-analysis

First author	Year	Ethnicity	Region	Source	Method	Diagnostic standard	Matching	Characteristics	Quality score
Hou *et al.*[25]	2000	Han	Shanxi	H-B	PCR-SSCP	SBP⩾140, DBP⩾90	Yes[a]	Cases were hypertensive patients with age at identification of <50-years old. Controls were healthy individuals without a family history of hypertension.	9
Jiang *et al.*[26]	2003	Han	Jiangsu	P-B	PCR-MS	SBP⩾140, DBP⩾90	No	Cases and controls (age at identification: 25–75 years) were all non-diabetic and randomly selected from the Jiangsu province.	10
Li *et al.*[27]	2004	Kazakh	Xinjiang	P-B	PCR-MS	SBP⩾140, DBP⩾90	Yes[a]	Cases were hypertensive patients free of DM and other cardiovascular disease. Controls were healthy individuals. There were no mixed marriages between the two groups.	8
Dou *et al.*[28]	2004	Han	Beijing	H-B	PCR-RFLP	SBP⩾140, DBP⩾90	Yes[a]	Cases were hypertensive patients free of any other chronic disease. Controls were matched 1:1 by sex and age at 3-years intervals to the cases.	11
Zhang *et al.*[29]	2005	Kazakh	Xinjiang	P-B	PCR-MS	SBP⩾160, DBP⩾95	Yes[b]	Unrelated subjects with no mixed marriages were included. Controls had a negative family history of hypertension. Individuals with BMI >33 $kg\,m^{-2}$ were excluded.	8
Xu *et al.*[30]	2005	Han	Beijing	H-B	PCR-MS	SBP⩾140, DBP⩾90	Yes[a]	Cases and controls were age, sex and area of residence matched. Both of the two groups were free of other cardiovascular disease and DM.	9
Zhao *et al.*[31]	2006	Han	Shanghai	H-B	PCR-MS	SBP⩾140, DBP⩾90	Yes[c]	Cases and controls were randomly selected from the region of Shanghai. Secondary hypertension, renal disease and other cardiovascular problems were excluded.	9
Jing *et al.*[32]	2006	Han	Beijing	H-B	PCR-MS	DBP: 95–109, SBP <180 mm Hg	Yes[a]	Hypertensive cases and healthy controls were randomly selected from Beijing area.	7
Hu *et al.*[33]	2006	Han	Shanghai	H-B	PCR-MS	SBP⩾140, DBP⩾90	Yes[a]	Secondary hypertension, DM, other cardiovascular and renal diseases were excluded between the cases and controls.	10
Dong *et al.*[34]	2006	Han	Guangdong	P-B	PCR-MS	SBP⩾140, DBP⩾90	Yes[a]	Cases were hypertensive patients and controls were age- and sex-matched healthy individuals with no family history of hypertension.	9
Zhan *et al.*[35]	2006	Han	Jiangsu	P-B	PCR-RFLP	SBP⩾140, DBP⩾90	Yes[b]	Cases were hypertensive individuals. Controls had no family history of hypertension, and any other chronic disease were excluded.	9
Huang *et al.*[36]	2007	Han	Shandong	P-B	PCR-RFLP	SBP⩾140, DBP⩾90	Yes[a]	Unrelated hypertensive cases and healthy controls free of secondary hypertension were selected. other cardiovascular disease and DM were excluded.	11
Wang *et al.*[37]	2007	Mongolian	Neimenggu	P-B	PCR-RFLP	SBP⩾140, DBP⩾90	Yes[a]	No mixed marriages occured among the cases and controls. Subjects suffering from chronic disease were excluded. There were no family history of hypertension in controls.	8
Li *et al.*[38]	2007	Han	Tianjin	H-B	PCR-MS	SBP⩾145, DBP⩾95	No	Subjects randomly selected from Tianjin area were included. Controls had a negative history of hypertension.	7

Table 1 (Continued)

First author	Year	Ethnicity	Region	Source	Method	Diagnostic standard	Matching	Characteristics	Quality score
Bian *et al.*[39]	2007	Han	Hebei	H-B	PCR-RFLP	SBP⩾140, DBP⩾90	Yes[c]	Cases or controls with secondary hypertension or DM were excluded. Controls were no familial hypertension history and frequency matched by sex.	9
Huang *et al.*[40]	2008	Kazakh	Xinjiang	P-B	PCR-MS	SBP⩾140, DBP⩾90	Yes[a]	Subjects with no mixed marriages were randomly selected from the region of Xinjiang. other cardiovascular disease and DM were excluded.	9
Lu *et al.*[41]	2008	Han	Fujian	H-B	PCR-RFLP	SBP⩾140, DBP⩾90	Yes[a]	Cases were hypertensive patients free of DM and other chronic disease. Controls were age- and sex-matched healthy individuals.	10
Gong *et al.*[42]	2009	Han	Henan	H-B	PCR-MS	SBP⩾140, DBP⩾90	Yes[a]	Hypertensive patients whose average daily salt intake exceed 6 g were included. Controls were healthy individuals and frequency matched by age and sex.	9
Lin *et al.*[43]	2009	Han	Fujian	H-B	PCR-RFLP	SBP⩾140, DBP⩾90	Yes[c]	Hypertensive cases and healthy controls (age at identification: 27–92 years) were selected. There were no other chronic disease in the subjects.	9
Niu *et al.*[44]	2010	Han	Shanghai	H-B	PCR-RFLP	SBP⩾140, DBP⩾90	Yes[a]	Unrelated hypertensive patients were recruited from the Shanghai area. Healthy controls were matched 1:1 by age, gender and area to the cases.	11

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure (mm Hg); DM, diabetes mellitus; H-B, hospital-based study; P-B, population-based study; PCR-SSCP, polymerase chain reaction and single strand conformation polymorphism; PCR-MS, polymerase chain reaction and mutagenically separated; PCR-RFLP, polymerase chain reaction and restriction fragment length polymorphism; SBP, systolic blood pressure (mm Hg).
[a]Age- and sex-matched.
[b]Age-matched.
[c]Sex-matched.

accurate data. The results of our quality score assessment varied between 7 and 11, indicating that most studies identified were of medium or high quality.

Of the 20 studies, 12 were hospital-based and 8 were population-based studies. The populations of these studies were as follows: 16 studies included Han subjects (4826 cases and 3777 controls), 3 included Kazakh subjects (636 cases and 462 controls) and 1 included Mongolian subjects (100 cases and 50 controls). The latter two ethnicities were Northern Chinese minorities. In total, 65% of the studies (13/20) stated that age and gender were well-matched between the case and control groups, 75% (15/20) were age-matched and 80% (16/20) were gender-matched. All studies used blood samples for genotyping. The detailed characteristics of all eligible studies in the present meta-analysis are described in Table 1, including the first author, year of publication, ethnicity, population location, source of subjects, genotyping method, diagnostic standard for hypertension, matching, main information extracted from each study and quality score. Sample sizes, genotype numbers, allele frequency in both cases and controls, and *P* values of HWE in controls are listed in Table 2.

Main meta results

In the meta-analysis for all subjects, the pooled overall frequency of the Trp allele was 49.96% for hypertensive cases and 47.74% for normotensive controls. For each study, we investigated the association between the α-*adducin* gene Gly460Trp polymorphism and EH risk, assuming different inheritance models of the 460Trp allele. The main results of this meta-analysis and the heterogeneity test are shown in Table 3. The random-effects model was used to pool the result, as the between-study heterogeneity was significant. Overall, a marginal significantly increased risk was found in the allele comparison (Trp *vs.* Gly: P=0.05, OR=1.08, 95% CI=1.00–1.17, $P_{heterogeneity}$=0.02 and I^2=42.3%) (Figure 1a), and a significant association was observed in the recessive genetic model (TrpTrp *vs.* GlyTrp+GlyGly: P=0.0009, OR=1.24, 95% CI=1.09–1.41, $P_{heterogeneity}$=0.04 and I^2=38.7%) (Figure 1b), as well as in the homozygote comparison (TrpTrp *vs.* GlyGly: P=0.006, OR=1.25, 95% CI=1.07–1.46, $P_{heterogeneity}$=0.03 and I^2=41.2%) (Figure 1c). However, we found no positive association in the dominant genetic model (GlyTrp+TrpTrp *vs.* GlyGly: P=0.22, OR=1.09, 95% CI=0.95–1.24, $P_{heterogeneity}$=0.01 and I^2=46.5%) (Table 3).

Subgroup analyses

In the subgroup analysis by ethnicity, the studies included were stratified as Han Chinese and Chinese Kazakhs, except for the Chinese Mongolians, among whom only one study with less data was performed. The Trp allele had a similar representation in cases and controls of Han Chinese (49.94 and 47.75%, respectively) and Chinese Kazakhs (48.74 and 47.94%, respectively). Although moderate heterogeneity existed among Han Chinese in all genetic models, significant association was maintained in some subgroup analyses, especially in the recessive genetic model (TrpTrp *vs.* GlyTrp+GlyGly: P=0.001, OR=1.25, 95% CI=1.09–1.42, $P_{heterogeneity}$=0.08 and I^2=35.3%) (Figure 2a) and in the homozygote comparison (TrpTrp *vs.* GlyGly: P=0.009, OR=1.26, 95% CI=1.06–1.50, $P_{heterogeneity}$=0.03 and I^2=44.7%) (Figure 2b). When the subgroup analysis was limited to Chinese Kazakhs, no heterogeneity was detected in any genetic model. No evidence for an association between the Gly460Trp polymorphism and hypertension risk was obtained (Table 3).

Furthermore, we also divided the Han Chinese into two subgroups: N-Han and S-Han. The fixed-effects model was used to pool

Table 2 Sample size of each study, the distribution of Gly460Trp genotypes and T allele frequencies of cases and controls and *P*-values of HWE in controls

	Sample size		*GG genotype*		*GT genotype*		*TT genotype*		*T allele frequency (%)*		*HWE P[a] value*
First author	*Cases*	*Controls*	*Cases*	*Controls*	*Cases*	*Controls*	*Cases*	*Controls*	*Cases*	*Controls*	*Controls*
Hou *et al.*[25]	183	129	54	36	84	66	45	27	47.54	46.51	0.75
Jiang *et al.*[26]	189	147	44	35	94	79	51	33	51.85	49.32	0.36
Li *et al.*[27]	235	132	56	35	119	62	60	35	50.85	50.00	0.49
Dou *et al.*[28]	234	234	42	66	113	117	79	51	42.09	46.79	0.95
Zhang *et al.*[29]	278	220	71	58	144	121	63	41	48.56	46.14	0.11
Xu *et al.*[30]	348	184	91	49	174	88	83	47	48.85	49.46	0.56
Zhao *et al.*[31]	278	231	117	64	72	112	89	55	44.96	48.05	0.66
Jing *et al.*[32]	463	260	110	70	262	136	91	54	47.95	46.92	0.42
Hu *et al.*[33]	396	214	114	59	200	104	82	51	45.96	48.13	0.70
Dong *et al.*[34]	97	87	23	21	49	40	25	26	51.03	52.87	0.47
Zhan *et al.*[35]	190	94	43	17	98	53	49	24	51.58	53.72	0.19
Huang *et al.*[36]	256	495	59	124	107	237	90	134	56.05	51.01	0.35
Wang *et al.*[37]	100	50	20	12	43	31	37	7	58.50	45.00	0.07
Li *et al.*[38]	80	80	17	25	42	38	21	17	52.50	45.00	0.72
Bian *et al.*[39]	160	151	35	40	79	69	46	42	53.44	50.66	0.29
Huang *et al.*[40]	123	110	42	31	51	50	30	29	45.12	49.09	0.34
Lu *et al.*[41]	200	200	50	73	102	96	48	31	49.50	39.50	0.95
Gong *et al.*[42]	196	192	38	42	84	101	74	49	59.18	51.82	0.46
Lin *et al.*[43]	1081	604	311	215	540	298	230	91	46.25	39.74	0.46
Niu *et al.*[44]	475	475	110	105	216	231	149	139	54.11	53.58	0.63
Total	5562	4289	1447	1177	2673	2129	1442	983	49.96	47.74	0.73

Abbreviation: HWE, Hardy–Weinberg equilibrium.
[a]The *P*-value of HWE determined by the χ^2 test.

the results in N-Han, as the between-study heterogeneity was insignificant. The results revealed significant associations for hypertension risk with the Gly460Trp polymorphism in the dominant genetic model (GlyTrp+TrpTrp *vs.* GlyGly: P=0.02, OR=1.21, 95% CI=1.03–1.41, $P_{heterogeneity}$=0.54 and I^2=0%), the recessive genetic model (TrpTrp *vs.* GlyTrp+GlyGly: P=0.002, OR=1.28, 95% CI=1.10–1.49, $P_{heterogeneity}$=0.12 and I^2=39.6%) and the homozygote comparison (TrpTrp *vs.* GlyGly: P=0.001, OR=1.36, 95% CI=1.13–1.64, $P_{heterogeneity}$=0.24 and I^2=23.9%) (Table 3). In S-Han, notable heterogeneity was discerned in all genetic models except for the recessive genetic model, in which a weak association was found (TrpTrp *vs.* GlyTrp+GlyGly: P=0.002, OR=1.24, 95% CI=1.08–1.42, $P_{heterogeneity}$=0.12 and I^2=39.0%) (Table 3).

In the analyses stratified by sample sources, significant associations were found both in population-based (TrpTrp *vs.* GlyTrp+GlyGly: P=0.02, OR=1.23, 95% CI=1.03–1.47, $P_{heterogeneity}$=0.15 and I^2=35.4%) and in hospital-based (TrpTrp *vs.* GlyTrp+GlyGly: P=0.005, OR=1.26, 95% CI=1.07–1.48, $P_{heterogeneity}$=0.04 and I^2=45.1%) subgroups under the recessive model (Table 3). In the homozygote comparison, significantly elevated risk was found in hospital-based studies (TrpTrp *vs.* GlyGly: P=0.02, OR=1.30, 95% CI=1.05–1.61, $P_{heterogeneity}$=0.01 and I^2=55.2%) (Table 3). Moreover, to uncover the potential influence of the studies whose diagnostic standards differed from others (Zhang *et al.*,[29] Jing *et al.*[32] and Li *et al.*[38]), repeated analyses have been conducted by omitting these three studies. Consistent with our previous findings, similar results were obtained when deleting the three studies. Significant associations were observed among the overall population, Han Chinese, N-Han and S-Han, and again, no positive association was found in Chinese Kazaks (data not shown).

Sensitivity analysis

Sensitivity analyses were conducted to assess whether each individual study affected the final results. Our analysis indicated that three independent studies by Dou *et al.*,[28] Zhao *et al.*[31] and Lin *et al.*[43] were the main cause of heterogeneity across all subjects and Han Chinese. The heterogeneity was effectively removed after the exclusion of these three studies (Dou *et al.*:[28] $P_{heterogeneity}$=0.10, I^2=30.7%, homozygote comparison; Zhao *et al.*:[31] $P_{heterogeneity}$=0.36, I^2=7.9%, dominant genetic model; and Lin *et al.*:[43] $P_{heterogeneity}$=0.10, I^2=30.5%, allele comparison) for total comparisons. Data for Han Chinese are not shown. In addition, sensitivity analyses suggested that the corresponding summary ORs were substantially altered among Han Chinese when excluding these three studies (data not shown). There was no single study that influenced the pooled OR qualitatively in the subgroup of Chinese Kazakhs.

Publication bias

Begg's funnel plot and Egger's test were used to evaluate the literature publication bias for allele comparison (Trp *vs.* Gly). The shape of the funnel plot did not reveal any evidence of obvious asymmetry (Figure 3), and Egger's test suggested the absence of publication bias among all the studies included (t=−0.56, P=0.581).

DISCUSSION

We performed a systematic review of the literature by means of a meta-analysis on the association between the *α-adducin* Gly460Trp polymorphism and EH, without evidence of publication bias for the outcome. On the basis of 5562 essential hypertensive individuals and 4289 controls in total, we concluded that the *α-adducin* gene Gly460Trp polymorphism appeared to be associated with an increased

Table 3 Summary ORs and 95% CI of Gly460Trp polymorphism and hypertension risk under various genetic contrasts

Genotype contrasts	*Population*	*Study numbers*	$P_{heterogeneity}$	P[a] *value*	*OR*	*95% CI*
Allele comparison	Overall	20	0.02[b]	0.05	1.08	1.00–1.17
(Trp vs. Gly)	Han	16	0.02[b]	0.08	1.08	0.99–1.18
	Kazakh	3	0.52[c]	0.81	1.02	0.86–1.21
	N-Han	8	0.21[c]	0.10	1.08	0.98–1.19
	S-Han	8	0.01[b]	0.34	1.07	0.93–1.23
	P-B	8	0.34[c]	0.11	1.09	0.98–1.22
	H-B	12	0.009[b]	0.15	1.08	0.97–1.21
Dominant model	Overall	20	0.01[b]	0.22	1.09	0.95–1.24
(GlyTrp+TrpTrp vs. GlyGly)	Han	16	0.004[b]	0.23	1.10	0.94–1.29
	Kazakh	3	0.51[c]	0.98	1.00	0.76–1.31
	N-Han	8	0.54[c]	0.02	1.21	1.03–1.41
	S-Han	8	0.0005[b]	0.97	1.00	0.76–1.30
	P-B	8	0.91[c]	0.83	1.02	0.85–1.22
	H-B	12	0.0008[b]	0.21	1.13	0.93–1.37
Recessive model	Overall	20	0.04[b]	0.0009	1.24	1.09–1.41
(TrpTrp vs. GlyTrp+GlyGly)	Han	16	0.08[b]	0.001	1.25	1.09–1.42
	Kazakh	3	0.55[c]	0.67	1.06	0.80–1.42
	N-Han	8	0.12[c]	0.002	1.28	1.10–1.49
	S-Han	8	0.12[c]	0.002	1.24	1.08–1.42
	P-B	8	0.15[c]	0.02	1.23	1.03–1.47
	H-B	12	0.04[b]	0.005	1.26	1.07–1.48
Homozygote comparison	Overall	20	0.03[b]	0.006	1.25	1.07–1.46
(TrpTrp vs. GlyGly)	Han	16	0.03[b]	0.009	1.26	1.06–1.50
	Kazakh	3	0.53[c]	0.76	1.05	0.75–1.48
	N-Han	8	0.24[c]	0.001	1.36	1.13–1.64
	S-Han	8	0.02[b]	0.29	1.16	0.88–1.52
	P-B	8	0.41[c]	0.14	1.18	0.95–1.46
	H-B	12	0.01[b]	0.02	1.30	1.05–1.61

Abbreviations: OR, odds ratio; CI, confidence interval; N-Han, northern Han Chinese; S-Han, southern Han Chinese; P-B, population-based study; H-B, hospital-based study.
[a]The *P*-value of OR determined by the Z test.
[b]Random effect estimate.
[c]Fixed effect estimate.

risk of hypertension in Chinese populations, especially in Han Chinese.

In 1997, Cusi *et al.*[7] first reported that the 460Trp allele of α-*adducin* was associated with hypertension in human subjects, particularly in a salt-sensitive form of EH. Subsequently, Iwai *et al.*[45] found that the Gly460Trp polymorphism seemed to be involved in the pathophysiology of hypertension among Japanese subjects. Nevertheless, some studies have shown inconsistent results as well as strong racial and regional disparities. A recent case-control study and meta-analysis by Niu *et al.*[44] failed to demonstrate the genetic association of the *ADD1* gene Gly460Trp polymorphism with hypertension. There are many possible reasons for this variability, and ethnic specificity and population structure may be the most important potential confounding factors. It is necessary to reconcile the conflicting findings in a genetically well-defined population. Our meta-analysis was focused on a single ethnicity or country, and accordingly eliminated racial differences. The results indicated that there was a significant association with increased risk among Chinese hypertensive individuals.

The molecular mechanism hypothesized for the Gly460Trp polymorphism is likely associated with a salt-sensitive form of hypertension. A previous study demonstrated that subjects bearing one Trp allele in α-*adducin* displayed increased renal tubular sodium reabsorption and retention as a result of a reduced renal pressure-natriuresis slope after sodium depletion or sodium load.[46] Further studies revealed similar results and demonstrated that the genetic variant modulated sodium transport through variations in sodium-potassium ATPase activity on the basolateral membrane and through modifications in the assembly of the actin cytoskeleton.[47] A systematic review by Beeks *et al.*[48] summarized seven studies addressing the association between the α-*adducin* Gly460Trp polymorphism and salt sensitivity. Five of these seven studies were performed by the same research group and showed evidence to support the significant association,[7,46,49–51] but one Polish study failed to obtain the same conclusion.[52] Another article by Grant *et al.*[53] reported that the systolic blood pressure response to sodium changes was significantly greater in subjects with homozygous 460Trp compared with subjects carrying other genotypes. In addition, a newly published meta-analysis investigating the association between the *ADD1* polymorphism (Gly460Trp) and the genetic predisposition to salt sensitivity confirmed that although the overall results showed no increased risk of salt sensitivity for adducin Trp allele carriers (P=0.08, OR=1.40 and 95% CI=0.96–2.04), they did show that the association between Gly460Trp and salt sensitivity is statistically significant in a subgroup of Asian people (P=0.02, OR=1.33 and 95% CI=1.06–1.69) (see ref. 54). This is consistent with our results. To summarize, it appears that the α-*adducin* gene mutation might affect overall body sodium metabolism and therefore trigger hypertension.

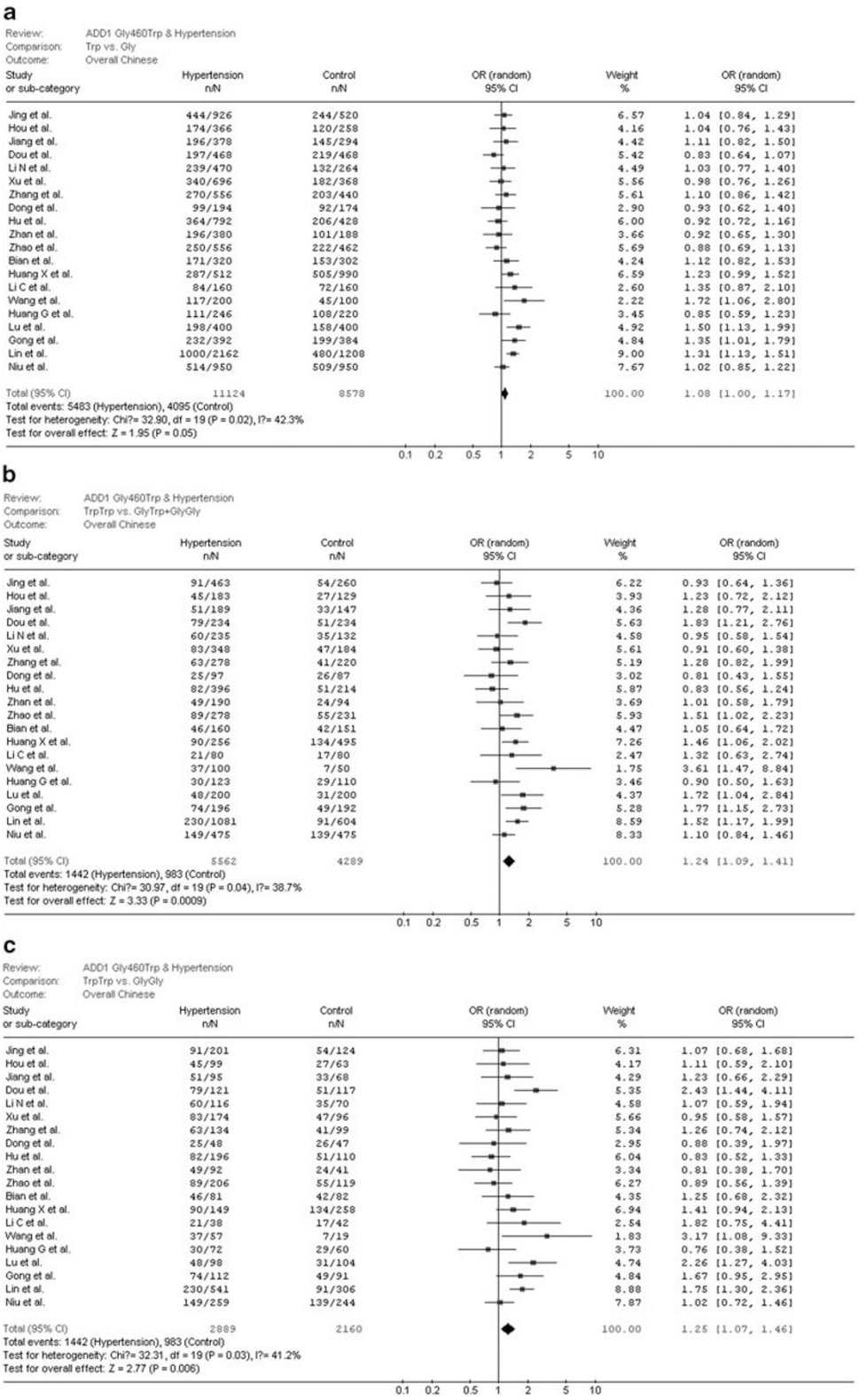

Figure 1 (**a**) Meta-analysis for the association between the Gly460Trp polymorphism and hypertension, contrasting Trp *vs.* Gly in Chinese populations using a random-effects model. N indicates the total number of Trp alleles plus Gly alleles and n indicates the total number of Trp alleles. (**b**) Meta-analysis for the association between the Gly460Trp polymorphism and hypertension under a recessive genetic model (TrpTrp *vs.* GlyTrp+GlyGly) in Chinese populations using a random-effects model. N indicates the total number of individuals and n indicates the total number of TrpTrp. (**c**) Meta-analysis for the association between Gly460Trp polymorphism and hypertension in the contrast of TrpTrp *vs.* GlyGly in Chinese individuals using a random-effects model. N indicates the total number of TrpTrp plus GlyGly and n indicates the total number of TrpTrp.

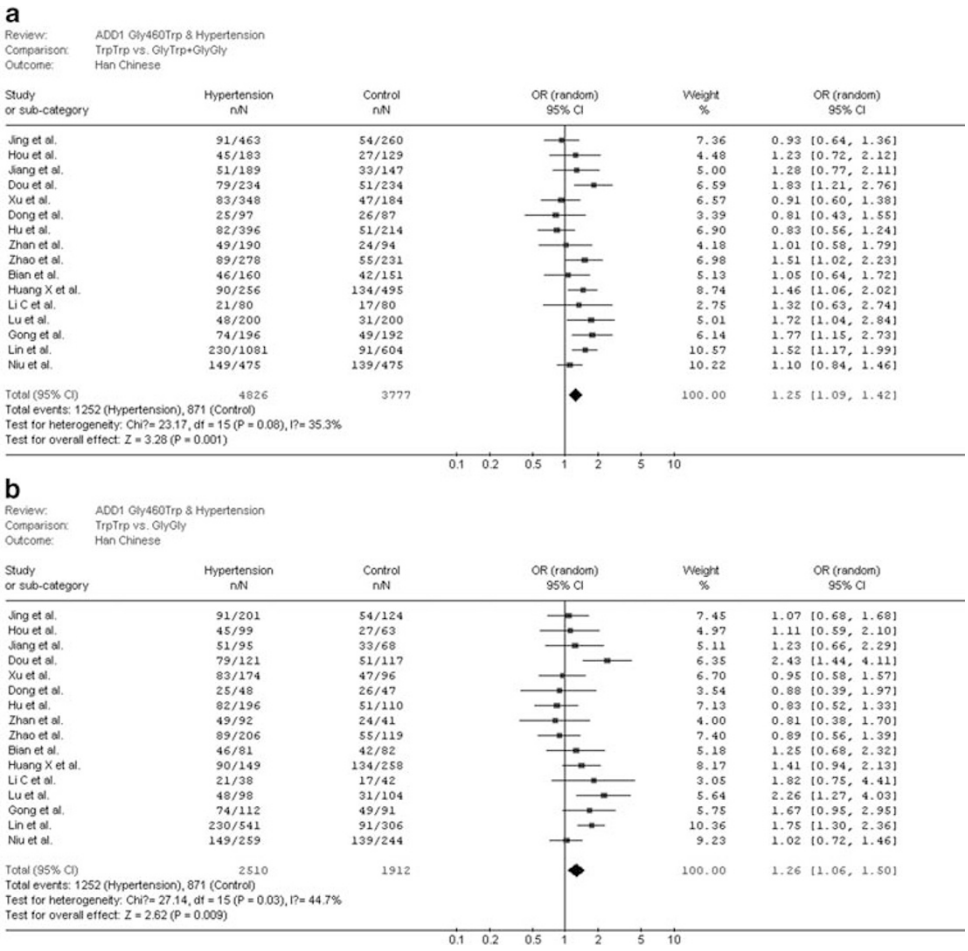

Figure 2 (**a**) Meta-analysis for the association between Gly460Trp polymorphism and hypertension under a recessive genetic model (TrpTrp *vs.* GlyTrp+GlyGly) in Han Chinese individuals using a random-effects model. N indicates the total number of Han Chinese individuals and n indicates the total number of TrpTrp. (**b**) Meta-analysis for the association between Gly460Trp polymorphism and hypertension in the contrast of TrpTrp *vs.* GlyGly in Han Chinese individuals using a random-effects model. N indicates the total number of TrpTrp plus GlyGly and n indicates the total number of TrpTrp.

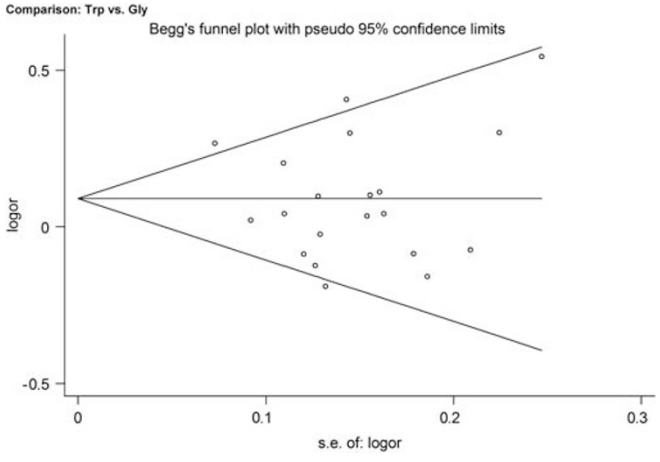

Figure 3 Begg's funnel plot of Egger's test of the Gly460Trp polymorphism allele comparison (Trp *vs.* Gly) for publication bias. No asymmetry was found, as indicated by the *P*-value of Egger's test.

China is the most populous country in the world, and the Han are the largest ethnicity. In the subgroup analyses, we divided all studies into two subgroups: Han Chinese and Chinese Kazakhs. Statistically significant associations were found among studies of Han Chinese. In the Han population, the individuals were further distinguished as N-Han and S-Han because they have different genetic backgrounds. This has been verified by a series of analyses of genetic markers,[21,55–57] anthropological data concerning somatometric and nonmetric features[58,59] and dermatoglyphic parameters.[22,60] In addition, environmental factors, such as geographical location, climate, eating habits and lifestyle all contribute to the North-South differences. In our current study, the Trp allele frequencies in the N-Han population (52.16% in cases and 49.10% in controls) were higher than those in the S-Han population (48.47% in cases and 46.61% in controls). The North-South differences were proven statistically significant using the chi-squared test (χ^2=12.613, $P<0.0001$ for cases and χ^2=4.652, P=0.031 for controls, respectively). When analyzing the gene-disease interaction, our research findings suggested that the *α-adducin* gene Gly460Trp polymorphism was associated with EH in both the N-Han and S-Han. Compared with the Han population, no association was detected under any genetic models of Chinese Kazakhs. Previous research showed that the genetic backgrounds of these two ethnic groups are very different. The Kazakh ethnic group resulted from genetic mixing of Caucasians and East Asians.[61–63] The genetic contribution of Caucasian in Chinese Kazakhs is 30.2% (see ref. 62). Our previous report showed that there is no association between hypertension and the *α-adducin* Gly460Trp polymorphism in the Caucasian population.[64] A series of studies suggested that the

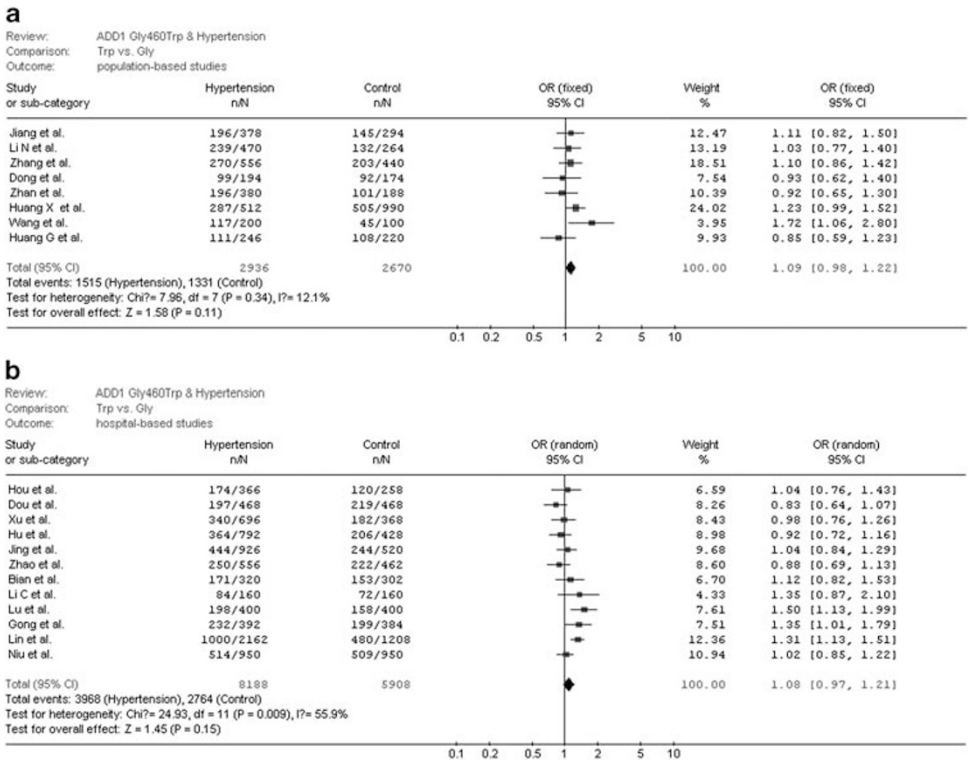

Figure 4 (**a**) Forest plot of *ADD1* Gly460Trp polymorphism and hypertension risk among population-based studies in allele comparison. (**b**) Forest plot of *ADD1* Gly460Trp polymorphism and hypertension risk among hospital-based studies in allele comparison.

frequencies of common genetic polymorphisms and haplotypes in Chinese Kazakhs were observed at frequencies intermediate between those seen in Chinese Hans and Caucasians.[65–67] Moreover, considering the limited studies and population of Kazakhs included in the meta-analysis, our results should be interpreted with caution. Future studies based on large-scale investigations are needed to further validate the association of the *ADD1* Gly460Trp polymorphism and hypertension risk in Kazakhs.

In the sensitivity analysis, three studies (Dou *et al*.,[28] Zhao *et al*.[31] and Lin *et al*.[43]) performed on Han Chinese individuals were recognized as the main cause of heterogeneity. Significant associations were obtained when each of these studies was excluded. The three independent studies were all hospital-based. The results from the analyses stratified by sample sources showed that no heterogeneity was detected for the genotype comparisons in the population-based studies, whereas considerable heterogeneity existed in the hospital-based studies. (Figure 4) In our meta-analysis, the number of subjects in hospital-based studies accounted for a much larger proportion (71.55%) than that in population-based studies (28.45%). Hospital-based studies usually have some selection biases that may affect the quality and reliability of individual studies. Controls in hospital-based studies might be ill-related population, and may not be representative of the general population. It is therefore necessary to interpret the selection bias that may be present in our results cautiously.

In summary, our meta-analysis suggested that the Gly460Trp polymorphism in *ADD1* is likely associated with hypertension susceptibility in Chinese individuals, especially in Han Chinese populations. The *α-adducin* gene Gly460Trp polymorphism may be a genetic indicator for Chinese hypertensive patients. Future studies examining gene–gene or gene–environment interactions are warranted to validate the risk identified in the current meta-analysis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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APPENDIX

Criteria of methodologic quality assessment for genetic association case/control study on hypertension

Criteria	*Quality score*
A. Representativeness of cases	
Consecutive/randomly selected from case population with clearly defined sampling frame	2
Consecutive/randomly selected from case population without clearly defined sampling frame or extensive inclusion/exclusion criteria	1
No method of selection described	0
B. Representativeness of controls	
Controls were consecutive/randomly drawn from the same sampling frame (ward/community) as cases	2
Controls were consecutive/randomly drawn from a different sampling frame than cases	1
No method of selection described	0
C. Ascertainment of hypertension cases	
Clearly described objective criteria for diagnosis of hypertension	2
Diagnosis of hypertension by patient self-report or by patient history	1
Not described	0
D. Ascertainment of controls	
Clinical examinations were performed on controls to prove that controls did not have hypertension	2
Article merely stated that controls were subjects who did not report hypertension; no objecting testing	1
Not described	0
E. Ascertainment of genotyping examination	
Genotyping done under ″blind″ conditions	1
Unblended or not mentioned	0
F. Test for Hardy–Weinberg equilibrium	
Hardy–Weinberg equilibrium in control group	2
Hardy–Weinberg disequilibrium in control group	1
Hardy–Weinberg equilibrium not checked	0
G. Association assessment	
Assessed association between genotypes and hypertension with appropriate statistics and adjustment for confounders	2
Assessed association between genotypes and hypertension with appropriate statistics without adjustment for confounders	1
Inappropriate statistic used	0
Total	13