The Influence of OLR1 and PCSK9 Gene Polymorphisms on Ischemic Stroke: Evidence from a Meta-Analysis

Anthony Au1, Lyn R. Griffiths2, Kian-Kai Cheng1,3, Cheah Wee Kooi4, Looi Irene5 & Loo Keat Wei6,7

Both OLR1 and PCSK9 genes are associated with atherosclerosis, cardiovascular disease and ischemic stroke. The overall prevalence of PCSK9 rs505151 and OLR1 rs11053646 variants in ischemic stroke were 0.005 and 0.116, respectively. However, to date, association between these polymorphisms and ischemic stroke remains inconclusive. Therefore, this first meta-analysis was carried out to clarify the presumed influence of these polymorphisms on ischemic stroke. All eligible case-control and cohort studies that met the search terms were retrieved in multiple databases. Demographic and genotyping data were extracted from each study, and the meta-analysis was performed using RevMan 5.3 and Metafor R 3.2.1. The pooled odd ratios (ORs) and 95% confidence intervals (CIs) were calculated using both fixed- and random-effect models. Seven case-control studies encompassing 1897 cases and 2119 controls were critically evaluated. Pooled results from the genetic models indicated that OLR1 rs11053646 dominant (OR = 1.33, 95% CI:1.11–1.58) and co-dominant models (OR = 1.24, 95% CI:1.02–1.51) were significantly associated with ischemic stroke. For the PCSK9 rs505151 polymorphism, the OR of co-dominant model (OR = 1.36, 95% CI:1.01–1.58) was found to be higher among ischemic stroke patients. In conclusion, the current meta-analysis highlighted that variant allele of OLR1 rs11053646 G > C and PCSK9 rs505151 A > G may contribute to the susceptibility risk of ischemic stroke.

Ischemic stroke is a heterogeneous group of neurovascular diseases and contributes to major morbidity and mortality in both developed and developing countries. The major risk factors for ischemic stroke such as obesity, diabetes mellitus, hypertension, hypercholesterolemia, dyslipidemia and atherosclerosis are well established. Nevertheless, the mechanism of ischemic stroke has not been fully elucidated and may involve a complex interplay between environmental and genetic factors, such as polymorphic variants of the genes that regulate cholesterol and lipid biosynthesis or degradation. Emerging lines of evidence revealed that lectin-like oxidized-low density lipoprotein receptor-1 (LOX-1) and proprotein convertase subtilisin/kexin 9 (PCSK9) play critical roles in hyperlipidemia and atherogenesis development, that ultimately leads to ischemic stroke.

LOX-1 is one of the major scavenger receptor for oxidized low density lipoprotein (ox-LDL), which encoded by human oxidized low density lipoprotein receptor 1 (OLR1) gene. This receptor protein mediates the recognition, internalization and degradation of ox-LDL. It is known that ox-LDL plays an important role during atherogenesis, by inducing vascular endothelial cell activation and dysfunction, results in pro-inflammatory responses, oxidative stress, necrosis and apoptosis. Endothelial cells apoptosis leads to an increased vascular permeability to cells and lipids, smooth muscle cell proliferation, increased coagulation and lipid accumulation, thus contributes to the

1Institute of Bioproduct Development and Department of Bioprocess Engineering, Faculty of Chemical Engineering, Universiti Teknologi Malaysia, 81300 Johor, Malaysia. 2Genomics Research Centre, Institute of Health and Biomedical Innovation, Queensland University of Technology, Musk Avenue, Kelvin Grove, QLD 4059, Australia. 3Innovation Centre in Agritechnology, Universiti Teknologi Malaysia, 81300 Johor, Malaysia. 4Department of Medicine, Taiping Hospital, Jalan Tamingsari, 34000 Taiping, Perak, Malaysia. 5Medical Department and Clinical Research Centre, Hospital Seberang Jaya, Jalan Tun Hussein Onn, 13700 Seberang Jaya, Pulau Pinang, Malaysia. 6Centre for Biodiversity Research, Universiti Tunku Abdul Rahman, Bandar Barat, 31900 Kampar, Perak, Malaysia. 7Department of Biological Science, Faculty of Science, Universiti Tunku Abdul Rahman, Bandar Barat, 31900 Kampar, Perak, Malaysia. Correspondence and requests for materials should be addressed to L.K.W. (email: wynnelkw@gmail.com)
development of atherosclerosis. It is suspected that LOX-1 upregulation may halt and reverse the atherosclerotic lesions, through the binding, endocytosis, and proteolytic degradation of oxLDL.

The OLR1 gene consists of six exons and five introns that spans over 7-kb, which located on chromosome 12p13.1-p12.3. Several single nucleotide polymorphisms (SNPs) in the OLR1 gene have been identified, including a c.501G>C transversion on exon 4, which results in an amino acidic substitution from lysine to asparagine at position 167 (p.K167N). This SNP was found to decrease binding and internalization of ox-LDL and has been associated with hypertension, myocardial infarction and carotid atherosclerosis. More importantly, this SNP is statistically linked to the risk of ischemic stroke, but discrepancies still exist between different populations.

PCSK9 was formerly known as neural apoptosis-regulated convertase 1 and characterized as the ninth member of the subtilisin family of kexin-like proconvertases. PCSK9 plays an essential role in the proteolytic maturation of several secretory proteins such as neuropeptides, growth factors, cytokines and pro-hormones. The PCSK9 plays an important role in modulating the plasma levels of low density lipoprotein cholesterol (LDL-C) through a post-transcriptional mechanism. PCSK9 binds to low density lipoprotein receptor (LDLR) and disrupts its endocytic recycling or directs it for lysosomal degradation. Therefore, PCSK9 activation can downregulate LDLR expression and inhibit the uptake of LDL-C, which in turns leading to hypercholesterolemia and ischemic stroke event.

The PCSK9 gene is located on chromosome 1p32.3 and is 22-kb in length. It comprises of 12 exons and 11 introns, which encodes for 692 amino acids. Previous studies have investigated the relationship between PCSK9 SNPs and their changes in circulating LDL-C levels. A common SNP - 23968A>C in exon 12, results in an amino acid substitution from glutamate to glycine at position 670 (p.E670G), is potentially associated with the altered enzyme activity of PCSK9. Moreover, this SNP has been reported to be associated with the risk of ischemic stroke, but the positive significance towards ischemic stroke event needs to be confirmed.

Both OLR1 and PCSK9 are positively linked, where the inhibition of PCSK9 can suppress the development of atherosclerosis by disrupting LOX-1 expression. Thus far, no meta-analysis has yet been conducted to investigate the relationship between OLR1 rs11053646 and PCSK9 rs505151 polymorphisms and ischemic stroke.

Results

Studies selection and characteristics. With regard to OLR1 rs11053646 and PCSK9 rs505151 polymorphisms, 84 and 176 studies were identified from the initial search (Fig. 1). Of these, six articles and one thesis were found to be related to the association between the studied polymorphisms and the risk of ischemic stroke. As for OLR1 rs11053646, one study was excluded due to insufficient information while another study was reporting on polymorphism other than our interest (Fig. 1). For PCSK9 rs505151, three studies were excluded due to (i) multiple studies from the same author (n = 1), (ii) insufficient information (n = 1), and (iii) study that reporting on polymorphism other than our interest (n = 1). No additional eligible article was found despite performing the extensive manual search on the references cited in the eligible publications and review articles. Therefore, a total of four (OLR1 rs11053646) and three articles (PCSK9 rs505151) encompassing 1138 and 759 cases as well as 1213 and 906 controls that met the inclusion criteria were included in the final meta-analysis model (Fig. 1). The detailed characteristics of all the selected studies were presented in Table 1. Meanwhile, the distribution of allele and genotype for each individual study were demonstrated in Table 2.

Quantitative synthesis of data. Since heterogeneity has been observed in the overall comparison, random-effect models were applied for all of the forest plots (Fig. 2a–c), except for the OLR1 rs11053646 dominant and co-dominant models (Fig. 2d–e). Ironically, the majority of genetic models for PCSK9 rs505151 were fixed-effect models, except for its recessive genetic model (Fig. 3a–e). Nevertheless, the dominant model of OLR1 rs11053646 was significantly increased the risks towards ischemic stroke with odd ratio (OR) 1.33 (95% CI:1.11–1.58, p = 0.002). Interestingly, both of the co-dominant models for OLR1 rs11053646 and PCSK9 rs505151 demonstrated similar odds towards ischemic stroke (OR = 1.24, 95% CI:1.02–1.51, p = 0.03; OR = 1.36, 95% CI:1.01–1.85, p < 0.05, respectively). Although it is not statistically significant, the GG genotype carriers of PCSK9 rs505151 had a higher risk of ischemic stroke as compared to the wild-type carriers (OR = 3.56, 95% CI:0.96–13.20, p = 0.06).

Heterogeneity and publication bias. The significance of inter-study heterogeneity in the overall comparison models is summarized as P<0.10 and I^2 > 50% (Table 3). Meanwhile, the potential publication bias was analyzed by performing both of the Begg’s and Egger’s tests. The shapes of funnel plots were relatively symmetry, except for the OLR1 rs11053646 homozgyous, heterozgyous and recessive models (Fig. 4) as well as the PCSK9 rs505151 co-dominant model (Fig. 5). However, the results from Egger’s test showed no significance of publication bias for all the tested genetic models (p > 0.05), suggesting that publication bias is not existed in this meta-analysis model. In particular, a non-significant p-value of 0.52 was observed for the co-dominant model of PCSK9 rs505151. Likewise, no significant evidence of publication bias were identified under OLR1 rs11053646 homozygous (p = 0.31) and recessive (p = 0.37) models, except for the heterozygous model (p = 0.002).

Discussion

To the best of our knowledge, this is the first meta-analysis that comprehensively assessed the association between OLR1 rs11053646 and PCSK9 rs505151 polymorphisms with the risk of ischemic stroke. In this study, a total of seven eligible articles comprising 1897 stroke cases and 2119 healthy controls were included. The present meta-analysis covered all the publications indexed in the major databases such as PubMed, Scopus and Web of Science, as well as other databases from China, Hong Kong, India, Japan, Korea, Malaysia, Russia and Latin America. The positive association between the allelic variant of the studied genes and increased ischemic stroke risk was confirmed in the current meta-analysis.
Figure 1. Flow chart of the study selection process for OLR1 rs11053646 (a) and PCSK9 rs505151 (b).
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**Table 1.** Main characteristic of the studies included in the current meta-analysis. a and b are from the same study, Han D et al. (2014).

| Author | Year | Country | Ethnicity | Total no. of | Case | Control | Case | Control | Sex (M/F) | Control Origin | Genotyping method |
|--------|------|---------|-----------|--------------|------|---------|------|---------|-----------|-----------------|-------------------|
| Abboud, S | 2007 | Belgium | Caucasians | 237 | 326 | 53.5 | 70.3 | 2.0 | 2.0 | Population-based | TaqMan SNP genotyping assay |
| Han, D | 2014 | China | Asians | 250 | 199 | 63.6 ± 11.3 | 62.4 ± 11.7 | 2.5 | 1.1 | Hospital-based | Single-base terminal extension |
| Han, D | 2014 | China | Caucasians | 158 | 149 | 59.4 ± 12.0 | 61.2 ± 11.5 | 1.6 | 1.2 | Hospital-based | Single-base terminal extension |
| Hattori, H | 2006 | Japan | Asians | 235 | 274 | 58.3 ± 7.8 | 59.1 ± 3.4 | 3.5 | 2.5 | Hospital-based | Single-nucleotide primer extension |
| Li, D | 2009 | China | Asians | 213 | 176 | 60.0 ± 13.8 | 59.0 ± 11.4 | 1.2 | 1.2 | Hospital-based | Restriction fragment length polymorphism |
| Liu, X | 2014 | China | Asians | 386 | 386 | 62.1 ± 9.9 | 61.9 ± 9.8 | 2.2 | 2.2 | Hospital-based | Ligation detection reaction |
| Slimani, A | 2014 | Tunisia | Caucasians | 114 | 232 | 66 (54.5–76.5) | 49.0 (45.0–55.0) | 1.4 | 2.9 | Hospital-based | Restriction fragment length polymorphism |
| Zhang, J | 2013 | China | Asians | 304 | 377 | 61.2 ± 7.1 | 61.1 ± 6.9 | 1.5 | 1.6 | Population-based | Restriction fragment length polymorphism |

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**Table 2.** The distribution of alleles and genotypes of OLR1 rs11053646 and PCSK9 rs505151 polymorphisms in the current meta-analysis. HWE: Hardy-Weinberg Equilibrium; W: wild type; H: heterozygous; V: variant; D: dominant allele frequency; M: minor allele frequency.

| Studied Polymorphisms | Author | Year | Sample size | Case | Control | W | H | V | D | M | p value |
|-----------------------|--------|------|-------------|------|---------|---|---|---|---|---|--------|
| OLR1 rs11053646       | Hattori, H | 2006 | 235/274 | 143/85 | 7/37 | 1/19 | 175/81 | 18/43 | 117/0.05 |
|                       | Li, D   | 2009 | 213/176 | 131/77 | 5/33 | 9/87 | 112/61 | 3/28 | 67/0.10 |
|                       | Liu, X  | 2014 | 386/386 | 239/135 | 12/613 | 59/159 | 274/97 | 15/645 | 127/0.09 |
|                       | Zhang, J | 2013 | 304/377 | 61/126 | 117/248 | 117/248 | 103/179 | 95/385 | 369/0.33 |
| PCSK9 rs505151        | Abboud, S | 2007 | 237/326 | 218/18 | 1/454 | 20/312 | 14/638 | 14/0.69 |
|                       | Han, D  | 2014 | 250/199 | 219/30 | 1/468 | 32/179 | 20/0 | 37/0 | 40/0.46 |
|                       | Han, D  | 2014 | 158/149 | 146/11 | 1/303 | 13/131 | 17/1 | 27/0 | 20/0.59 |
|                       | Slimani, A | 2014 | 114/232 | 90/20 | 1/260 | 4/199 | 32/1 | 43/0 | 34/0.81 |

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risks represent the major findings of this meta-analysis. Present study has extended our previous knowledge on the participation of a large number of candidate genes in the development of ischemic stroke, particularly the genes involved in the coagulation, homocysteine and lipid signaling pathways.

The clinical impact of OLR1 in the pathogenesis of atherosclerosis and ischemic stroke has been investigated. A higher LOX index was reported to be positively associated with ischemic stroke risk. It has been demonstrated that amino acids substitution of p.K167N (c.501G>T) may reduce the binding affinity of the OLR1 receptor and reduce the LOX-1 expression. In human subjects, the CC variant possesses a lower binding affinity towards ox-LDL and reduced its mRNA expression, which can lead to increased inflammation and affect the atherogenic process in the carotid artery. In contrast, an 11-year follow-up study reported that plasma soluble LOX-1 levels were elevated in the CC genotype carriers as compared to GG genotype among Japanese. In this meta-analysis, OLR1 rs11053646 C allele is associated with ischemic stroke in the dominant model and/or in the recessive model. Consistent with this phenomenon, Liu and colleagues reported that the CC + GC genotype and C allele of this SNP increased the risks of ischemic stroke in Chinese population (OR = 1.51, p < 0.001; OR = 1.32, p = 0.04, respectively). Similarly, Zhang et al. found that C allele (OR = 1.52, p < 0.001) and CC genotype (OR = 2.08, p = 0.001) were significantly higher among Chinese patients with ischemic stroke. However, other studies have shown a lack of association between this SNP and ischemic stroke. Hattori et al. suggested the CC + GC genotype and C allele were less likely to be associated with ischemic stroke (OR = 1.14, p = 0.48; OR = 0.98, p = 0.91, respectively). Likewise, a relatively small number of study has indicated the higher frequencies of CC genotype (OR = 1.33) and C allele (OR = 1.09) among ischemic stroke patients, but the differences with controls were not statistically significant (p = 0.66 and p = 0.63). Hence, the presence of genetic heterogeneity and small sample size could possibly explain the divergent results of these studies.

Increasing evidence has indicated the critical roles of PCSK9 in the risk of hypercholesterolemia and ischemic stroke. Recently, PCSK9 inhibitor shows promising results for the treatment of familial hypercholesterolemia and significantly reduced the LDL-C levels. It is generally well accepted that genetic polymorphisms in PCSK9 gene can contribute to the variable expression and affect the enzyme activity of PCSK9. Nevertheless, the association between p.E670G and LDL-C still remains controversial. Some studies have reported positive associations between G allele and increased levels of LDL-C, whereas other study has shown contrary finding. Moreover, PCSK9 670 GG variant is associated with higher LDL-C levels and increased intima-media thickness progression.
in the presence of ApoE4 allele. In contrast, several studies suggested that LDL-C levels are not mediated by this SNP. With regards to the association of disease, this meta-analysis revealed that the co-dominant model of PCSK9 rs505151 is associated with ischemic stroke, where the distribution of G allele is higher among ischemic stroke patients. Among the included studies, Abboud and colleagues first demonstrated a potential association

Figure 2. Forest plot of odds ratios for the association between OLR1 rs11053646 and ischemic stroke risk. (a) under homozygous model (CC vs GG); (b) under heterogeneous model (GC vs GG); (c) under recessive model (GG + GC vs CC); (d) under dominant model (GC + CC vs GG); (e) under co-dominant model (C vs G).
of this SNP with ischemic stroke, especially the large-vessel atherosclerosis stroke. The odd ratios of G allele and AG + GG genotype were higher among Belgian ischemic stroke subjects (OR = 2.10, p = 0.047; OR = 2.01, p = 0.045 respectively). This observation is further supported by a Tunisian case-control study, where the incidence of G alleles (OR = 1.77, p = 0.032) tends to be higher towards ischemic stroke risk21. Interestingly, patients with

**Figure 3. Forest plot of odds ratios for the association between PCSK9 rs505151 and ischemic stroke risk.**

(a) under homozygous model (GG vs AA); (b) under heterogeneous model (AG vs AA); (c) under recessive model (AA + AG vs GG); (d) under dominant model (AG + GG vs AA); (e) under co-dominant model (G vs A).
| Variables          | No. of study | Sample size (cases/controls) | Homozygous OR (95% CI) | Heterozygous OR (95% CI) | Recessive OR (95% CI) | Dominant OR (95% CI) | Co-dominant OR (95% CI) |
|-------------------|-------------|-----------------------------|-----------------------|------------------------|----------------------|----------------------|------------------------|
|                   |             |                             | CC vs. GG P P heter f² (%) | GC vs. GG P P heter f² (%) | CC vs. (GG + GC) P P heter f² (%) | GC vs. CC + GC P P heter f² (%) | C vs. G P P heter f² (%) |
| OLR1 rs11053646   | 4           | 1138/1213                   | 1.00 (0.47–2.12) 1.00 | 0.00 0.74 1.16 (0.83–1.62) 0.39 0.01 74 | 1.00 (0.47–2.11) 1.00 | 0.00 0.74 1.33 (1.11–1.58) 0.002 0.45 74 | 1.24 (1.02–1.51) 0.003 0.11 51 |
| G > C             |             |                             |                       |                        |                      |                     |                        |
| PCSK9 rs505151    | 3           | 759/906                     | 3.56 (0.96–13.20) 0.06 0.68 0 | 1.19 (0.86–1.66) 0.30 0.20 35 | 1.61 (0.04–67.16) 0.06 0.68 0 | 1.29 (0.94–1.79) 0.12 0.13 47 | 1.36 (1.01–1.85) 0.05 0.09 54 |
| A > G             |             |                             |                       |                        |                      |                     |                        |

Table 3. Meta-analysis of the association between OLR1 rs11053646 and PCSK9 rs505151 with ischemic stroke. P, p-value for Z test; P heter, p-value for Cochran’s Q test.

AG and AA genotypes have their LDL-C levels twice as high as the normal control subjects, which indicated the association between this SNP and the risk of ischemic stroke is mediated by increased levels of LDL-C. However, the odd ratios of G allele were divergently reported among Hans (0.73) and Uygur (0.63) populations22. Their study suggested that there is no significant association between rs505151 and ischemic stroke, but the LDL-C levels have not been determined22. It is noteworthy that the true effect of this SNP could be masked by other genes or environmental factors.

A pertinent source of bias in the meta-analysis is that the source of selected study may be skewed due to the tendency of journals in selectively publishing studies with positive findings. Begg’s funnel plots demonstrated the existence of publication bias in different genetic models of OLR1 rs11053646 and PCSK9 rs505151. However, the non-significant P-value of Egger’s test (p > 0.05) indicated that the overall pooled results are unbiased, except for the OLR1 rs11053646 heterozygous model. Therefore, the results of this model shall be interpreted with caution. The observed heterogeneity may be attributed by the different ethnicities, sample size, study design, genotyping methods, and other environmental factors. Moreover, population heterogeneity may be derived from the genetic diversity of individual studies, i.e. genetic diversity may still exist even though the studied subjects are derived from the same population, ethnicity, country and district.

However, there are several limitations in the current meta-analysis. For instance, we did not perform stratification analysis according to ischemic stroke subtypes since TOAST classification was not been reported in any of the eligible studies. In addition, the subgroup analysis has not been carried out in this meta-analysis. The population data of PCSK9 rs505151 are limited for the subgroup analysis, since only a single Asian population was presented in this meta-analysis. Despite these limitations, our meta-analysis covered the most available association case-control studies, which involving both the hospital- and population-based. Furthermore, the present study has provided a better understanding on the association between the studied SNPs and the risk of ischemic stroke for the first time.

In conclusion, the current meta-analysis suggested that the variant alleles of OLR1 rs11053646 and PCSK9 rs505151 may confer an increased risk of ischemic stroke. Therefore, these SNPs may be used as genetic biomarkers in relation to the burden of ischemic stroke, and serve as potential targets for diagnostic and therapeutic implications, and could thus have potential benefit for translational research in ischemic stroke. Further investigations with larger number of samples from more countries are needed, in order to facilitate the translation of genetic biomarkers into clinical practice.

Methods

Search strategy. This meta-analysis followed the Cochrane Collaboration definition and PRISMA 2009 guidelines for meta-analysis and systematic review. We performed a comprehensive literature search throughout PubMed, Scopus, Web of Science, Google scholar, WHO Global Health Library, VHL, Istatge, KoreaMed, Korean Science Citation Index, POPLINE, New York Academy of Medicine Grey Literature Report, Indian Citation Index, System for Information on Grey Literature in Europe, IMSEAR, MJM, Mycite, WPRIM and CNKI to retrieve the genetic association studies of ischemic stroke. The medical subject heading and keywords terms "lectin-like oxidized LDL receptor-1", "LOX-1", "OLR1", "proprotein convertase subtilisin/kexin type 9", "PCSK9", "neural apoptosis-regulated convertase 1", "NARC1", "ischemic stroke", "cerebrovascular disease", "cerebrovascular accident", "brain infarction", "brain ischemia", "cerebral ischemia", "polymorphism", "variant", "gene mutation", "single nucleotide polymorphism (SNP)", "gene variation" and the related Chinese characters were used as the criteria for searching. There was no limitation in language, where articles written in English, Japanese, Korean, Spanish, Russian or Chinese were retrieved. In addition, the time period for literature searching was from the first available article until July 2015.

Study selection and data abstraction. The inclusion criteria for the gene association studies in the final meta-analysis were as follows: (i) case-control and/or cohort studies; (ii) contained SNP genotype data; and (iii) adequate data for the calculation of odds ratios (ORs) and 95% confidence intervals (CIs).

Data abstraction was performed independently by two authors (A.A. and L.K.W.). This meta-analysis was conducted when the data of three unduplicated studies are available. The following information from each study was summarized: (i) first author; (ii) publication year; (iii) province of study population; (iv) ethnicity; (v) number of cases and controls; (vi) mean age and sex ratio; and (vii) genotyping method.
Figure 4. Funnel plot of publication bias for the association between OLR1 rs11053646 and ischemic stroke risk. (a) under homozygous model (CC vs GG); (b) under heterogeneous model (GC vs GG); (c) under recessive model (GG + GC vs CC); (d) under dominant model (GC + CC vs GG); (e) under co-dominant model (C vs G).
Figure 5. Funnel plot of publication bias for the association between PCSK9 rs505151 and ischemic stroke risk. (a) under homozygous model (GG vs AA); (b) under heterogeneous model (AG vs AA); (c) under recessive model (AA + AG vs GG); (d) under dominant model (AG + GG vs AA); (e) under co-dominant model (G vs A).
Statistical analysis. The genotypic distributions for studied polymorphisms were compared against the controls for any possible deviations from the Hardy-Weinberg equilibrium. Crude OR and 95% confident interval (CIs) were calculated to test the strength of associations between studied polymorphisms and ischemic stroke. The significance of the pooled ORs was determined by Z test for polymorphisms under different genetic models (homozygous, heterozygous, recessive, dominant and co–dominant) for OLR1 rs11053646 and PCSK9 rs505151. The heterogeneity for all the included studies was evaluated using Cochran’s Q test and F statistics. The random–effects model was chosen when significant heterogeneous exist (I2 > 50%); otherwise, fixed–effects model would be adopted. Potential publication bias was tested with Begg’s funnel plot and Egger’s regression test. The statistical tests were performed using the Review manager version 5.3 and Metafor package in R version 3.2.1. All statistics were two–sided and p < 0.05 was considered statistically significant.

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