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*Review Article*

# Association of Klotho single nucleotide polymorphisms with cardiovascular diseases: a systematic review and meta-analysis

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**Abstract:** Objectives: A systematic review and meta-analysis was performed to confirm the relationship between the Klotho single nucleotide polymorphisms (SNPs) and the risk of cardiovascular disease (CVD). Methods: Electronic databases of PubMed, EMBASE, the Cochrane Library and China National Knowledge Infrastructure were searched. Case-control studies or observational studies were included. Data synthesis and analysis were performed using the statistical software STATA. Results: Sixteen studies including 9585 participants provided the valid data for exploring the correlation between the 4 Klotho SNPs and the risk of 3 kinds of CVDs. Overall results showed that the significant correlation was observed between the risk of CVDs and G-395A SNP under the additive genetic models (OR=1.23, 95% CI=1.08-1.40, P=0.001), dominant model (OR=1.24, 95% CI=1.05-1.45, P=0.009) and recessive model (OR=1.61, 95% CI=1.30-2.00, P<0.001). No significant associations were found between the risk of CVDs and C1818T, F352V and C370S SNPs (all P>0.05). Conclusion: This meta-analysis can indicate the G-395A, C1818T SNPs could be a risk factor for coronary heart disease, while the G-395A and F352V SNPs might be a risk for hypertension. No association between the risk of ischemic stroke and the G-395A, F352V and C1818T SNPs could be established from our meta-analysis.

**Keywords:** Cardiovascular diseases, Klotho protein, single nucleotide polymorphism, gene, meta-analysis

## Introduction

Cardiovascular disease (CVD) comprises a class of diseases involving the heart or blood vessels. Specifically, CVD refers to any disease that affects the cardiovascular system, including ischemic heart disease (IHD), stroke, hypertension, atrial fibrillation, congenital heart disease (CHD), endocarditis, and peripheral artery disease (PAD), et al. It is estimated that CVDs account for approximately a third of all deaths worldwide. Moreover, CVDs are characterized by high incidence rates, morbidity, mortality, disability and other complications [1]. In China, a report from the National Center for Cardiovascular Disease indicated that more than 290 million people (22.3% of the population) experience CVDs and the total mortality resulting from CVDs has increased from 240.03 to 268.92 per one hundred thousand over the period from 2004 to 2010 [2].

Klotho, a peptide hormone with anti-aging activities, was first identified in 1997 by Kuro et al. The Klotho gene was named after Klotho or Clotho, one of the Fates in Greek mythology, the goddess who spins the thread of life [3]. The human Klotho gene, located on chromosome 13q12 and spanning over 50 kb in length is composed of 5 exons and 4 introns. More than 10 mutations or single nucleotide polymorphisms (SNPs) in the human KLOTHO gene have been distinguished. Among these, six SNPs, which are known as KL-VS, have been associated with latent coronary artery disease [4]. As a result of this association, a current area of investigation has been directed toward examining the relationship between the risk of CVDs

and Klotho SNPs. Specifically, the SNPs of F352V (rs9536314), C370S (rs9527025) and C1818T (rs564481) in the Exon and G-395A (rs1207568) located in the promoter have been reported to be related to CVDs. located in promoter have been reported to be related to CVDs. Imamura et al. and Jo et al. indicated that the Klotho G-395A polymorphism might represent a genetic risk factor for coronary artery disease (CAD) [5, 6]. In partial support of this conjecture are the findings of Rhee et al. who reported a significant correlation between the systolic blood pressure and the G-395 polymorphism, but this finding was not consistent with the study of Wang [7, 8]. Findings from the Tavakkoly-Bazzaz et al. indicated that a functional variant of the Klotho gene (termed KL-VS) was not associated with CAD risk, at least as examined within an Iranian sample of patients [9]. Moreover, Majumdar and Kim reported no significant association between the Klotho C1818T SNP and risk of ischemic stroke (IS) [10, 11]; and, Liu et al. reported that the SNPs C370S and F352V were not related with essential hypertension in the aged [12, 13].

Obviously, attempts at establishing relationships between Klotho SNPs and CVDs have lacked consistency. Recently, we have found two comprehensive compilations regarding Klotho [14, 15]. Findings from a meta-analysis as performed by Zhai et al. revealed that Klotho G-395A was a susceptible factor for CAD in East-Asia populations. A systematic review from Di Bona D et al. indicated that the KL-VS variant was associated with healthy aging [15]. In an approach to establish a more precise evaluation of the relationship between Klotho SNPs and CVD risk, we conducted a meta-analysis of all available case-control and observational studies related to this issue.

## Material and methods

Our study followed the guidelines of MOOSE (Meta-analysis Of Observational Studies in Epidemiology), a criterion which was employed to report this systematic review and meta-analysis [16].

### *Search methods for identification of studies*

Key words and search strategies were developed by two authors (Y.X.S. and H.M.Z.). The search strategies were set after performing several iterations in the electronic databases. Studies were obtained by searching multiple electronic bibliographic databases including PubMed, EMBASE, the Cochrane Library and China National Knowledge Infrastructure (CNKI) from the initiation of each their databases until January of 2015. In order to access all relevant studies, the following algorithm was used: “Klotho” with (Text+Mesh): “Klotho protein” [Supplementary Concept] OR “Klotho protein” OR “Klotho protein, human” OR KL “protein, human OR Klotho gene”. Reference lists within each of the identified articles were subsequently hand-searched to identify any additional eligible studies. Unpublished studies were identified by retrieving SIGLE (System for Information on Grey Literature) database.

### *Inclusion criteria*

All publications and screened studies that satisfied the following criteria were selected: Types of studies Case-control studies or any observational studies that included two study groups exploring the association between Klotho SNPs and risk of CVDs. The search language included both English and Chinese and no restrictions were imposed regarding nationality of the publication.

Types of participants Studies with two comparison groups, one of which consisted of participants experiencing at least one type of CVD and the second composed of healthy individuals. Healthy individuals were identified as those satisfying standard diagnostic criteria and showing no evidence of any relevant CVDs. CVDs included coronary heart disease (CHD), CAD, stroke, hypertension or atherosclerosis. CHD was identified according to American Heart Association/American College of Cardiology (AHA/ACC) guidelines; CAD was diagnosed by coronary angiography with diameter stenosis being ≥50%; Ischemic stroke was diagnosed by CT or MRI scans; Hypertension was defined as a systolic blood pressure of ≥140 mmHg and a diastolic blood pressure of ≥90 mmHg; Atherosclerosis was diagnosed by brachial ankle pulse wave velocity.

### *Exclusion criterion*

Methodology Quality of Studies rated as being less than four stars were excluded from this meta-analysis.

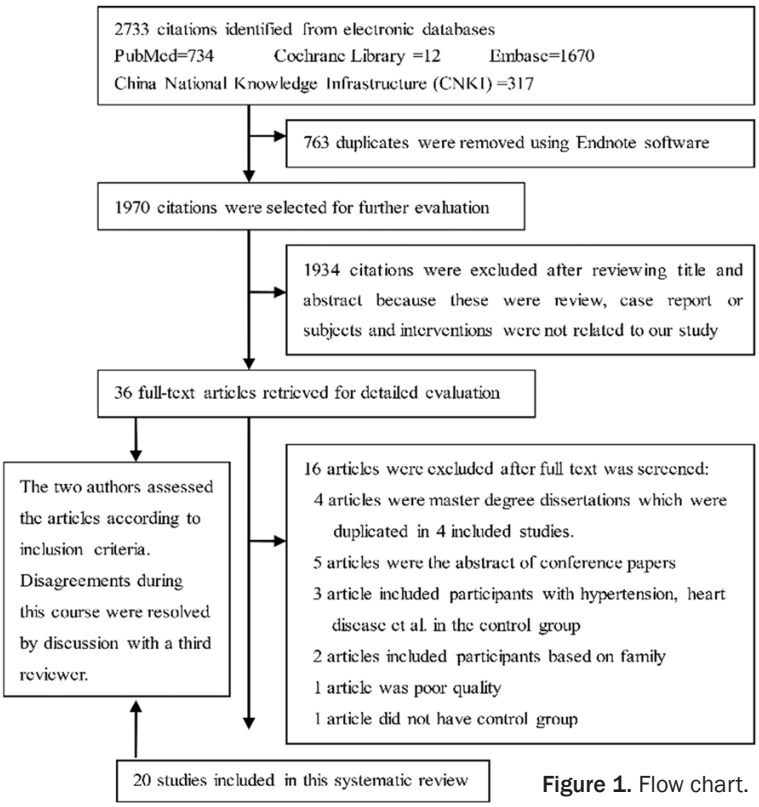


**Figure 1.** Flow chart.

*Outcomes*

The primary outcomes of CVD cases were those who were diagnosed with the CHD, CAD, stroke, hypertension or atherosclerosis. If more than one outcome was reported, a broader concept was chosen [eg, CAD rather than vasospastic angina (VSA)]. The meta-analysis was executed for those studies which provided original data and sufficient information to estimate odds ratio (OR) and corresponding 95% confidence interval (CI).

*Selection of literature*

All references from the database retrieval were imported into Endnote software. After excluding duplicates, studies were independently selected by two authors (S.H.C. and H.M.Z.) according to the inclusion criteria. Article titles and abstracts were first reviewed as a means to provide an initial exclusion of studies that did not meet the inclusion criteria. A full text of any published article that potentially met the inclusion criteria was then obtained for further evaluation. In cases where information contained within the report was deficient or additional critical data were required, the authors of the original study were contacted by email. Any disagreements regarding the selection of studies to be included were resolved by discussion with a third reviewer (F.K.M.).

*Data extraction*

A form was designed by the authors as a means to extract relevant information and included the following information: basic information (author, year of publication, country of origin), source of controls, sample size, age, ethnic group of participants, genotype of the Klotho, outcome measure included in the original research, material used for assessment of Klotho gene, Hardy-Weinberg equilibrium (HWE) of the control group and comparability between the two groups. Two reviewers (S.H.C. and H.M.Z.) independently reviewed the eligible studies, with a third reviewer (Y.X.S.) used, if necessary, to resolve disagreements of opinion. When information of included studies was lacking, we contacted the authors of the original study.

*Methodological quality assessment*

The methodological quality assessment was completed by 2 independent reviewers (S.H.C. and H.M.Z.). The Newcastle-Ottawa quality assessment scale (NOS) was adopted to assess the quality of the studies [17]. The NOS contains 3 classes that include 8 items: selection (4 items), comparability (1 item) and exposure for case-control studies (3 items). A star system was used as a means to provide a semi-quantitative assessment of study quality. Each item can achieve a maximum of one star, with the exception of the comparability item, which can receive a maximum of two stars. As a result, the number stars can range between 0-9. Seven or more stars correspond to a high quality study, 4-6 stars to medium quality, while studies achieving less than 4 stars were considered of poor quality.

**Table 1.** Characteristics of included studies

| Studies: author, year, country | Published language | Source of controls | Study type | Sample size | | Year of data collection | Ethnicity | Age: mean ± SD | Genotype of the klotho | Primary outcome | Blood sample | Comparability* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | C1 | C2 | | | | | | | |
| Low, 2005, USA | English | Hospital | Case control | 295 | 145 | 1999~2003 | American | C1: 47.4±7.1<br>C2: 54.3±10.8 | KL-VS | PCAD | Peripheral venous blood | Age, BMI, Diabetes mellitus, Hypertension, Smoking |
| Kim, 2006, Korea | English | Unclear | Case control | 273 | 455 | Unclear | Korean | C1#: M 62.5, F 62.1<br>C2#: M 67.8, F 68.9 | G395A<br>C1818T<br>C2298T | IS | Peripheral blood cell | Age, Hyperlipidemia, Hypertension |
| Imamura, 2006, Japan | English | Annual physical examinations | Case control | 197 | 331 | Unclear | Japanese | C1: 63.5±9.8<br>C2: 53.6±11.4 | G395A | CAD | Peripheral blood leukocytes | Age, BMI, Diabetes mellitus, Hypertension, Smoking |
| He, 2008, China | Chinese | Unclear | Unclear | 272 | 139 | Nov 2003~Feb 2007 | Han people in China | C1: 71.12±6.78<br>C2: 68.88±6.69 | C1818T | ACS | Peripheral blood leukocytes | Age, Sex, GLU Smoking, LDL |
| Jin, 2008, China | Chinese | Health checkup | Case control | 146 | 235 | Nov 2003~Feb 2007 | Han people in China | C1: 65.82±9.47<br>C2: 61.58±10.93 | C1818T | UAP | Peripheral blood leukocytes | Age, Sex, Smoking, HDL, |
| Wang, 2008, China | Chinese | Health checkup | Case control | 130 | 102 | Oco 2006~Mar 2007 | Han people in China | C1: 53.1±10.1<br>C2: 50.5±11.1 | G395A | Arterio-sclerosis | Peripheral venous blood | BMI, BP, Diabetes mellitus, Smoking |
| Jo, 2009, Korea | English | Hospital | Unclear | 225 | 209 | Nov 2004~Aug 2005 | Korean | C1: 64.1±11.5<br>C2: 59.3±12.1 | G395A | CAD | Peripheral blood mononuclear cell | Age, Sex, LVEF, Diabetes mellitus, Smoking, Total cholesterol, Hypertension |
| Majumdar, 2010, India | English | Healthy Volunteer | Case control | 460 | 574 | Unclear | Indian ancestry | C1: 41.76±16.34<br>C2: 40.36±11.42 | F352V<br>C1818T | IS | Peripheral blood leukocytes | BMI, SBP, DBP, Total cholesterol, GLU, HDL, LDL, Smoking, Hypertension |
| Wang, 2010, China | Chinese | Health checkup | Unclear | 215 | 220 | Feb 2006~May 2008 | Han people in China | C1: 57.2±11.3<br>C2: 49.9±10.1 | G395A | Essential Hypertension | Peripheral blood leukocytes | Age, BMI, Diabetes mellitus, Smoking |
| Oguro, 2010, Japan | English | Health checkup | Case control | 853 | 1783 | Unclear | Japanese | C1: 65.20±10.40<br>C2: 64.80±11.20 | rs3752472<br>rs9536314<br>rs650439 | Carotid athero-sclerosis | Peripheral blood leukocytes | Sex, BMI, SBP, DBP, Diabetes mellitus, HBA1c, hyperlipidemia, Smoking, HDL, mean IMT, Number of subject with IMT thickening |
| Liu, 2010, China | Chinese | Unclear | Unclear | 692 | 460 | Unclear | Han people in China | C1: 72.94±6.89<br>C2: 66.49±5.71 | G395A<br>F352V<br>C370S | Hypertension | Peripheral blood leukocytes | Unclear |
| He, 2010, China (1) | Chinese | Health checkup | Case control | 375 | 235 | Nov 2003~Feb 2007 | Han people in China | C1: 65.85±10.75<br>C2: 61.62±10.94 | C1818T | ACS | Peripheral blood leukocytes | Age, Sex, GLU, Smoking, HDL, LDL |
| He, 2010, China (2) | Chinese | Health checkup | Case control | 326 | 219 | Nov 2003~Feb 2008 | Han people in China | C1: 65.81±11.03<br>C2: 62.03±10.60 | G395A | ACS | Peripheral blood leukocytes | Age, Sex, GLU, Smoking, LDL |
| He, 2011, China | Chinese | Unclear | Case control | 229 | 235 | Nov 2003~Feb 2007 | Han people in China | C1: 65.87±11.53<br>C2: 61.58±10.93 | C 1818T | AMI | Peripheral blood | Age, Sex, GLU, Smoking |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Liu, 2011, China | Chinese | Health checkup | Unclear | 775 | 339 | Jul 2007~Aug 2009 | Han people in China | C1: 73.65±8.37<br>C2: 73.87±10.46 | G395A<br>F352V<br>C370S | Hypertension | Peripheral blood leukocytes | Yes |
| Tavakkoly-Bazzaz, 2011, Iran | English | Hospital | Case control | 54 | 53 | Feb 2008~Mar 2010 | Iranian | C1: 63±8.5<br>C2: 55±11 | F352V | CAD | Anticoagulated blood | Age, Sex, Diabetes mellitus, Smoking, Hyperlipidemia |
| You, 2012, China | Chinese | Unclear | Unclear | 317 | 301 | Mar 2007~Sep 2009 | Han people in China | C1: 68.90±7.97<br>C2: 67.69±9.97 | G395A<br>C370S<br>F352V | CHD | Peripheral blood leukocytes | TG, HDL, LDL, GLU |
| Li, 2013, China | Chinese | Unclear | Unclear | 76 | 69 | Jul 2012~Aug 2012 | Chinese | Unclear | G395A | Hypertension | Peripheral blood leukocytes | Yes |
| Tian, 2013, China | Chinese | Health checkup | Case control | 389 | 388 | Sep 2011~May 2012 | Chinese | C1: 62.02±9.78<br>C2: 62.06±9.78 | G395A | IS | Peripheral blood leukocytes | Hyperlipidemia, Diabetes mellitus, Smoking, Drinking, SBP, DBP |
| Cai, 2013, China | Chinese | Hospital | Case control | 340 | 280 | Mar 2011~Jun 2012 | Han people in China | C1: 63.16±12.11<br>C2: 64.36±11.98 | G395A | CAD | Peripheral venous blood | Sex, GLU, Drinking, TG, Smoking, FPG, TC, LDL, family history of coronary heart disease, SBP, DBP |

Abbreviations: CAD, coronary artery disease; PCAD, premature coronary artery disease; IS, ischemic stroke; UAP, unstable angina pectoris; CHD, Coronary heart disease; AMI, acute myocardial infarct ion; ACS, acute coronary syndrome. SD, standard deviation; BMI, body mass index; GLU, glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triacylglycerol; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; CKD, chronic kidney disease; CKD, chronic kidney disease; GFR, glomerular filtration rate; IMT, intima-media thickness. #Using mean age; M, male; F, female. *If baseline characteristics were shown to be significantly different between the two groups, these outcomes were shown in the blanks, otherwise, "Yes" represents the comparable baseline characteristics. C1: case group; C2: control group.

**Table 2.** Methodological quality of included studies

| Studies: author, year, country | Selection of Participants | | | | Comparability | Exposure | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | Item 1 | Item 2 | Item 3 | Item 4 | Item 5 | Item 6 | Item 7 | Item 8 | |
| Low, 2005, USA | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 5 |
| Kim, 2006, Korean | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 6 |
| Imamura, 2006, Japan | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 6 |
| He, 2008, China | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 5 |
| Jin, 2008, China | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 5 |
| Wang, 2008, China | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 6 |
| Jo, 2009, South Korea | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 7 |
| Majumdar, 2010, India | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 4 |
| Wang, 2010, China | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 6 |
| Oguro, 2010, Japan | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 7 |
| Liu, 2010, China | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 5 |
| He, 2010, China (1) | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 5 |
| He, 2010, China (2) | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 5 |
| He, 2011, China | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 4 |
| Liu, 2011, China | 1 | 1 | 1 | 1 | 2 | 0 | 1 | 0 | 7 |
| Tavakkoly, 2011, Iran | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 5 |
| You, 2012, China | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 5 |
| Li, 2013, China | 1 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 4 |
| Tian, 2013, China | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 6 |
| Cai, 2013, China | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 6 |

Selection: item 1, is the case definition adequate? (if some independent validation was required, 1 star); item 2, representativeness of the cases (if yes, 1 star); item 3, selection of controls (if they were from community controls, 1 star); 4, definition of controls (if they had no history of CVDs and new occurrence, 1 star). Comparability: item 5, comparability of cases and controls on the basis of design or analysis: item 1, ethnicity (if yes, 1 star); 2, age, body mass index, GLU, glucose/diabetes mellitus and smoking (if yes, one star). Exposure: item 1, ascertainment of exposure (if in reliable method, 1 star); 2, same method of ascertainment for cases and controls (if yes, 1 star); Item 3, non-response rate (if they were the same between cases and controls, one star).

*Data synthesis and analysis*

Data synthesis and analysis were performed using the statistical software STATA (version 12.0). The pooled odds ratios (OR) and 95% confidence interval (CI) were adopted to describe the association between CVDs and Klotho SNPs. Statistical heterogeneity among included studies was evaluated with use of the Q statistic with a significance level set at $P<0.1$. Heterogeneity was considered low, moderate or high as based upon $I^2$ values of 25%, 50%, or 75%, respectively. When heterogeneity showed no statistical significance, we used the fixed effects model ($I^2<50\%$; $P>0.1$), for all other cases the random effects model was applied. A sensitivity analysis was also included to investigate further the source of heterogeneity. The sensitivity analysis was conducted to evaluate those studies that deviated from Hardy-Weinberg equilibrium (HWE). Further, subgroup analyses were performed with regard to participants' ethnicity/race and outcomes. Publication bias was assessed by a funnel plot using both Begg's funnel plot and Egger's linear regression test [18].

We assessed the overall correlation between Klotho SNPs and the risk of CVDs using the following three genetic models [19]: a) an additive genetic model (A vs a), b) a dominant genetic model (AA+Aa vs aa) and c) a recessive genetic model (aa vs AA+Aa). We calculated unadjusted OR and their 95% CI from raw genotype frequency data. All *P* values were 2-sided at a level of significance set at $\alpha \leq 0.05$. HWE was tested using the Chi-squared test and was considered statistically significant when $P<0.05$.

## Results

*Characteristics of included studies and participants*

A total of 2733 references were screened for eligibility. Among these, 763 duplicates were

**Table 3.** Genotype frequencies of the G395A, C1818T, F352V, C370S in the case and control group

| Studies: author, year, country | | GG | | AG | | AA | | G | | A | | HWE* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | n | % | n | % | n | % | n | % | n | % | $\chi^2$ | n |
| Kim, 2006, Korean | Case | 183 | 67.03 | 80 | 29.00 | 10 | 3.70 | 446 | 81.70 | 100 | 18.30 | 0.73 | 0.70 |
| | Control | 325 | 71.40 | 118 | 25.93 | 12 | 2.60 | 768 | 84.40 | 142 | 15.6 | | |
| Imamura, 2006, Japan | Case | 138 | 70.10 | 59 | 29.90 | 0 | 0.00 | 335 | 85.05 | 59 | 14.95 | 1.74 | 0.42 |
| | Control | 268 | 81.00 | 62 | 18.70 | 1 | 0.30 | 598 | 90.35 | 64 | 9.65 | | |
| Wang, 2008, China | Case | 87 | 66.92 | -- | -- | 43 | 33.08 | -- | -- | -- | -- | -- | -- |
| | Control | 53 | 51.96 | -- | -- | 49 | 48.04 | -- | -- | -- | -- | | |
| Jo, 2009, South Korea | Case | 157 | 69.70 | 60 | 26.70 | 8 | 3.60 | 374 | 83.05 | 76 | 16.95 | 3.48 | 0.18 |
| | Control | 164 | 78.50 | 39 | 18.70 | 6 | 2.90 | 367 | 87.85 | 51 | 12.25 | | |
| Wang, 2010, China | Case | 155 | 72.10 | 52 | 24.20 | 8 | 3.70 | 362 | 84.20 | 68 | 15.80 | 0.34 | 0.84 |
| | Control | 133 | 60.50 | 78 | 35.50 | 9 | 4.10 | 344 | 78.25 | 96 | 21.85 | | |
| Liu, 2010, China | Case | 295 | 42.63 | 305 | 44.07 | 92 | 13.29 | 895 | 64.67 | 489 | 35.33 | 0.80 | 0.67 |
| | Control | 235 | 50.09 | 182 | 39.56 | 43 | 9.35 | 652 | 69.87 | 268 | 29.13 | | |
| He, 2010, China(2) | Case | 240 | 73.60 | 79 | 24.20 | 7 | 2.10 | 559 | 85.70 | 93 | 14.30 | 0.40 | 0.82 |
| | Control | 162 | 74.00 | 54 | 24.70 | 3 | 1.40 | 378 | 86.35 | 60 | 13.75 | | |
| Liu, 2011, China | Case | 341 | 44.00 | 333 | 42.97 | 101 | 13.03 | 1015 | 65.49 | 535 | 34.52 | 1.86 | 0.39 |
| | Control | 178 | 52.51 | 142 | 41.89 | 19 | 5.60 | 498 | 73.46 | 180 | 26.55 | | |
| You, 2012, China | Case | 150 | 47.32 | 124 | 39.12 | 43 | 13.56 | 96 | 63.16 | 56 | 36.84 | 0.65 | 0.72 |
| | Control | 150 | 49.83 | 129 | 42.86 | 22 | 7.31 | 102 | 73.91 | 36 | 26.09 | | |
| Li, 2013, China | Case | 30 | 39.47 | 36 | 47.37 | 10 | 13.15 | 424 | 66.88 | 210 | 33.12 | 0.04 | 0.98 |
| | Control | 38 | 55.07 | 26 | 37.68 | 5 | 7.25 | 429 | 71.26 | 173 | 28.74 | | |
| Tian, 2013, China | Case | 256 | 65.80 | 118 | 30.30 | 15 | 3.90 | 630 | 80.95 | 148 | 19.05 | 0.95 | 0.10 |
| | Control | 266 | 68.60 | 110 | 28.40 | 12 | 3.00 | 642 | 82.80 | 134 | 17.20 | | |
| Cai, 2013, China | Case | 235 | 69.10 | 99 | 29.10 | 6 | 1.80 | 569 | 83.65 | 111 | 16.35 | 0.77 | 0.68 |
| | Control | 205 | 73.20 | 67 | 23.90 | 8 | 2.90 | 477 | 85.15 | 83 | 14.85 | | |
| | | CC | | CT | | TT | | C | | T | | | |
| Kim, 2006, Korean | Case | 183 | 67.00 | 83 | 30.40 | 7 | 2.60 | 449 | 82.20 | 97 | 17.80 | 0.00 | 1.00 |
| | Control | 305 | 67.00 | 135 | 29.70 | 15 | 3.30 | 745 | 81.90 | 165 | 18.10 | | |
| Jin, 2008, China | Case | 100 | 68.50 | 40 | 27.40 | 6 | 4.10 | 240 | 82.20 | 52 | 17.80 | 3.80 | 0.15 |
| | Control | 174 | 74.00 | 52 | 22.10 | 9 | 3.80 | 400 | 85.10 | 70 | 14.90 | | |
| Majumdar, 2010, India | Case | 256 | 57.40 | 166 | 37.22 | 24 | 5.38 | 678 | 76.01 | 214 | 24.00 | 0.02 | 1.00 |
| | Control | 315 | 55.17 | 219 | 38.35 | 37 | 6.48 | 849 | 74.34 | 293 | 25.66 | | |
| He, 2010, China (1) | Case | 255 | 68.00 | 106 | 28.30 | 14 | 3.70 | 616 | 82.15 | 134 | 17.85 | 3.80 | 0.15 |
| | Control | 174 | 74.00 | 52 | 22.10 | 9 | 3.80 | 400 | 85.05 | 70 | 14.85 | | |
| He, 2011, China | Case | 155 | 67.70 | 66 | 28.80 | 8 | 3.50 | 376 | 82.10 | 82 | 17.90 | 3.80 | 0.15 |
| | Control | 174 | 74.00 | 52 | 22.10 | 9 | 3.80 | 400 | 85.05 | 70 | 14.85 | | |
| | | FF | | FV | | VV | | F | | V | | | |
| Majumdar, 2010, India | Case | 359 | 78.04 | 89 | 19.35 | 12 | 2.61 | 807 | 87.72 | 113 | 12.29 | 0.19 | 0.91 |
| | Control | 411 | 71.60 | 151 | 26.30 | 12 | 2.10 | 973 | 84.75 | 175 | 15.25 | | |
| Liu, 2010, China | Case | 233 | 33.67 | 433 | 62.57 | 26 | 3.76 | 899 | 64.96 | 485 | 35.05 | 46.90 | <0.01 |
| | Control | 184 | 40.00 | 262 | 56.90 | 14 | 3.04 | 630 | 68.45 | 290 | 31.49 | | |
| Liu, 2011, China | Case | 272 | 35.10 | 471 | 60.77 | 32 | 4.13 | 1015 | 65.49 | 535 | 34.51 | 34.47 | <0.01 |
| | Control | 131 | 38.64 | 196 | 57.82 | 12 | 3.54 | 458 | 67.55 | 220 | 32.45 | | |
| Tavakkoly-Bazzaz, 2011, Iran | Case | 54 | 100.00 | 0 | 0.00 | 0 | 0.00 | 108 | 100.00 | 0 | 0.00 | -- | -- |
| | Control | 53 | 100.00 | 0 | 0.00 | 0 | 0.00 | 106 | 100.00 | 0 | 0.00 | | |
| You, 2012, China | Case | 115 | 36.28 | 184 | 58.04 | 18 | 5.68 | 414 | 65.30 | 220 | 34.70 | 14.46 | <0.01 |
| | Control | 121 | 40.20 | 162 | 53.82 | 18 | 5.98 | 404 | 67.11 | 198 | 32.89 | | |
| | | CC | | CS | | SS | | C | | S | | | |
| Liu, 2010, China | Case | 283 | 40.89 | 386 | 55.78 | 23 | 3.32 | 952 | 68.78 | 432 | 31.21 | 31.47 | <0.01 |
| | Control | 189 | 40.48 | 251 | 54.56 | 20 | 2.17 | 629 | 67.76 | 291 | 29.45 | | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Liu, 2011, China | Case | 335 | 43.23 | 416 | 53.68 | 24 | 3.10 | 1086 | 70.07 | 464 | 29.94 | 22.67 | <0.01 |
| | Control | 148 | 43.66 | 179 | 52.80 | 12 | 3.54 | 475 | 70.06 | 203 | 29.94 | | |
| You, 2012, China | Case | 156 | 49.21 | 152 | 47.95 | 9 | 2.84 | 464 | 73.19 | 170 | 26.82 | 36.34 | <0.01 |
| | Control | 99 | 32.89 | 187 | 62.13 | 15 | 4.98 | 385 | 63.96 | 217 | 36.05 | | |

removed with Endnote software while 1934 citations were excluded after title and abstract screening. Thirty six full-text articles were retrieved for a more detailed assessment. Of these 36 articles, 16 were excluded [7, 20-34]: 4 were master's degree dissertations [20-23], whose data reappeared within a separate group of 7 articles that were then included in our analysis [12, 13, 32, 35-38]; 5 articles were abstracts from reports presented at conferences [24-28]; in 3 articles, participants of the control group experienced various conditions such as hypertension and heart disease [29-31]; 2 articles consisted of family-based studies [33, 34]; and the remaining 2 articles were excluded due to either poor methodological quality [32] or being part of an uncontrolled study [7] respectively. Finally, 20 studies that met the inclusion criteria were included in this meta-analysis [5, 6, 8-13, 35-46]. **Figure 1** shows Flow diagram.

Twelve articles were clearly defined as case-control studies [5, 9-11, 35, 36, 39, 41, 42, 44-46], while 8 articles failed to indicate the type of study performed [6, 8, 12, 13, 37, 38, 40, 43]. We contacted the authors of the original studies, but only one replied to our email indicating that they adopted a case control study design [43]. Therefore, the exact protocols used in 7 of the studies remain unknown [6, 8, 12, 13, 37, 38, 40]. However, as these studies satisfied our inclusion criteria, it was decided to include them within our analyses. **Table 1** provides a summary of the characteristics of the studies included.

The studies included in this meta-analysis involved 9 SNPs of the Klotho gene. Klotho G-395A was reported within 12 studies [5, 6, 8, 11-13, 35, 37, 38, 42, 45, 46], C1818T in 5 studies [10, 11, 36, 41, 44] and there were 5 references for F352V and 3 references for C370S [9, 10, 12, 13, 38]. One article contained 3 additional SNPs (rs3752472, rs9536314, rs650439) [43], while 2 other articles also reported KL-VS and C2298T [11, 39]. In this meta-analysis, we extracted data from all reported Klotho SNPs used within the included studies, but could only combine the data from the four most common SNPs: G-395A, C1818T, F352V and C370S (**Table 3**).

Among the included studies, 16 contained detailed data involving 9585 (case/control: 5035/4550) participants (**Table 3**) [5, 6, 8-10, 12, 13, 35-38, 40, 41, 44-46]. The actual average age, rather than >47.4. Eleven studies included the Han people in China [8, 12, 13, 35, 36, 38-44, 46] and 2 study with Japanese [5, 43] and 2 with Korean subjects [6, 11]. Recruited subjects were involved with 2 studies involving Chinese as their subject [37, 45], as well as in individual studies with American [39], Indian [10] and Iranian [9] subjects. Some variables closely related to CVD were not comparable between the groups. Six studies reported that body mass index was greater in the case versus control group [5, 8, 10, 39, 42, 43]. In 14 studies, the number of participants showing complications associated with diabetes were significantly greater in the case versus control group [5, 6, 8-10, 35, 36, 38, 40, 42-46]. Results from 15 studies indicated that a significantly greater number of the participants were smokers [5, 6, 8-10, 35, 36, 38, 40-46]. This meta-analysis included outcomes from a variety of CVDs, including coronary heart disease (CHD) [38], coronary artery disease (CAD) [5, 6, 9, 46], premature coronary artery disease (PCAD) [39], acute coronary syndrome (ACS) [35, 36, 40], ischemic stroke (IS) [10, 11, 45], unstable angina pectoris (UAP) [35], acute myocardial infarction (AMI) [44], hypertension [8, 12, 13, 37], atherosclerosis [43] and arteriosclerosis [42]. The CAD, PCAD, ACS, UAP and AMI were categorized as CHD in this meta-analysis when the subgroup analysis was performed (**Table 1**). F352V and C370S in 3 studies were not consistent with HWE in the control group after using the Chi-squared test (**Table 3**) [12, 13, 38].

*Methodological quality*

**Table 2** presents the results of the risk of bias assessment. Three studies with equal to 7

**Table 4.** Meta-analysis of the association between the Klotho polymorphism and CVDs risk in three genetic models

| Allele and genotype | Country | Outcome | Number of studies | OR (95% CI) | $I^2$ (%) | $P_h$ | Analysis model | *P* |
|---|---|---|---|---|---|---|---|---|
| 395A allele vs 395G allele (additive model) | All | All | 11 | 1.23 (1.08, 1.40) | 52.8 | 0.020 | Random | 0.001 |
| | Korean | All | 2 | 1.29 (1.03, 1.62) | 0 | 0.440 | Random | 0.025 |
| | Han people in China | All | 8 | 1.18 (1.01, 1.38) | 61.5 | 0.011 | Random | 0.004 |
| | All | IS | 2 | 1.16 (0.96, 1.41) | 0 | 0.702 | Random | 0.116 |
| | All | CHD | 5 | 1.25 (1.08, 1.45) | 3.5 | 0.387 | Random | 0.002 |
| | All | Hypertension | 4 | 1.21 (0.88, 1.66) | 81.5 | 0.001 | Random | 0.236 |
| 395AA+395AG vs 395GG (dominant model) | All | All | 11 | 1.24 (1.05, 1.45) | 56.3 | 0.011 | Random | 0.009 |
| | Korean | All | 2 | 1.34 (1.04, 1.74) | 0 | 0.367 | Random | 0.026 |
| | Han people in China | All | 8 | 1.16 (0.96, 1.40) | 60.9 | 0.012 | Random | 0.131 |
| | All | IS | 2 | 1.18 (0.94, 1.47) | 0 | 0.716 | Random | 0.148 |
| | All | CHD | 5 | 1.29 (1.05, 1.58) | 32.4 | 0.205 | Random | 0.015 |
| | All | Hypertension | 4 | 1.20 (0.80, 1.79) | 81.1 | 0.001 | Random | 0.384 |
| 395AA vs 395AG+395GG (recessive model) | All | All | 11 | 1.61 (1.30, 2.00) | 0 | 0.485 | Fixed | <0.001 |
| | Korean | All | 2 | 1.34 (0.69, 2.62) | 0 | 0.866 | Fixed | 0.392 |
| | Han people in China | All | 8 | 1.66 (1.32, 2.07) | 20.2 | 0.269 | Fixed | <0.001 |
| | All | IS | 2 | 1.32 (0.74, 2.34) | 0 | 0.851 | Fixed | 0.341 |
| | All | CHD | 5 | 1.49 (0.99, 2.24) | 5.7 | 0.374 | Fixed | 0.055 |
| | All | Hypertension | 4 | 1.75 (1.32, 2.31) | 33.1 | 0.214 | Fixed | <0.001 |
| 1818T allele vs 1818C allele (additive model) | All (11) | All | 11 | 1.05 (0.92, 1.19) | 15.8 | 0.314 | Fixed | 0.468 |
| | Han people in China | All | 3 | 1.24 (1.02, 1.52) | 0 | 1.000 | Fixed | 0.034 |
| | All | IS | 2 | 0.94 (0.79, 1.10) | 0 | 0.713 | Fixed | 0.424 |
| | All | CHD | 3 | 1.24 (1.02, 1.52) | 0 | 1.000 | Fixed | 0.034 |
| 1818TT+1818CT vs 1818CC (dominant model) | All | All | 5 | 1.09 (0.94, 1.27) | 24.2 | 0.260 | Fixed | 0.236 |
| | Han people in China | All | 3 | 1.34 (1.06, 1.69) | 0 | 0.993 | Fixed | 0.013 |
| | All | IS | 2 | 0.95 (0.78, 1.15) | 0 | 0.661 | Fixed | 0.575 |
| | All | CHD | 3 | 1.34 (1.06, 1.69) | 0 | 0.993 | Fixed | 0.013 |
| 1818TT vs 1818CT+1818CC (recessive model) | All | All | 5 | 0.87 (0.62, 1.24) | 0 | 0.987 | Fixed | 0.448 |
| | Han people in China | All | 3 | 0.98 (0.57, 1.69) | 0 | 0.987 | Fixed | 0.464 |
| | All | IS | 2 | 0.81 (0.51, 1.28) | 0 | 0.909 | Fixed | 0.361 |
| | All | CHD | 3 | 0.98 (0.57, 1.69) | 0 | 0.974 | Fixed | 0.938 |
| 352V allele vs 352F allele (additive model) | All | All | 4 | 1.04 (0.88, 1.22) | 57.4 | 0.070 | Random | 0.660 |
| | Han people in China | All | 3 | 1.12 (1.00, 1.26) | 0 | 0.834 | Random | 0.044 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | All | Hypertension | 2 | 1.14 (1.00, 1.30) | 0 | 0.622 | Random | 0.054 |
| 352VV+352FV vs 352FF (dominant model) | All | All | 4 | 1.07 (0.81, 1.40) | 73.6 | 0.010 | Random | 0.637 |
| | Han people in China | All | 3 | 1.23 (1.05, 1.44) | 0 | 0.778 | Random | 0.010 |
| | All | Hypertension | 2 | 1.23 (1.04, 1.49) | 0 | 0.513 | Random | 0.018 |
| 352VV vs 352FV+352FF (recessive model) | All | All | 4 | 1.14 (0.81, 1.62) | 0 | 0.936 | Fixed | 0.459 |
| | Han people in China | All | 3 | 1.12 (0.76, 1.64) | 0 | 0.838 | Fixed | 0.574 |
| | All | Hypertension | 2 | 1.21 (0.75, 1.94) | 0 | 0.904 | Fixed | 0.432 |
| 370S allele vs 370C allele (additive model) | All | All | 3 | 0.87 (0.68, 1.12) | 77.6 | 0.012 | Random | 0.275 |
| | All | Hypertension | 2 | 0.99 (0.87, 1.13) | 0 | 0.889 | Random | 0.875 |
| 370SS+370CS vs 370CC (dominant model) | All | All | 3 | 0.81 (0.54, 1.23) | 85.2 | 0.001 | Random | 0.324 |
| | All | Hypertension | 2 | 1.01 (0.85, 1.21) | | 0.957 | Random | 0.890 |
| 370SS vs 370CS+370CC (recessive model) | All | All | 3 | 0.74 (0.49, 1.10) | 0 | 0.724 | Fixed | 0.134 |
| | All | Hypertension | 2 | 0.80 (0.51, 1.27) | 0 | 0.747 | Fixed | 0.352 |

Abbreviation: $P_h$ the *P* value of heterogeneity.

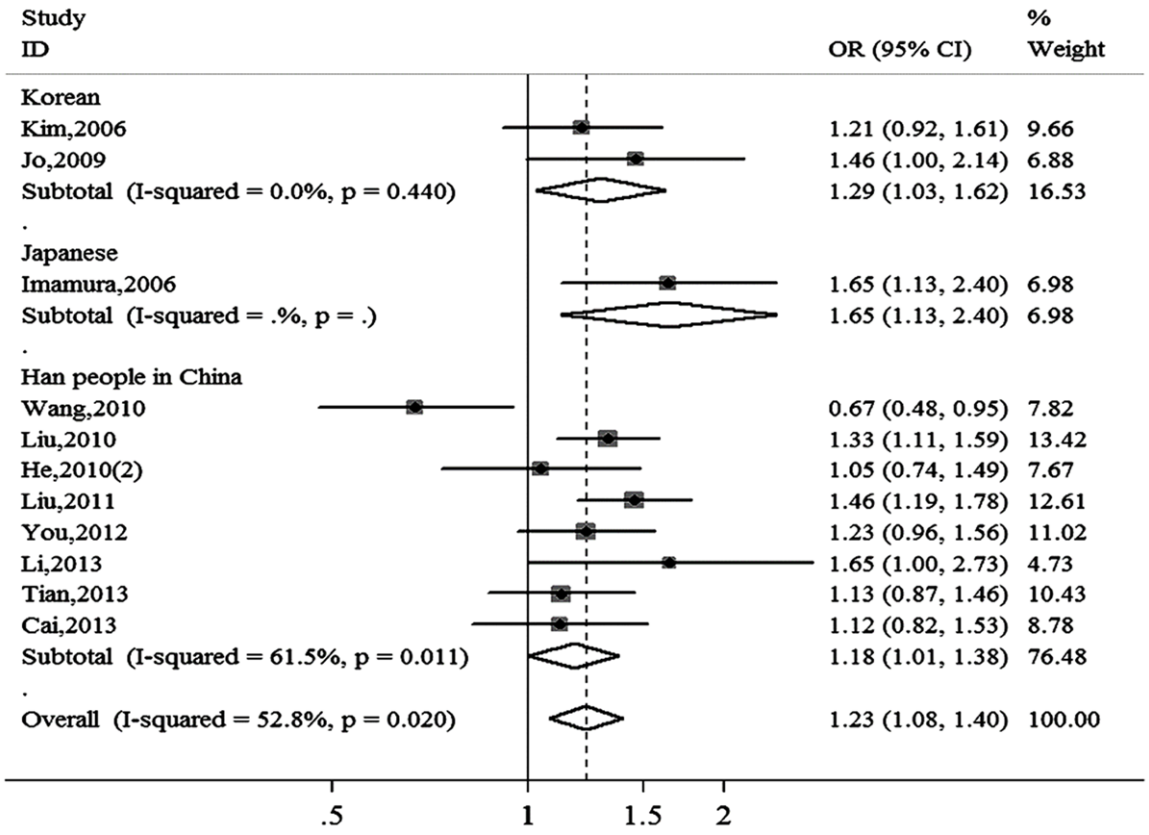


Figure 2. Forest plot of ORs and 95% CIs for the association between G395A and CVDs under the additive genetic model, stratified by country (Analyzed by random effects model).

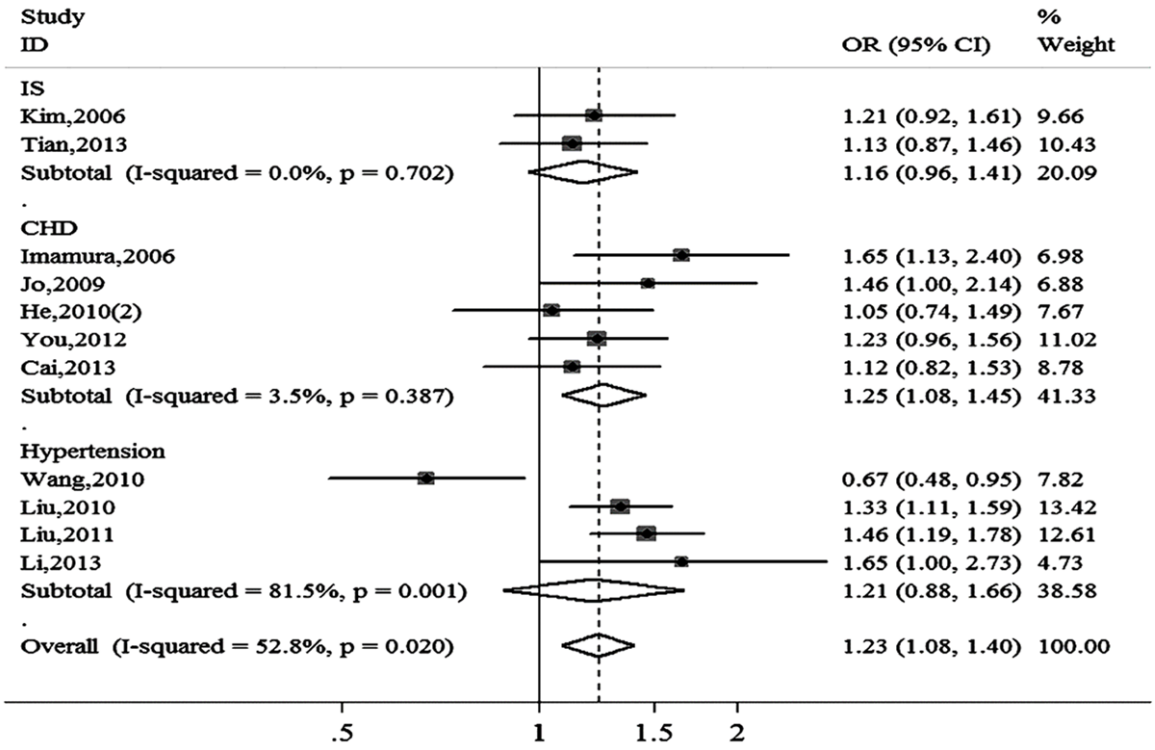

Figure 3. Forest plot of ORs and 95% CIs for the association between G395A and CVDs under the additive genetic model, stratified by outcome (Analyzed by random effects model).

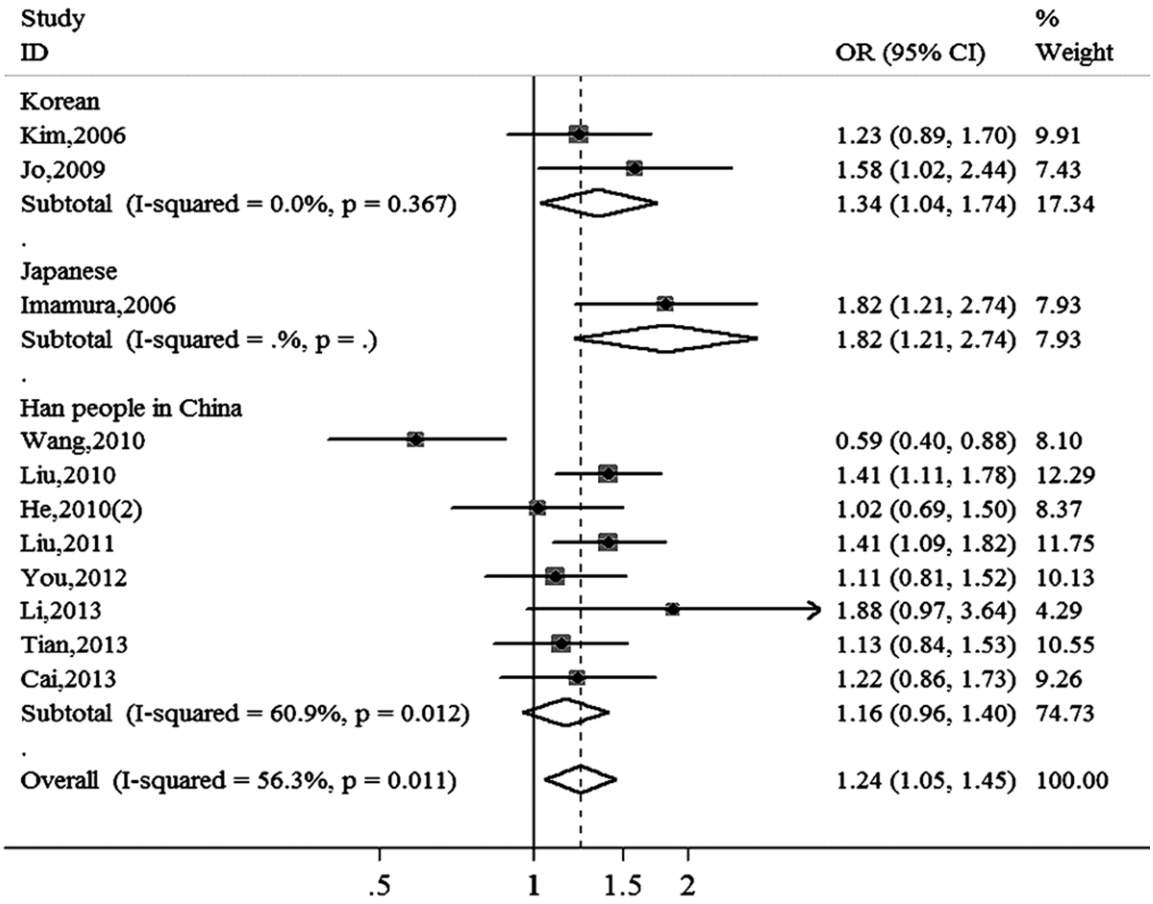


Figure 4. Forest plot of ORs and 95% CIs for the association between G395A and CVDs under the dominant genetic model, stratified by country (Analyzed by random effects model).

stars were defined as high quality [6, 12, 43] and 17 studies rated with 4-6 stars were classified as medium quality [5, 8-11, 13, 35-42, 44-46]. In the Hua's study, the determination of exposures was the only information available to assess their methodological quality. As detailed data were not presented in this study, it was excluded from our meta-analysis [32].

*Meta-analysis of the association between G-395A and CVDs risk*

Of the 12 studies that included an examination of Klotho G-395A [5, 6, 8, 11-13, 35, 37, 38, 42, 45, 46], and one of them did not provide the full data of gene frequency [42], so the data from the remaining 11 studies were combined. A random-effects model was adopted for the additive genetic and dominant genetic models, as a statistical heterogeneity was present among studies with these two models ($I^2$=52.8, $P$=0.020; **Table 4** and **Figures 2**, **3**) and ($I^2$= 56.3, $P$=0.011), respectively (**Table 4** and **Figures 4**, **5**). A fixed-effects model was performed for the recessive genetic model ($I^2$=0, $P$=0.485; **Table 4** and **Figures 6**, **7**). Overall, results from this analysis showed that individuals carrying the Klotho 395A allele showed a significant increase in the risk for CVDs as compared with those carrying the 395G allele in the additive genetic model (OR=1.23, 95% CI=1.08, 1.40; **Table 4** and **Figures 2**, **3**). Similarly, this statistically significant difference was also observed between participants with AA+AG and those with GG in the dominant genetic

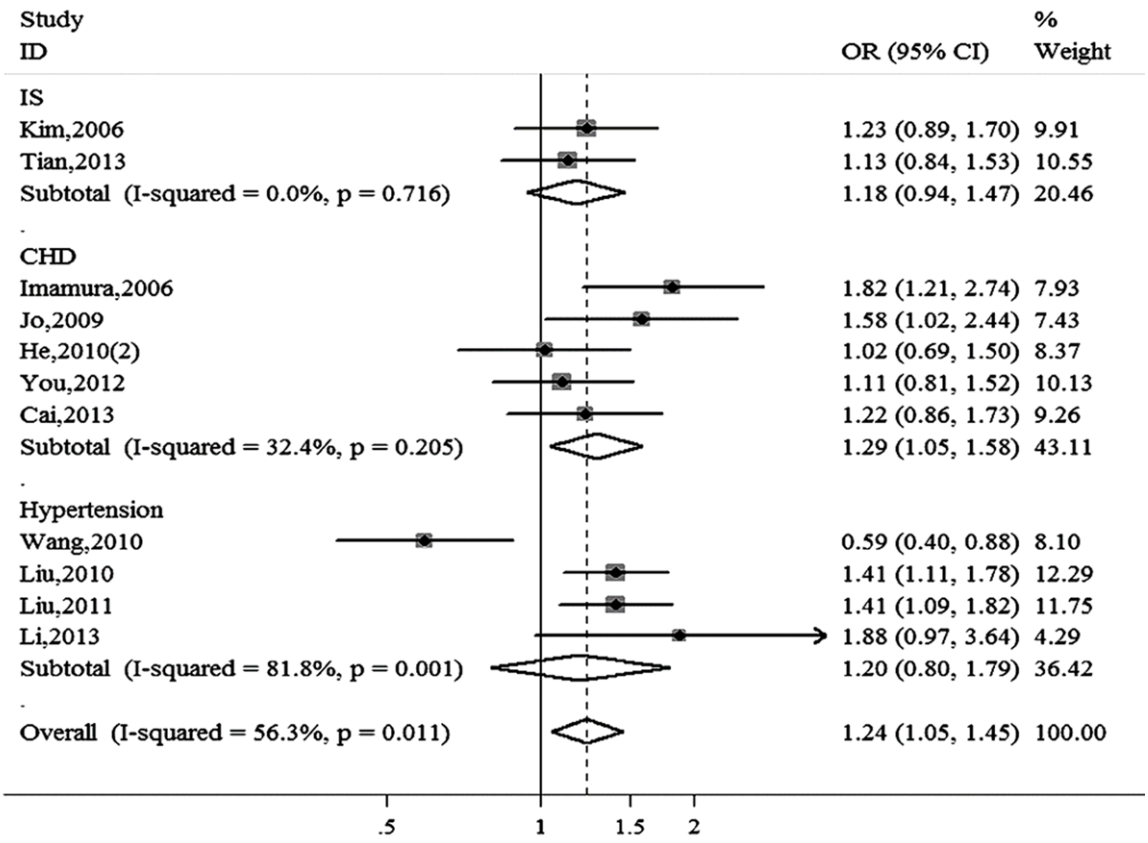


**Figure 5.** Forest plot of ORs and 95% CIs for the association between G395A and CVDs under the dominant genetic model, stratified by outcome (analyzed by random effects model).

model (OR=1.24, 95% CI=1.05, 1.45; **Table 4** and **Figures 4**, **5**) and AA vs GG+AG in the recessive genetic model (OR=1.61, 95% CI=1.30, 2.00; **Table 4** and **Figures 6**, **7**).

In addition, subgroup analyses were performed on different races to explore further the association between Klotho G-395A SNPs and CVDs. A significant association was observed in Koreans, as assessed with the additive genetic model (OR=1.29, 95% CI=1.03, 1.62; **Figures 2**, **3**) and the dominant genetic model (OR=1.34, 95% CI=1.04, 1.74; **Figures 4**, **5**). And, a significant association was found in Han Chinese, as determined with the additive model (OR=1.18, 95% CI=1.01, 1.38; **Figures 2**, **3**) and the recessive model (OR=1.66, 95% CI=1.32, 2.07; **Figures 6**, **7**).

A subgroup analysis was carried out with respect to the outcomes reported. Results indicated that the G-395A SNP was associated with a significantly greater risk of CHD, but not IS, as determined within each of the three models. However, a significant association between an increased risk of hypertension and the G-395A SNP was only observed with the recessive model (OR=1.75, 95% CI=1.32, 2.31; **Table 4** and **Figures 6**, **7**).

Due to statistical heterogeneity, a sensitivity analysis was performed. **Figure 8** shows that the study of Wang et al. 2010 contributed the greatest amount of this heterogeneity [8]. After excluding this study, the statistical heterogeneity was reduced ($I^2$=0%, $P$=0.531), but the overall results did not change (**Table 5**).

*Meta-analysis of the association between C1818T and CVDs risk*

Five studies included within our analyses contained detailed data on the C1818T SNP [10, 11, 36, 41, 44]. As a small amount of statistical heterogeneity was present within these studies, a fixed-effects model was used with all three genetic models (**Table 4**). Pooled analyses of the overall results failed to identify any

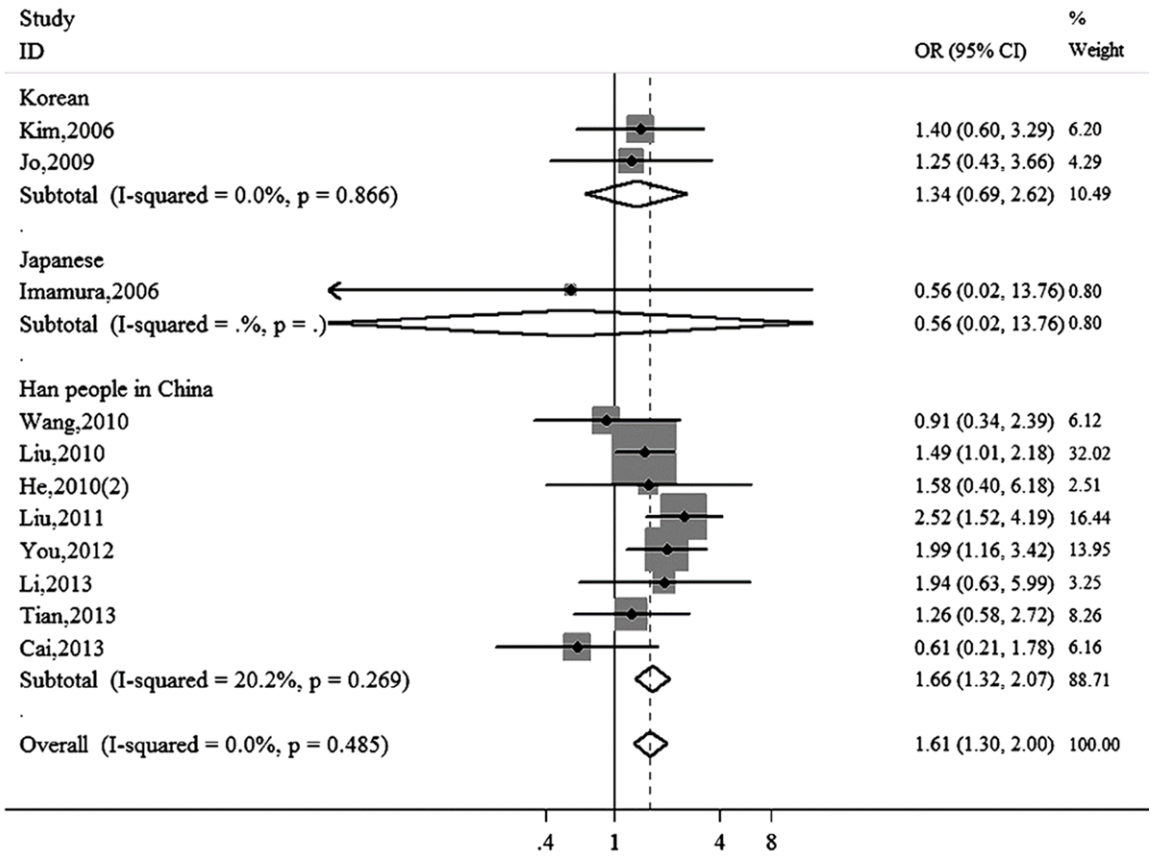


**Figure 6.** Forest plot of ORs and 95% CIs for the association between G395A and CVDs under the recessive genetic model, stratified by country. (Analyzed by fixed effects model).

association between the risk of CVDs and the C1818T SNP for none of the models: additive genetic model (OR=1.05, 95% CI=0.92, 1.19; **Table 4**), dominant genetic model (OR=1.09, 95% CI=0.94, 1.27; **Table 4**) and the recessive genetic model (OR=0.87, 95% CI=0.62, 1.24; **Table 4**). However, results obtained from the subgroup analyses revealed a significant association between the C1818T SNP and CVDs within the Han Chinese, as determined with the additive genetic and the dominant genetic models (**Table 4**). This was also evidence for an association between the C1818T SNP and an increased risk of CHD within these same models (**Table 4**).

*Meta-analysis of the association between F352V and CVDs risk*

Five included studies reported the F352V SNP [9, 10, 12, 13, 38], and it was possible to combine the data from 4 of these studies [10, 12, 13, 38]. The random-effects model was adopted for the additive genetic and dominant genetic models, due to a limited degree of statistical heterogeneity within these two models, ($I^2$= 57.4, $P$=0.07) and ($I^2$=73.6, $P$=0.01), respectively (**Table 4**). The fixed-effects model was adopted with the recessive genetic model ($I^2$=0, $P$=0.936; **Table 4**). An overall analysis with all 3 genetic models was performed. Results of this pooled analyses failed to indicate any statistically significant association with the additive genetic model (OR=1.04, 95% CI=0.88, 1.22; **Table 4**), the dominant genetic model (OR=1.07, 95% CI=0.81, 1.40; **Table 4**) or the recessive genetic model (OR=1.14, 95% CI=0.81, 1.62; **Table 4**). Attempts at performing a subgroup analysis as stratified by race and outcome only contained Han Chinese and hypertension. A significant correlation was obtained for both of these subgroups within the additive genetic model (Han Chinese: OR=1.12, 95% CI=1.00, 1.26; hypertension: OR=1.14, 95% CI=1.00, 1.30; **Table 4**) and the dominant genetic model

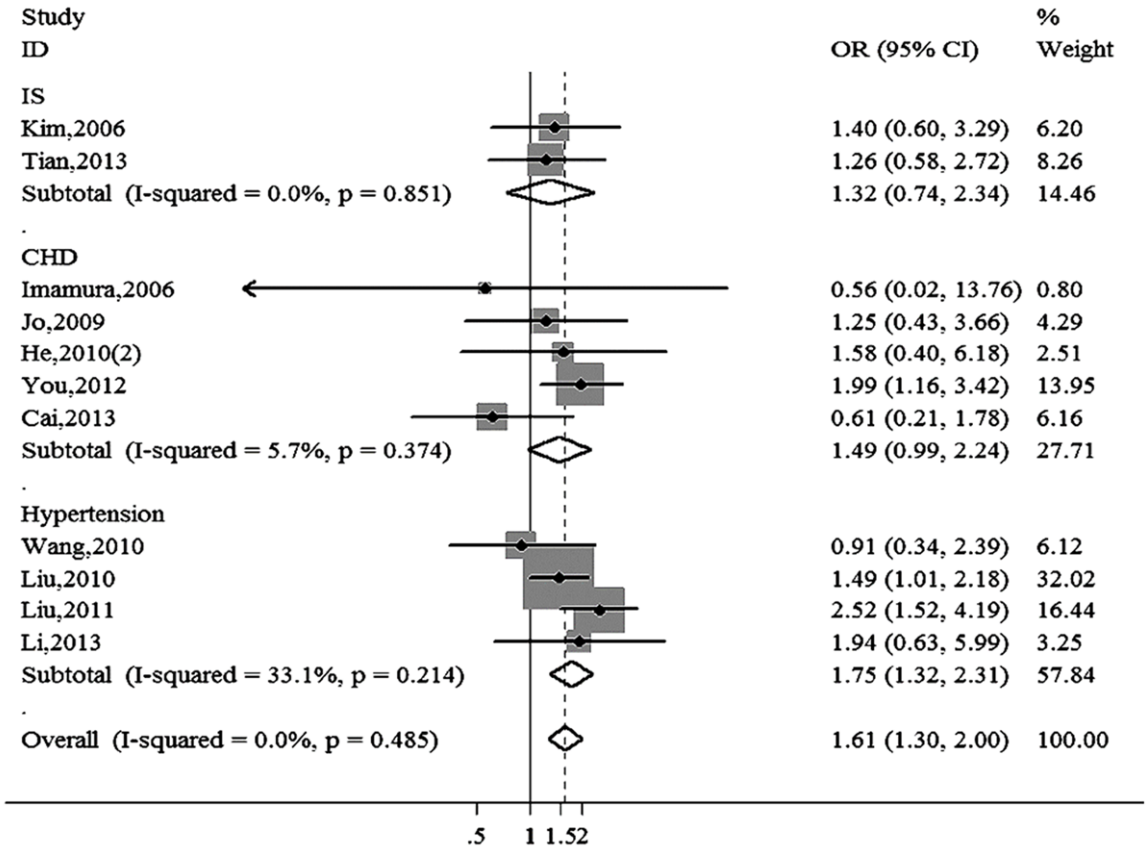


**Figure 7.** Forest plot of ORs and 95% CIs for the association between G395A and CVDs under the recessive genetic model, stratified by outcome (analyzed by fixed effects model).

(Han Chinese: OR=1.23, 95% CI=1.05, 1.44; hypertension: OR=1.07, 95% CI=0.81, 1.40; **Table 4**).

A sensitivity analysis was performed as a means to identify and reduce the statistical heterogeneity in the additive genetic and dominant genetic models. Results from this sensitivity analysis displayed that the study of Majumdar et al. contributed to the greatest degree of this heterogeneity [10]. With the exclusion of this study, statistical heterogeneity was reduced (**Table 5**) and a significant correlation was now observed with the additive genetic model (OR=1.12, 95% CI=1.00, 1.26) and dominant genetic model (OR=1.23, 95% CI=1.05, 1.44) (**Table 5**).

*Meta-analysis of the association between C370S and CVDs risk*

C370S SNPs were reported within 3 Chinese studies [12, 13, 38]. A random-effects model was adopted with the additive genetic and dominant genetic models due to the limited degree of statistical heterogeneity within these two models, ($I^2$=77.6, $P$=0.012) and ($I^2$=85.2, $P$=0.001), respectively. A fixed-effects model was adopted with the recessive genetic model ($I^2$=0, $P$=0.724). No association was found between the risk of CVDs and C370S SNP for any of the models: additive genetic model (OR=0.87, 95% CI=0.68, 1.12), dominant genetic model (OR=0.81, 95% CI=0.54, 1.23) or the recessive genetic model (OR=0.74, 95% CI= 0.49, 1.10). A subgroup analysis was not possible due to the limited number of studies (**Table 4**).

Sensitivity analysis displayed that the study of You et al. was the greatest source for the heterogeneity in these studies [22]. Exclusion of this study and re-analysis of the pooled data, resulted in a reduction of the statistical heterogeneity ($I^2$=0%, $P$=0.531), but the overall results were not changed (**Table 5**).

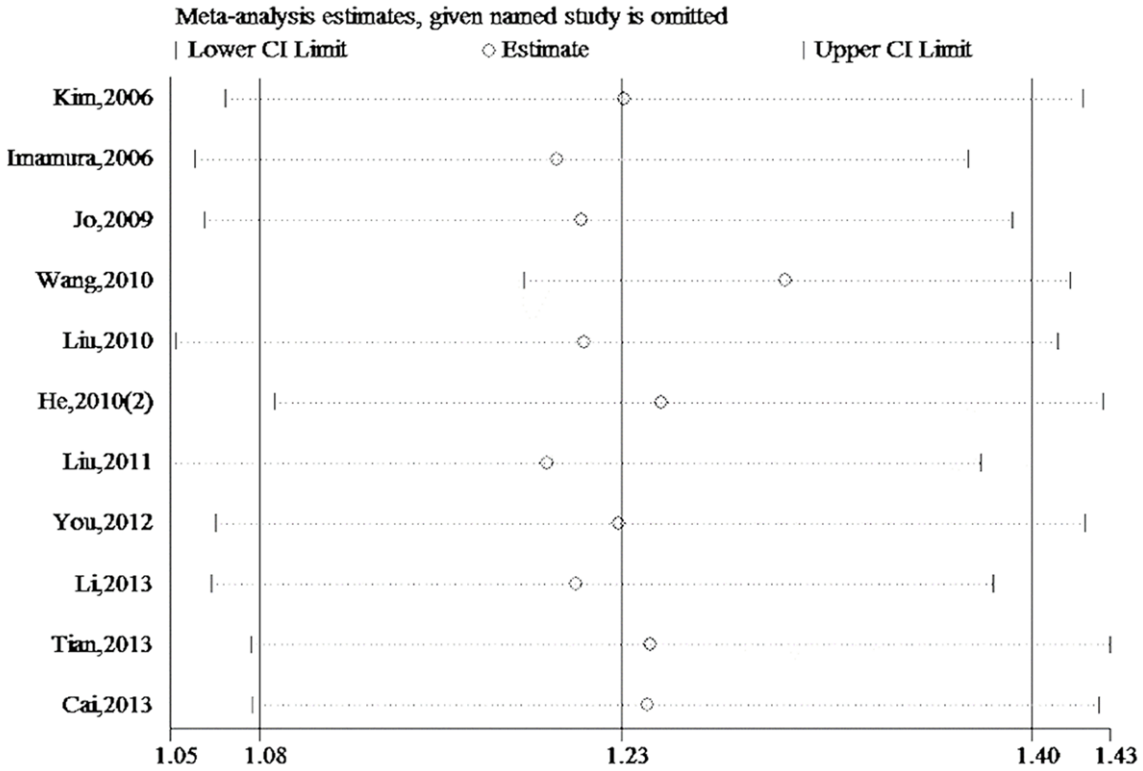


**Figure 8.** Sensitivity analysis of G-395A SNP under the additive genetic model. Each circle represents a separate study.

**Table 5.** Sensitivity analysis of the association between the Klotho polymorphism and CVDs risk

| Allele and genotype | Study numbers | OR (95% CI) | $I^2$ (%) | $P_h$ | Analysis model | *P* |
|---|---|---|---|---|---|---|
| 395A allele vs 395G allele (additive model) | 10 | 1.30 (1.19, 1.41) | 0 | 0.531 | Fixed | <0.001 |
| 395AA+395AG vs 395GG (dominant model) | 10 | 1.31 (1.18, 1.45) | 0 | 0.450 | Fixed | <0.001 |
| 352V allele vs 352F allele (additive model) | 3 | 1.12 (1.00, 1.26) | 0 | 0.834 | Fixed | 0.044 |
| 352VV+352FV vs 352FF (dominant model) | 3 | 1.23 (1.05, 1.44) | 0 | 0.778 | Fixed | 0.010 |
| 370S allele vs 370C allele (additive model) | 2 | 0.98 (0.87, 1.13) | 0 | 0.889 | Fixed | 0.875 |
| 370SS+370CS vs 370CC (dominant model) | 2 | 1.01 (0.85, 1.21) | 0 | 0.957 | Fixed | 0.890 |

Abbreviation: $P_h$ the *P* value of heterogeneity. *Sensitivity analysis was conducted by excluding the study that was not consistent with HWE in the control group.

*Publication bias*

We performed Begg's funnel plot and Egger's test to evaluate the potential for any publication bias. With respect to the association of the G-395A SNP and risk of CVDs with the three genetic models, the results of the Begg's funnel plot suggested no publication bias was present within any of these three models (*P*=0.876; **Figures 9-11**). Similarly, the results of the Egger's test failed to show any statistical evidence for publication bias as assessed with the additive genetic model (*P*=0.780), dominant genetic model (*P*=0.810) or recessive genetic model (*P*=0.218).

## Discussion

Twenty case-control or observational studies based on case and control groups were identified, which involved examining the association between Klotho SNPs and the risk of CVDs [5, 6, 8-13, 35-46]. This meta-analysis included detailed data from 16 studies including 9585

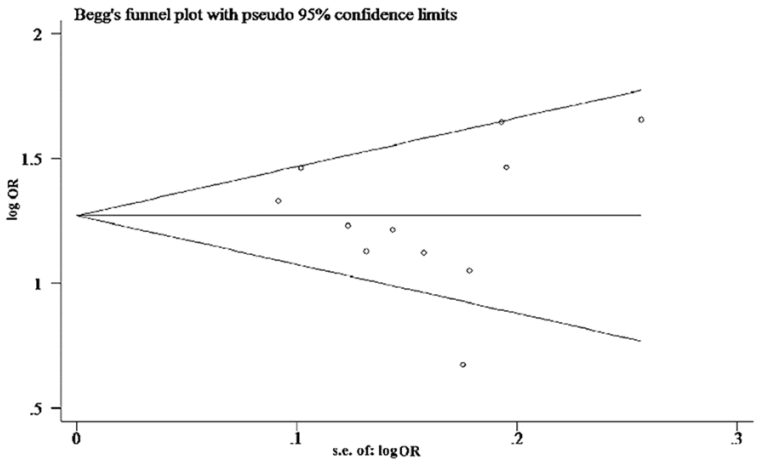


Figure 9. Begg's funnel plot for the publication bias on the association between G-395A SNP and CVDs risk in the additive genetic model.

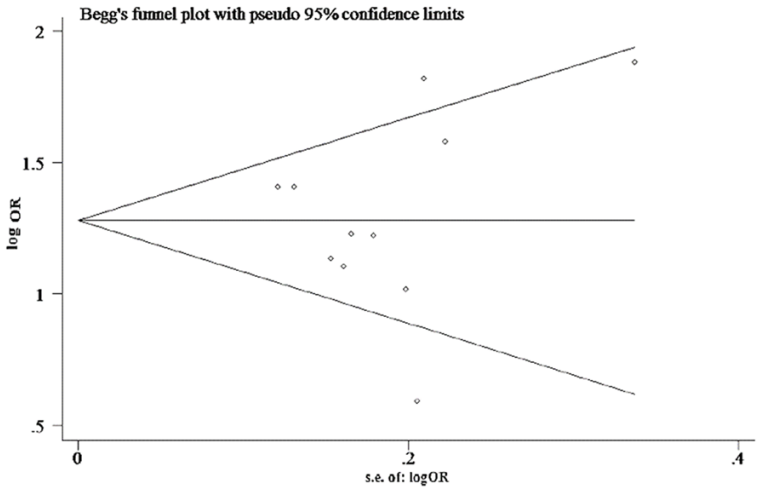


Figure 10. Begg's funnel plot for the publication bias on the association between G-395A SNP and CVDs risk in the dominant genetic model.

(case/control: 5035/4550) participants, 3 types of CVDs (CHD, IS and hypertension) and 4 Klotho gene SNPs (G-395A, C1818T, F352V and C370S). Studies judged as being of poor quality were excluded, while those deemed as being of middle to high quality with regard to their methodology were included within our analyses.

Currently, a considerable amount of attention has been focused on genetic susceptibility of CVDs and a number of studies have reported relationships between Klotho G395-A, C1818T, F352V and C370S and CVDs. Among the 4 Klotho gene SNPs, G-395A is located within the promoter area and its polymorphism has been reported to influence promoter function, while the G→A substitution has the potential to affect Klotho expression and thus change its physiological effects [6]. Findings from several studies have revealed that G-395A is an independent risk factor for CHD [6, 35]. In this meta-analysis we demonstrate that the G-395A polymorphism was significantly associated with the incidence of CVDs, principally CHD. In particular, those subjects with the 395A allele show an increased risk as compared with that of 395G carriers, who show no increase for the risk of CVDs. The remaining three Klotho SNPs (C1818T, F352V and C370S) are located on the Exon, and have been shown to be associated with CHD, atherosclerotic and hypertension. For the C1818T polymorphism, Rhee et al. reported that subjects with the allele T showed a lower prevalence of CAD than those with the CC genotype [47]. However, within studies conducted in China, the TT genotype was reported as a susceptibility factor for CHD [41]. You et al. reported that CC carriers of the Klotho C370S polymorphism were more likely to experience CHD, but they did not find any association between F352V and CVDs risk [22]. Our meta-analysis indicated that C1818T, F352V and C370S Klotho SNPs were not risk factors for CVDs. However, following sensitivity analysis, an association between CVDs and the F352V polymorphism was disclosed. It should be noted that results obtained in the 3 studies involved with examining F352V using sensitivity analysis were not consistent with HWE in the control group [12, 13, 38]. Accordingly, any conclusions regarding this relationship need to be considered with caution. It is well established that the Klotho gene has an important anti-

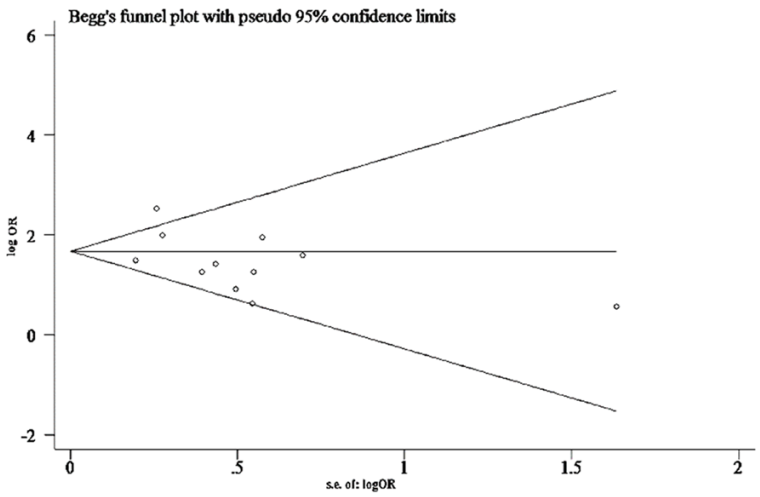


**Figure 11.** Begg's funnel plot for the publication bias on the association between G-395A SNP and CVDs risk in the regressive genetic model.

aging function, which may contribute to its protection of the cardiovascular system. However, SNPs of Klotho could alter this function and accelerate the aging process to then become a risk factor for CVDs.

It has been reported that the distribution of Klotho gene alleles show considerable ethnic variation. For example, Tavakkoly-Bazzaz reported that F352V and C370S SNPs show no associated risk of CHD in Iranians [9]; and the Klotho allele 395A, which is a risk allele for Chinese and Japanese, is a protective allele for Koreans [5, 13, 47]. In this study, the data were stratified by race and analyzed, but a subgroup analysis was only conducted on the participants from Korea and China carrying the Klotho G-395A SNP. A significant increase in risk was detected in Koreans with the additive genetic and dominant genetic models, while a statistically significant association for Han Chinese participants was obtained with the additive genetic, recessive genetic and dominant models, but only after sensitivity analysis. In Zhai's study, only the association between Klotho G-395A and the risk of CAD was tested. The meta-analysis performed in that study revealed that G-395A was a susceptible factor of CAD in East-Asia populations [14].

Three types of CVDs (CHD, IS and hypertension) were analyzed in this meta-analysis. Our results indicated that the G-395A and C1818T SNPs were risk factors for CHD risk, but no evidence was obtained for such a relationship involving the F352V and C370S SNPs. A relationship between hypertension and the G-395A, F352V and C370S SNPs was detected in the studies included within our analyses. A statistically significant relationship was obtained with the additive genetic and dominant genetic models for the F352V SNPs and with the recessive model for G-395A. Based on the results of our current meta-analysis, C370S does not appear to be a risk factor for CVDs, especially for hypertension. Kim et al. reported that there was no significant association between the risk of IS and the G-395A and C1818T SNPs, as determined within Koreans [11]. Similarly, Majumdar found that the C1818T polymorphism was not a risk factor for IS, but F352V could contribute to an early onset of stroke within Indian participants [10]. Our meta-analysis demonstrated that none of the four Klotho SNPs examined seem to contribute to IS.

To our knowledge, this is the first meta-analysis to examine relationships between the Klotho gene SNPs and the risk of CVDs. A comprehensive search using several databases was used to identify eligible articles. Subsequently, reference lists of included studies were then tracked and SIGLE databases were searched for other possible or unpublished articles. Two reviewers independently scanned the search output, extracted pertinent information, inputted the data and assessed the methodological quality of each included study in order to reduce the potential for bias.

Several limitations were present with regard to the methodological quality of the included studies. Some studies failed to describe the study design, source of participants in the control group, as well as the control of confounding factors while, others lacked a complete presentation of the data which precluded our attempts to combine results among the studies. Within the 20 studies, 16 of them from 5 countries provided useful data. However, detailed data

for meta-analysis were only available from several Asian countries, especially China. Accordingly, the findings presented may restrict the universality of these findings. In addition, small sample sizes within studies may be associated with insufficient power. Although the statistical heterogeneity was reduced following sensitivity analysis, some of the results obtained were altered, which suggests that conclusions resulting from these analyses require caution.

In summary, the results of our meta-analysis indicate that the G-395A and C1818T SNPs could be a risk factor for CHD, while the G-395A and F352V SNPs might be a risk for hypertension. No association between the risk of IS and the G-395A, F352V and C1818T SNPs could be established from our meta-analysis. The evidence for Klotho SNPs within different countries/ethnic groups remains unclear and requires further research.

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## Disclosure of conflict of interest

None.

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