Apolipoprotein A5 Gene Promoter Region-1131T/C Polymorphism Is Associated with Risk of Ischemic Stroke and Elevated Triglyceride Levels: A Meta-Analysis

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Key Words
Apolipoprotein A5 · Ischemic stroke · Triglyceride · Polymorphism · Meta-analysis

Abstract

Background: The association between polymorphism -1131T/C in the promoter region of apolipoprotein A5 (APOA5) and ischemic stroke and plasma triglyceride (TG) levels remains controversial. To better clarify the association between APOA5-1131T/C and risk of ischemic stroke and plasma TG levels, we performed a meta-analysis to examine the allele and genotype of APOA5-1131T/C polymorphism in ischemic stroke cases and controls. Methods: Based on the search of PubMed, Embase, MEDLINE, CNKI (National Knowledge Infrastructure) and CBM (Chinese BioMedical Literature Database) databases, we identified and abstracted outcome data from all articles to evaluate the association between APOA5 and ischemic stroke/plasma TG levels. The pooled odds ratios (ORs) with 95% confidence intervals (CIs) were performed in dominant model (CC + TC vs. TT), recessive model (CC vs. TC + TT), homozygote comparison (CC vs. TT) and heterozygote comparison (TC vs. TT). The association between dominant model (CC + TC vs. TT) and plasma TG/total cholesterol/high-density lipoprotein cholesterol levels was measured by a weighted mean difference (WMD) with its corresponding 95% CI. To evaluate the ethnicity-specific effects, subgroup analyses were performed by ethnic group. Results: A meta-analysis containing 2,294 ischemic stroke cases and 1,858 controls from 8 case-control studies was performed. The results showed that APOA5-1131T/C polymorphism was significantly associated with ischemic stroke in all comparison models (CC + TC vs. TT, OR = 1.70, 95% CI = 1.24–2.32; CC vs. TC + TT, OR = 1.36, 95% CI = 0.98–1.90; CC vs. TT, OR = 1.73, 95% CI = 1.34–2.23; TC vs. TT, OR = 1.67, 95% CI = 1.19–2.36). On subgroup analysis by ethnicity, similarly significant associations were found in both Asians and Europeans, and the Europeans possessed a higher risk of ischemic stroke, especially in CC versus TT model (OR = 4.47, 95% CI = 1.33–15.06). Significant association between the C allele and elevated TG levels was detected in both ischemic stroke cases and controls; the TG levels were higher in the ischemic stroke cases and controls carrying the APOA5-1131C allele than in the noncarriers (CC + TC vs. TT, cases WMD = 0.43, 95% CI = 0.27–0.59; controls WMD = 0.51, 95% CI = 0.35–0.66). Similar within-group comparison of the total cholesterol and high-density lipoprotein cholesterol levels did not show any difference. Conclusions: Our meta-analysis revealed that the APOA5-1131T/C polymorphism is associated

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with a significant risk of ischemic stroke and elevated TG levels. The CC genotype and C allele might be a genetic risk factor that increases susceptibility of ischemic stroke and elevates plasma TG levels, and might be a useful target for clinical therapeutic intervention.

Introduction

Stroke is the third leading cause of death and the leading cause of long-term disability worldwide [1]. The common sporadic form of ischemic stroke is underpinned by environmental and genetic risk factors [2]. Although environmental and clinical risk factors (such as hypertension, diabetes mellitus, hyperlipemia, atherosclerosis, smoking, excessive alcohol drinking, and so on) are considered important [3, 4], nowadays a growing spectrum of genetic susceptibility factors have been identified [5, 6]. With the help of robust genome-wide association studies, the biological plausibility and epidemiological evidence suggest that the elevated triglyceride (TG) level potentially contributes to an increased risk for ischemic stroke [7–9].

In 2001, a new apolipoprotein gene, apolipoprotein A5 (APOA5), was first reported [10] and found to be strongly associated with plasma TG levels [11]. The APOA5 gene is located on chromosome 11q23 and presents in the APOA1-C3-A4 gene cluster. Human and mouse data consistently show that APOA5 is a factor that apparently reduces plasma TG levels [10]. Compared with controls, transgenic mice overexpressed human APOA5 and produced one third lower plasma TG levels, while APOA5 knockout mice had fourfold higher plasma TG levels. APOA5-1131T/C, as a polymorphism site in the promoter region of APOA5, has been found to modify the susceptibility of ischemic stroke and shown to be associated with elevated TG levels [12–19].

A variety of molecular epidemiological studies have focused on the association between APOA5-1131T/C polymorphism and ischemic stroke and TG levels. However, results in different studies have been inconsistent. Two meta-analysis articles on the association between APOA5-1131T/C and coronary artery disease have just been performed [20, 21], but currently there is no meta-analysis article on APOA5-1131T/C and ischemic stroke. To draw a more stable and reliable conclusion about the association between APOA5-1131T/C and risk of ischemic stroke and plasma TG levels, we performed a meta-analysis to examine the allele, genotype of APOA5-1131T/C polymorphism in cases and controls.

Materials and Methods

Literature Search

Two independent investigators screened an electronic literature from PubMed, Embase, MEDLINE, CNKI (National Knowledge Infrastructure) and CBM (Chinese BioMedical Literature Database) databases up to 2011. The initial search used the MeSH terms ‘apolipoprotein A5’ OR ‘APOA5’, ‘ischemic stroke’ OR ‘cerebral infarction’ OR ‘stroke’ in combination with ‘polymorphism’ OR ‘variant’ OR ‘mutation’. We reviewed the bibliographies of all selection articles to identify additional relevant studies.

Inclusion and Exclusion Criteria

The inclusion criteria were: (1) case-control studies to evaluate the association between APOA5-1131T/C and risk of ischemic stroke, (2) useful data including genotype number or frequency given, (3) studies clearly describe ischemic stroke diagnoses and the sources of cases and controls, (4) studies written in English and Chinese with full-text, (5) genotype distribution of controls in Hardy-Weinberg equilibrium (HWE). The exclusion criteria were: (1) not case-control studies, (2) studies without available genotype number or frequency, (3) genotype distribution of controls not in HWE, (4) animal studies, reviews, case reports, abstracts and repeated literature.

Data Extraction

Two independent investigators extracted the following data: first author, year of publication, characteristics of cases and controls (age, gender, body mass index, total cholesterol (TC) levels, high-density lipoprotein cholesterol (HDL-C) levels and TG levels), study population, ethnicity, genotyping methods and genotype number in cases and controls. The results were compared, and disagreements were resolved by consensus. Different ethnicity descents were categorized as Asians and Europeans.

Statistical Analysis

Pooled odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were used to assess the association between the polymorphism APOA5-1131T/C and risk of ischemic stroke. Four different genetic models, including dominant model (CC + TC vs. TT), recessive model (CC vs. TC + TT), homozygote comparison (CC vs. TT) and heterozygote comparison (TC vs. TT), were estimated in our analysis. The association between dominant model (CC + TC vs. TT) and plasma TG/TC/HDL-C levels was measured by a weighted mean difference (WMD) with its corresponding 95% CI. Heterogeneity assumption was assessed by the Q test and I² statistics. If the result of the Q test was $p_0 \geq 0.1$ and $I^2 < 50\%$, the fixed-effects model was used to calculate the pooled ORs [22]; otherwise, the random-effects model was used [23]. Moreover, Galbraith plot was used to detect the potential sources of heterogeneity. To evaluate the ethnicity-specific effects, subgroup analyses were performed by ethnic group. Publication bias was tested by Begg’s funnel plot and Egger’s test [24, 25]. All analyses were performed using Review Manager 5.0 and Stata 10.0. Statistical significance was set at two-sided $p < 0.05$. 

A5-1131T/C Polymorphism Is Associated with Ischemic Stroke and TG Level
Results

Study Characteristics

The initial search identified fifty potentially relevant studies, eight of which met the inclusion criteria in the final, including 2,294 ischemic stroke cases and 1,858 controls (fig. 1). The main characteristics of the studies are summarized in table 1. Of the eight studies, five were conducted in Asians and three in Europeans. Six studies (1,828 cases and 1,231 controls) were available for the meta-analysis of APOA5-1131T/C polymorphism and TG levels [12, 13, 16–19].

All the cases of ischemic stroke were diagnosed based on the WHO criteria. Most of the controls received routine health examinations. In all of the selected studies, the genomic DNA was extracted from peripheral blood samples and the gene polymorphisms were detected by polymerase chain reaction restriction fragment length polymorphism. In all of the included studies, the /H9273 2 test was used to compare the different genotypes between groups, and the ORs derived from multiple logistic regression were used to evaluate the impact of the APOA5 -1131T/C genotype on the risk of ischemic stroke at 95% CIs. Three of six studies used the Mann-Whitney test to assess the different TG levels between groups. In the remaining three studies, the unpaired Student’s t test was used.

Meta-Analysis Results

The meta-analyses suggested that APOA5-1131T/C polymorphism was significantly associated with ischemic stroke in all comparison models (CC + TC vs. TT, OR = 1.70, 95% CI = 1.24–2.32; CC vs. TC + TT, OR = 1.36, 95% CI = 0.98–1.90; CC vs. TT, OR = 1.73, 95% CI = 1.34–2.23; TC vs. TT, OR = 1.67, 95% CI = 1.19–2.36). Forest plots on the basis of all studies are shown in figure 2. The results showed that the carriers of the C allele have higher risk of ischemic stroke than the noncarriers.

On subgroup analysis by ethnicity of study population, significant association between the C allele and ischemic stroke risk was detected in both Asians and Europeans. Compared with Asians, the Europeans had a higher risk of ischemic stroke, especially in CC versus TT (OR = 4.47, 95% CI = 1.33–15.06; table 2).

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

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Ethnicity</th>
<th>Genotyping method</th>
<th>Males in cases/controls, %</th>
<th>Mean age of cases/controls, years</th>
<th>IS cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Havasi et al.</td>
<td>Hungary</td>
<td>European</td>
<td>PCR-RFLP</td>
<td>52.98/54.33</td>
<td>NG/54.6±13.3</td>
<td>TT: 60 5 302</td>
<td>TT: 261 27s</td>
</tr>
<tr>
<td>Xu et al.</td>
<td>China</td>
<td>Asian</td>
<td>PCR-RFLP</td>
<td>60.96/60.50</td>
<td>64.58±11.83/61.85±12.27</td>
<td>99 40 14 153</td>
<td>105 77 18 200</td>
</tr>
<tr>
<td>Zhang et al.</td>
<td>China</td>
<td>Asian</td>
<td>PCR-RFLP</td>
<td>60.44/60.13</td>
<td>65.39±10.94/61.17±10.17</td>
<td>130 115 27 272</td>
<td>177 123 16 316</td>
</tr>
<tr>
<td>Li et al.</td>
<td>China</td>
<td>Asian</td>
<td>PCR-RFLP</td>
<td>63.02/65.46</td>
<td>63.92±9.90/62.25±9.61</td>
<td>77 63 54 194</td>
<td>172 77 62 311</td>
</tr>
<tr>
<td>Maasz et al.</td>
<td>Hungary</td>
<td>European</td>
<td>PCR-RFLP</td>
<td>39.68/31.30</td>
<td>66.1±0.74/58.1±1.51</td>
<td>309 58s 11s 378</td>
<td>119 11s 1s 131</td>
</tr>
<tr>
<td>Chen et al.</td>
<td>China</td>
<td>Asian</td>
<td>PCR-RFLP</td>
<td>39.68/31.30</td>
<td>67.76±10.86/65.54±11.10</td>
<td>43 76s 21s 140</td>
<td>60 43s 26s 129</td>
</tr>
<tr>
<td>Járomi et al.</td>
<td>Hungary</td>
<td>European</td>
<td>PCR-RFLP</td>
<td>40.35/28.49</td>
<td>65.1±0.62/56.5±2.20</td>
<td>434 68 11 513</td>
<td>159 12 1 172</td>
</tr>
<tr>
<td>Li et al.</td>
<td>China</td>
<td>Asian</td>
<td>PCR-RFLP</td>
<td>55.56/56.13</td>
<td>65.35±10.52/63.87±11.23</td>
<td>125 181 36 342</td>
<td>167 115 28 310</td>
</tr>
</tbody>
</table>

PCR-RFLP = Polymerase chain reaction restriction fragment length polymorphism; NG = not given; IS = ischemic stroke.

a Calculated value.

Fig. 1. Flowchart of the current study selection.
Significant association between the C allele and elevated TG levels was detected in both ischemic stroke cases and controls (1,828 cases and 1,231 controls). The TG levels were higher in the ischemic stroke cases and controls carrying the APOA5-1131C allele than in the noncarriers (CC + TC vs. TT, cases WMD = 0.43, 95% CI = 0.27–0.59; controls WMD = 0.51, 95% CI = 0.35–0.66; fig. 3). Similar within-group comparison of the TC and HDL-C levels did not show any difference.

Heterogeneity Analysis
The heterogeneity of CC versus TT, TC versus TT, CC + TC versus TT and CC versus TC + TT was analyzed for eight case-control studies. The results in CC versus TT (p = 0.28, I^2 = 19) and CC versus TC + TT (p = 0.17, I^2 = 33) had no heterogeneity, the other two models (TC vs. TT, p = 0.0001, I^2 = 77; CC + TC vs. TT, p = 0.0001, I^2 = 76) indicated significant heterogeneity. To detect the source of heterogeneity, we performed subgroup analyses stratified by the characteristics of the
When classified by ethnicity, the heterogeneity in the Europeans was ruled out. We determined that ethnicity might substantially influence the initial heterogeneity. By using Galbraith plot, one study [11] was identified as the main contributor of heterogeneity on TC versus TT and CC versus TC + TT (fig. 4). After excluding the outlier study, the heterogeneity was effectively removed or decreased. The pooled ORs were recalculated after removal of the outlier study, and the pooled ORs were similar to those when the outlier study was included (fig. 5).

Table 2. Subgroup analysis of APOA5-1131T/C polymorphism and risk of ischemic stroke in four genetic models

<table>
<thead>
<tr>
<th>Category</th>
<th>CC + TC versus TT</th>
<th>CC versus TC + TT</th>
<th>CC versus TT</th>
<th>TC versus TT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>I^2</td>
<td>OR (95% CI)</td>
<td>I^2</td>
</tr>
<tr>
<td>Overall</td>
<td>1.70 (1.24, 2.32)</td>
<td>76</td>
<td>1.38 (1.08, 1.75)</td>
<td>33</td>
</tr>
<tr>
<td>Asians</td>
<td>1.45 (0.97, 2.18)</td>
<td>83</td>
<td>1.28 (1.00, 1.64)</td>
<td>42</td>
</tr>
<tr>
<td>Europeans</td>
<td>2.36 (1.70, 3.28)</td>
<td>0</td>
<td>4.09 (1.22, 13.72)</td>
<td>0</td>
</tr>
</tbody>
</table>

Publication Bias
Publication bias was assayed by funnel plot inspection and Egger’s test. The shape of the funnel plot did not reveal obvious asymmetry in dominant model (CC + TC vs. TT). Then, Egger’s test was used to provide statistical evidence of funnel plot symmetry. The results still did not indicate any evidence of publication bias (p = 0.721 for CC + TC vs. TT).

Fig. 3. Forest plot for APOA5-1131T/C polymorphism (CC + TC vs. TT) and TG levels in both ischemic stroke cases and controls.
Ischemic stroke is a multifactorial and polygenic disorder disease which is thought to be the result of complex gene-gene and gene-environment interactions. Both environmental and common genetic factors lead to the development of ischemic stroke. As demonstrated by previous studies, the elevated TG level may be an independent risk factor for ischemic stroke. Human and animal data consistently show that the newly identified APOA5 gene may play an important role in the development of ischemic stroke and TG metabolism.

The association between APOA5 polymorphisms (T-1131C, IVS3+G476A, C56G, and T1259C) and the risk of ischemic stroke has been investigated, but the limited number of studies and small sample sizes make it difficult to draw definitive conclusions. However, the findings from these studies suggest that the T-1131C polymorphism may be associated with an increased risk of ischemic stroke.

**Fig. 4.** Galbraith plot of APOA5-T1131C polymorphism and risk of ischemic stroke in dominant model and heterozygote comparison. 

- The study by Xu et al. [11] was the outlier in dominant model (CC + TC vs. TT).
- The study by Xu et al. [11] was the outlier in heterozygote comparison (TC vs. TT).

**Fig. 5.** Forest plot for APOA5-T1131C polymorphism and risk of ischemic stroke after excluding the outlier study in dominant model and heterozygote comparison. 

- Dominant model (CC + TC vs. TT).
- Heterozygote comparison (TC vs. TT).

**Discussion**

Ischemic stroke is a multifactorial and polygenic disorder disease which is thought to be the result of complex gene-gene and gene-environment interactions. Both environmental and common genetic factors lead to the development of ischemic stroke. As demonstrated by previous studies, the elevated TG level may be an independent risk factor for ischemic stroke. Human and animal data consistently show that the newly identified APOA5 gene may play an important role in the development of ischemic stroke and TG metabolism.

The association between APOA5 polymorphisms (T-1131C, IVS3+G476A, C56G, and T1259C) and the risk of ischemic stroke has been investigated, but the limited number of studies and small sample sizes make it difficult to draw definitive conclusions. However, the findings from these studies suggest that the T-1131C polymorphism may be associated with an increased risk of ischemic stroke.
data are available about the role of some polymorphisms (IVS3+G476A, C56G, and T1259C) [16, 30]. APOA5-
1131T/C, as one polymorphism site of APOA5, has been widely studied in ischemic stroke susceptibility. However,
inconclusive results have been obtained. Some studies supported the conclusion that risk for ischemic stroke was associated with the polymorphism, whereas other studies drew converse conclusions. To better explain the association between APOA5-1131T/C and risk of ischemic stroke and plasma TG levels, we performed a meta-analysis to examine the allele and genotype of APOA5-1131T/C polymorphism in cases and controls.

The results of our meta-analysis confirmed that the CC genotype and C allele of the APOA5-1131T/C polymorphism could increase the risk of ischemic stroke and elevate plasma TG levels. Compared with TT subjects, the CC had a 73% increased risk of ischemic stroke, the TC had a 67% increased risk, and the C carriers (CC + TC) had a 70% increased risk. As far as TG levels are concerned, there were 45 and 51% increased risks for C carriers (CC + TC) compared with TT subjects in both ischemic stroke cases and controls, respectively. This association was still significant after excluding the outlier study. When stratifying by ethnicity, we obtained similar results in both Asians and Europeans. Moreover, the association was more significant in Europeans. Thus, the C allele might be a genetic risk factor that increases susceptibility to ischemic stroke and elevates plasma TG levels; this finding is consistent with the results of previous studies [17, 19].

In view of the complex effect of genetic polymorphisms on disease progression, the mechanism behind the association between the APOA5-1131T/C polymorphism and ischemic stroke risk may be that the APOA5-1131T/C polymorphism influences the function of the protein transcript, which could modify secondarily the interaction of APOA5 with the lipoprotein lipase and ultimately lead to increased circulating TG levels [10, 31, 32]. This process can be involved in the abnormal accumulation of lipids in the endothelial cells under pathologic conditions, eventually in the formation of atherogenic plaques that are elements in obstructive vascular diseases (such as ischemic stroke) [33, 34]. Prieur et al. [35] identified a peroxisome proliferator response element (PPRE) in the promoter region of the APOA5 gene and demonstrated that the APOA5 gene is a highly responsive peroxisome proliferator-activated receptor-α (PPARα) target gene. The APOA5 gene expression could be enhanced markedly by fibrates through the PPARα pathway, whose signal transmission required the participation of the PPRE. Considering this, it is hypothesized that APOA5-1131T/C might change the affinity of this or the binding of other unknown regulatory elements, leading to reductions of gene expression. The presence of naturally existing APOA5-1131T/C confers a risk for ischemic stroke through elevating plasma TG levels [29, 30].

Some limitations of our meta-analysis should be noted. First, the small number of studies and sample size limited the ability to draw more solid conclusions. So, more convincing evidence, such as larger sample size, number of studies, and ethnicity, is required to confirm our conclusions. Second, several human APOA5 gene single-nucleotide polymorphisms have been identified; we only included the APOA5-1131T/C polymorphism in this meta-analysis because this polymorphism was most extensively studied. Meta-analyses that investigate the association of other polymorphisms of the APOA5 gene with ischemic stroke and plasma TG levels should be performed later. Third, the influence of bias in this analysis could not be completely excluded; as it was very difficult to get full papers published in various languages, we only included the studies published in English and Chinese. Moreover, ischemic stroke is known to be a complex disease, and the gene-environment interactions should be considered.

In spite of the limitations, our meta-analysis has some key advantages. First, to the best of our knowledge, this is the first meta-analysis to explain the association between APOA5-1131T/C polymorphism and ischemic stroke. The results should be more reliable than those from a single study, as cases and controls were pooled from different studies, and statistical power of analysis was significantly increased. Second, no publication bias was found. After excluding one outlier study, the heterogeneity was effectively removed or decreased, no significant changes and reversal of results were found, which suggested the result of the present meta-analysis was stable and reliable.

In conclusion, our meta-analysis has revealed that the -1131T/C polymorphism in the promoter region of the APOA5 gene was associated with a significant risk of ischemic stroke and elevated plasma TG levels. The CC genotype and C allele may have a promoting effect on ischemic stroke and elevated TG levels. These findings highlighted the importance of the APOA5 gene in ischemic stroke and plasma TG levels, which might be a useful target for clinical therapeutic intervention.
Acknowledgments

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References


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Disclosure Statement

None.