Endothelial Nitric Oxide Synthase G894T Gene Polymorphism and Essential Hypertension in the Chinese Population: a Meta-Analysis Involving 11,248 Subjects

Li Yan-yan

Abstract

Background The endothelial nitric oxide synthase (eNOS) G894T gene polymorphism has been suggested to be linked to the risk of essential hypertension (EH), however the results are still debatable.

Objective and Methods To assess the association between eNOS G894T gene polymorphism and EH, such electronic databases as Pubmed, Embase, Web of Science, China Biological Medicine Database (CBMD) and China National Knowledge Infrastructure (CNKI) were searched. The selection criteria were as follows: a) Evaluation of the association of eNOS G894T gene polymorphism and EH. b) EH diagnosis in compliance with EH diagnosis criteria of the World Health Organization (WHO) in 1999. c) The study results were in line with the Hardy-Weinberg equilibrium (HWE). In 23 separate studies with 11,248 subjects the relation between eNOS G894T gene polymorphism and EH was analyzed by current meta-analysis. Random effect model was used to calculate the pooled odds ratio (ORs) and its corresponding 95% confidence interval (95% CI).

Results In this eNOS G894T gene polymorphism and EH meta-analysis in the Chinese population, the distribution of T allele frequency was 0.154 for EH group and 0.128 for the control group. A significant association was found between G894T gene polymorphism and EH (p=0.0007). The pooled OR for the distribution frequency of T allele was 1.33 (95% CI:1.13-1.56, P_{heterogeneity} <0.00001). In the stratified analysis by ethnicity, there was a significant association in Han subgroup (p=0.005). The pooled OR for the distribution frequency of T allele was 1.31 (95% CI:1.09-1.59, P_{heterogeneity} <0.00001). No significant increased risk for EH was found in the non-Han subgroup (p=0.08).

Conclusion In the current meta-analysis, T allele of eNOS G894T gene was suggested to be related to the increased risk of EH in the Chinese population, particularly in those of Han ethnicity.

Key words: endothelial nitric oxide synthase, gene polymorphism, G894T, hypertension, meta-analysis

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Introduction

Essential hypertension (EH) is a polygenic disease by the interaction of environment and heredity. The pathogenesis is complex involving nervous and humoral regulation. The effect of nitric oxide (NO) in regulating blood pressure has received more and more attention recently. NO is generated by nitric oxide synthase (NOS) catalyzing the substrates of L-arginine and oxygen molecule with the help of other cofactors. NO has a functions in vessel relaxation, inhibition of the vascular smooth muscle cell proliferation and blood pressure (BP) regulation.

Endothelial nitric oxide synthase (eNOS), which is a limiting-velocity factor for endothelial cells to produce NO, belongs to NOS family. The eNOS gene, located in 7q35-36, spans 21 kb with 26 exons and 25 introns. There are approximately 10 distributed polymorphism loci in promoter, exons and introns of eNOS gene. Among these loci, the mutation of guanine (G base) substituting for thymine (T base).
in the 894th base of the 7th exon of the eNOS gene results in the Glu being supplanted by Asp in the 298th of the corresponding amino acid sequence. This gene variant decreases NO production and subsequently influences the development of EH and atherosclerosis (1).

There has been a great divergence in the research results on the association of eNOS G894T gene polymorphism and EH. In 1998, Lacolley et al reported that the non-mutation G gene of eNOS G894T was the susceptible gene for EH in Caucasians of France (2). In contrast to the result, Miyamoto et al (3) and Yasujima et al found that T allelic gene of eNOS G894T was the risk factor for the EH in Japanese (4). However, Kato et al found that there was no significant association between eNOS G894T gene polymorphism and EH in Japanese (5).

The research results were different in the Chinese population. In 2009, Wang et al performed a meta-analysis involving 1,900 cases and 1,216 controls from 10 studies and found that 894G→T mutation in the eNOS appeared to be related to EH in Chinese Han population (6). Nevertheless, in 2010, Wang et al performed a meta-analysis with respect to eNOS gene polymorphism and EH of 8 studies and found a marginal association for allele contrast in Han Chinese (7). So that, whether there is an association of eNOS G894T gene polymorphism and EH or not still remains unclarified. It is well known that China is a multi-ethnic country and the work of Wang et al failed to present results separately by ethnicity. The present meta-analysis which included studies of poor research quality, providing little or insufficient data, violating the inclusion criteria, the repeated publications. If the same research result appeared in different articles, the result was only adopted once in the present meta-analysis. The data extracted from the studies included such details as the first author, publication year, region, ethnicity, number of genotypes, geno-typing method, study design, matching criteria, total number of cases and controls and weighting factors. All of the data are shown in Table 1.

Statistical methods

The odds ratio (OR) corresponding to the 95% confidence interval (95% CI) was used to assess the intensity of association between eNOS G894T gene polymorphism and EH. The heterogeneity assumption was checked by Chi-square-based Q-test (significance set at <p<0.05) (8). If heterogeneity existed among the studies, the pooled OR was estimated by the random-effects model (the DerSimonian and Laird method) (9). Alternately, the fixed-effects model was used to estimate the pooled OR (the Mantel-Haenszel method) (10). A random effects meta-regression of the logarithm OR was used to examine putative sources of heterogeneity. The restricted maximum likelihood approach was used to estimate the between-study variance (11). Publication year, ethnicity, control quantity total sample size, ratio of EH and control quantity were assigned as predictors. The Han ethnicity was set as 1 and the non-Han was set as 2.

The Hardy-Weinberg equilibrium (HWE) was assessed by Fisher's exact test (significance set at <p<0.10). The funnel plot was used to estimate the potential publication bias. The asymmetry of the funnel plot was assessed by Egger's linear regression test on the natural logarithm scale of the OR (significance set at <p<0.05) (12). The statistical analyses were performed by STATA 10.0 software (Stata Corp., College Station, TX).

Results

Materials and Methods

Publication search and inclusion criteria

All of the studies published in such electronic databases, such as PubMed, Embase, Web of Science, China Biological Medicine Database (CBMD), China National Knowledge Infrastructure (CNKI) were searched in the current meta-analysis using the 'MeSH terms' of 'hypertension', 'endothelial nitric oxide synthase' and 'polymorphism' (last research was updated on May 15, 2011) within a range of published years from 2002 to 2010. In order to identify the relevant publications, the references cited in the research papers were also scanned.

The data was included in the current meta-analysis if the studies met the following criteria: a) Evaluation of the association of eNOS G894T gene polymorphism and EH. b) The EH diagnosis complied with the 1999EH diagnosis criteria of the World Health Organization (WHO). Secondary hypertension was excluded from the present investigation. c) The study results satisfied the Hardy-Weinberg equilibrium (HWE).

Study characteristics and meta-analysis results

A total of 35 papers were reviewed, of which 23 papers were included in the present meta-analysis. Of the 12 excluded studies, 4 papers were reviews, 3 papers had nothing on the eNOS G894T gene polymorphism, 2 papers lacked control populations, and 3 papers were duplications. The total data of the 23 studies were gathered from 5,917 EH patients and 5,331 controls including 7 ethnic groups (Han, Mongolian, Kazakh, Dongxiang, Uygur, Hani and Yi). The
Table 1. Characteristics of the Investigated Studies of the Association between eNOS G894T Gene Polymorphism and Essential Hypertension

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Region</th>
<th>Ethnicity</th>
<th>Study design</th>
<th>Matching criteria</th>
<th>sample size (HP/control)</th>
<th>Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liu HZ[13]</td>
<td>2002</td>
<td>Hubei</td>
<td>Han</td>
<td>Case-control</td>
<td>Age, sex, ethnicity, BMI</td>
<td>103/74</td>
<td>3.67</td>
</tr>
<tr>
<td>Chen W[14]</td>
<td>2003</td>
<td>Guangxi</td>
<td>Han</td>
<td>Case-control</td>
<td>Age, sex, ethnicity</td>
<td>126/96</td>
<td>3.79</td>
</tr>
<tr>
<td>Jia CQ[15]</td>
<td>2003</td>
<td>Shandong</td>
<td>Han</td>
<td>Case-control</td>
<td>Age, sex, ethnicity, BMI</td>
<td>116/136</td>
<td>3.82</td>
</tr>
<tr>
<td>Zhan Y[16]</td>
<td>2003</td>
<td>Jiangsu</td>
<td>Han</td>
<td>Case-control</td>
<td>Age, sex, ethnicity, BMI</td>
<td>95/95</td>
<td>2.92</td>
</tr>
<tr>
<td>Li DB[17]</td>
<td>2004</td>
<td>Beijing</td>
<td>Han</td>
<td>Case-control</td>
<td>Age, sex, ethnicity</td>
<td>310/151</td>
<td>4.35</td>
</tr>
<tr>
<td>Tan JC[18]</td>
<td>2004</td>
<td>Chongqing</td>
<td>Han</td>
<td>Case-control</td>
<td>Age, sex, ethnicity</td>
<td>226/226</td>
<td>5.29</td>
</tr>
<tr>
<td>Mu HS[19]</td>
<td>2005</td>
<td>Chongqing</td>
<td>Han</td>
<td>Case-control</td>
<td>Age, sex, ethnicity</td>
<td>384/244</td>
<td>5.23</td>
</tr>
<tr>
<td>Zhao YX[20]</td>
<td>2006</td>
<td>Shandong</td>
<td>Han</td>
<td>Case-control</td>
<td>Age, sex, ethnicity, BMI</td>
<td>150/150</td>
<td>4.33</td>
</tr>
<tr>
<td>Liang Q[21]</td>
<td>2006</td>
<td>Guangdong</td>
<td>Han</td>
<td>Case-control</td>
<td>Age, sex, ethnicity</td>
<td>124/100</td>
<td>3.11</td>
</tr>
<tr>
<td>Wang Z[22]</td>
<td>2006</td>
<td>Liaoning</td>
<td>Han</td>
<td>Case-control</td>
<td>Ethnicity</td>
<td>277/547</td>
<td>4.96</td>
</tr>
<tr>
<td>Zhao F[23]</td>
<td>2006</td>
<td>Guangdong</td>
<td>Han</td>
<td>Case-control</td>
<td>Age, sex, ethnicity, BMI</td>
<td>150/70</td>
<td>3.46</td>
</tr>
<tr>
<td>Zhao Q[24]</td>
<td>2006</td>
<td>Beijing</td>
<td>Han</td>
<td>Case-control</td>
<td>Age, sex, ethnicity</td>
<td>501/489</td>
<td>5.51</td>
</tr>
<tr>
<td>Li DJ[25]</td>
<td>2009</td>
<td>Guangdong</td>
<td>Han</td>
<td>Case-control</td>
<td>Age, sex, ethnicity, BMI</td>
<td>235/240</td>
<td>4.26</td>
</tr>
<tr>
<td>Liu HZ[26]</td>
<td>2009</td>
<td>Hubei</td>
<td>Han</td>
<td>Case-control</td>
<td>Age, sex, ethnicity, BMI</td>
<td>129/117</td>
<td>4.31</td>
</tr>
<tr>
<td>Liu HZ[26]</td>
<td>2009</td>
<td>Hubei</td>
<td>Han</td>
<td>Case-control</td>
<td>Age, sex, ethnicity, BMI</td>
<td>129/117</td>
<td>4.31</td>
</tr>
<tr>
<td>Niu WQ[27]</td>
<td>2009</td>
<td>Beijing</td>
<td>Han</td>
<td>Case-control</td>
<td>Age, sex, ethnicity, BMI</td>
<td>130/154</td>
<td>6.10</td>
</tr>
<tr>
<td>Wang ZQ[28]</td>
<td>2009</td>
<td>Shandong</td>
<td>Han</td>
<td>Case-control</td>
<td>Age, sex, ethnicity</td>
<td>230/186</td>
<td>5.05</td>
</tr>
<tr>
<td>Zhou JZ[29]</td>
<td>2010</td>
<td>Xinjiang</td>
<td>Han</td>
<td>Case-control</td>
<td>Ethnicity</td>
<td>176/131</td>
<td>4.18</td>
</tr>
<tr>
<td>Zhao SG[31]</td>
<td>2008</td>
<td>Neimenggu</td>
<td>Mongolian</td>
<td>Case-control</td>
<td>Sex, ethnicity, BMI</td>
<td>174/112</td>
<td>5.89</td>
</tr>
<tr>
<td>Xu JF[32]</td>
<td>2004</td>
<td>Xinjiang</td>
<td>Kazakh</td>
<td>Case-control</td>
<td>Age, sex, ethnicity, BMI</td>
<td>203/190</td>
<td>2.38</td>
</tr>
<tr>
<td>Zhang HF[33]</td>
<td>2009</td>
<td>Gansu</td>
<td>Dongxiang</td>
<td>Case-control</td>
<td>Age, sex, ethnicity</td>
<td>349/214</td>
<td>5.56</td>
</tr>
<tr>
<td>Zhang LP[34]</td>
<td>2006</td>
<td>Xinjiang</td>
<td>Uygur</td>
<td>Case-control</td>
<td>Sex, ethnicity</td>
<td>375/414</td>
<td>2.50</td>
</tr>
<tr>
<td>Tang WR[35]</td>
<td>2008</td>
<td>Yunnan</td>
<td>Hani</td>
<td>Case-control</td>
<td>Age, sex, ethnicity</td>
<td>271/267</td>
<td>4.81</td>
</tr>
</tbody>
</table>

PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism
BMI: body mass index

Minority groups were analyzed and merged into the non-Han subgroup. The OR values were different among the 23 studies, some of which held out that T allele of G894T gene increased the EH risk. However, the results of other studies were opposed to this conclusion. Hence, these results had to be analyzed comprehensively to obtain a reasonable conclusion. The flow diagram of article selection process is shown in Fig. 1.

In this eNOS G894T gene polymorphism and EH meta-analysis, the distribution of T allelic frequency was 0.154 in the EH group and 0.128 for control group. Figure 2 showed the summary OR for distribution of T allelic frequency which was 1.33 (95% CI:1.13-1.56, P_{hetogeneity} <0.00001) by random effects model.

There was a significant difference between EH and control group (p=0.0007). The heterogeneity comparison was also significant (p<0.0001, I^2=73.3%). In the stratified analysis by ethnicity, no significant risks were found in the non-Han subgroup (p=0.08). There was a significant association in Han subgroup (p=0.005). The pooled OR for the distribution frequency of T allele was 1.31 (95% CI:1.09-1.59, P_{hetogeneity} <0.00001). The weighting factor of the Han subgroup possessed 74.37% and that of non-Han subgroup was 25.63% (Table 2).

Taking the significant heterogeneity into account, the meta-regression was readily conducted with the dependent variable logarithm OR. In meta-regression, the heterogeneity was closely related to the ‘publication year’ variable (p=0.012). By contrast, ethnicity, control group quantity, ratio of EH and control quantity or total sample size were not correlated with the heterogeneity (p>0.05) (Table 3).

The subsection analysis was performed by publication year in the Han ethnic group. All 17 studies of the Han ethnic group were divided into 2 subsections once again. The 12 studies published between 2002 and 2006 were defined as subsection 1 and other 5 studies published between 2006 and 2010 were referred to as subsection 2. The estimated pooled OR for the distribution frequency of T allele was 1.44 (95% CI:1.17-1.77, P_{hetogeneity} =0.005) in the subsection 1. Among the 5 studies of subsection 2, the estimated pooled OR was 1.06 (95% CI:0.71-1.59, P_{hetogeneity} <0.0001) (Table 4).
Figure 1. Flow diagram of articles selection process for endothelial nitric oxide synthase G894T gene polymorphism and EH risk meta-analysis.

Bias diagnostics

No publication bias was found visually from the funnel plot (Fig. 3). No significant difference was found in the Egger’s test which implied that there was a low publication bias in the current meta-analysis (p>0.05). There was no bias in HWE in the population.

Discussion

It has been discovered that the release of basal NO mediated by eNOS plays a key role in the regulation of BP and blood flow. The basal blood flow would be decreased 50% by inhibiting NO synthesis. On the other hand, the BP would be elevated by the inhibition or deficiency of eNOS and the suffocating of NO synthesis. Node et al found that the NO concentration in serum or urine of EH patients is lower than normal individuals (36). The reactivity of vessels to acetylcholine is decreased in the descendants of EH patients. This suggests that there is less release of basal NO and restricted endothelium-dependent diastolic function in the EH suffers. However, this phenomena could not be explained by lack of substrate, endothelial injury secondary to EH or the weakened effect of NO on peripheral vessels.

Based on the advances in eNOS gene polymorphism research, the restricted endothelium-dependent diastolic function was probably associated with eNOS gene mutation. In 1998, Yoshimura et al first reported that the Glu being supplanted by Asp in the 298th led to the alteration of α helix to tight fold which influenced the activity of eNOS and increased the EH risk (37). In 2008, Periaswamy et al carried out a case control study in 438 hypertensive patients and 444 healthy control subjects in a south Indian Tamilian population and found that the eNOS G894T gene polymorphism is a candidate gene for hypertension and the association is gender specific for females (38). In 2010, Sandrim et al examined whether eNOS G894T gene polymorphisms affected the therapeutic responses of women with gestational hypertension (GH) or preeclampsia (PE), they found that eNOS TT haplotype affected the responsiveness to antihypertensive therapy in PE in Brazil (39).

In this eNOS G894T gene polymorphism and EH meta-analysis, the distribution of T allelic frequency was 0.154 for EH group and 0.128 for control group. The summary
The pooled OR for the distribution frequency of T allele was a significant association in the Han subgroup (p=0.005). There were found in the non-Han subgroup (p=0.08). There was no significant difference between EH and control group (p=0.12). Other confounding factors, such as ethnicity, control group quantity, ratio of EH and control quantity or total sample size were not related to the heterogeneity (p>0.05). It was shown by the publication year subgroup analysis in the Han ethnic group that the pooled OR for the distribution frequency of T allele was 1.44 (95% CI:1.17-1.77, \( \phi_{\text{heterogeneity}} =0.005 \)) in the subsection 1 among the 12 studies published between 2002 and 2006. Among the 5 studies published between 2006 and 2010 of the subsection 2, the pooled OR was 1.06 (95% CI: 0.71-1.59, \( \phi_{\text{heterogeneity}}<0.0001 \)). It was indicated that in the EH patients compared to control individuals, T allele increased the EH risk 0.44 times in subsection 1 and 0.06 times in subsection 2. The subsection analysis result suggested that the heterogeneity in the subsection 2 was higher than in subsection 1 and the research quality of subsection 2 needed to be further improved.

The current research results were generally consistent with the two previous studies by Wang et al (6, 7), but there was still a difference between the present study and that of Wang et al because they only found a marginally significant association between eNOS G894T gene polymorphisms and...
Table 3. The Meta-regression Results among 23 Studies

<table>
<thead>
<tr>
<th>Item</th>
<th>Coefficient</th>
<th>Standard Error</th>
<th>T value</th>
<th>p value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Publication year</td>
<td>-0.0498115</td>
<td>0.0177683</td>
<td>-2.8</td>
<td>0.012</td>
<td>-0.0872994 to -0.0123237</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>0.1334667</td>
<td>0.0898598</td>
<td>1.49</td>
<td>0.156</td>
<td>-0.0561408 to 0.3230343</td>
</tr>
<tr>
<td>Control quantity</td>
<td>-0.0010565</td>
<td>0.0015102</td>
<td>-0.7</td>
<td>0.494</td>
<td>-0.0042428 to 0.0021297</td>
</tr>
<tr>
<td>Total sample size</td>
<td>0.0005053</td>
<td>0.0007197</td>
<td>0.7</td>
<td>0.492</td>
<td>-0.0010131 to 0.0020237</td>
</tr>
<tr>
<td>Ratio of EH and control quantity</td>
<td>-0.213995</td>
<td>0.1583178</td>
<td>-1.35</td>
<td>0.194</td>
<td>-0.5480165 to 0.1200264</td>
</tr>
<tr>
<td>Summation</td>
<td>100.1838</td>
<td>35.60323</td>
<td>2.81</td>
<td>0.012</td>
<td>25.06753 to 175.3</td>
</tr>
</tbody>
</table>

*: p<0.05

Table 4. Summary of Subsection Analysis Stratified by Publication Year in Han Ethnicity

<table>
<thead>
<tr>
<th>Subsection by publication year</th>
<th>Literature number</th>
<th>Weight (%)</th>
<th>Pooled OR (95% CI)</th>
<th>Z(P)</th>
<th>F(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subsection 1: 2002-2006</td>
<td>12</td>
<td>50.45</td>
<td>1.44 (1.17–1.77)</td>
<td>3.46</td>
<td>58.8</td>
</tr>
<tr>
<td>subsection 2: 2009-2010</td>
<td>5</td>
<td>23.91</td>
<td>1.06 (0.71–1.59)</td>
<td>0.29</td>
<td>83.4</td>
</tr>
<tr>
<td>whole Han population</td>
<td>17</td>
<td>74.36</td>
<td>1.31 (1.09–1.59)</td>
<td>2.83</td>
<td>73.3</td>
</tr>
</tbody>
</table>

Figure 3. Funnel plot for studies of the association of essential hypertension and eNOS G894T gene polymorphism (distribution of T allelic frequency of eNOS G894T). The horizontal and vertical axis correspond to the OR and confidence limits. OR: odds ratio, SE: standard error

EH (p=0.01) (7). The differing results were probably involved with the insufficient selection studies number. In the current meta-analysis, 17 studies published from 2002 to 2010 were included for the Han subgroup and a significant association was found between them (p=0.005). In contrast, only 8 studies published from 2002 to 2009 were eligible for the research of Wang et al. That is to say, the conclusion currently reached should be more objective and reasonable than the others.

The limitations for the differing research results might include the following aspects: 1) the racial difference. There was a rather great difference in the eNOS G894T genotype distribution frequency among the different racial lines. 2) The basic character of the population as the environmental factor and lifestyle, for example, the body mass index (BMI). In 2010, Souza-Costa et al found that the eNOS haplotype, Glu of Glu298Asp was associated with hypertension in obese children and adolescents of Brazil (40). 3) Other factors, such as the research methods, the statistical methods and the sample size. In the current meta-analysis, the non-Han subgroup sample size was relatively small and the corresponding result might be indistinct. Further studies involving a larger sample size would be warranted.

In conclusion, the current meta-analysis suggested that T allele of eNOS G894T gene polymorphism was involved with the susceptibility of EH, particularly in the Han ethnic group. It is worthy to mention that in consideration of the age, sex, family history, environmental factors, eNOS gene expression, serum eNOS level, the investigation sample size should be further expanded. Due to the mentioned limitations, further research should be performed soon to verify the above inference.

The authors state that they have no Conflict of Interest (COI).

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