

Original Article

Polymorphisms in the *PTX1* may not be associated with ischemic stroke susceptibility

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Abstract: Objective: Ischemic stroke is a global health burden due to the increasingly higher incidence rate and mortality rate. Etiological research into the role of genetics in this heterogeneous disease may have diagnostic and prognostic implications. The present study was designed to assess the association between *PTX1* SNPs: -717A>G and -286C>T>A, and ischemic stroke risk. Methods: Risk of ischemic stroke was estimated using summary ORs. The fixed effects model was performed in calculating the pooled ORs. All statistical data were analyzed with STATA software. Results: We combined 4,604 subjects for SNP -717A>G and 3,093 subjects for SNP -286C>T>A. SNP -717A>G was not found to be significantly associated with ischemic stroke risk (GG vs. AA, OR = 1.12, 95% CI = 0.83-1.50, P_{Het} = 0.207; GG + GA vs. AA, OR = 1.04, 95% CI = 0.93-1.17, P_{Het} = 0.533; GG vs. GA + AA, OR = 1.10, 95% CI = 0.82-1.47, P_{Het} = 0.220). Meta-analysis of SNP -286C>T>A also demonstrated no statistical evidence of a significant association with ischemic stroke (AA vs. CC, OR = 0.86, 95% CI = 0.59-1.25, P_{Het} = 0.348; AA vs. CC, OR = 0.92, 95% CI = 0.80-1.06, P_{Het} = 0.609; AA vs. CC, OR = 0.89, 95% CI = 0.62-1.30, P_{Het} = 0.374). Conclusions: These results suggest that the *PTX1* gene polymorphisms may not be associated with a predisposition to ischemic stroke.

Keywords: *PTX1*, polymorphism, ischemic stroke

Introduction

Ischemic stroke constitutes approximately 85% of all strokes and has caused a large number of deaths globally [1, 2]. Much effort has been taken over the past decade to gain new insights into the pathogenesis of ischemic stroke, with massive numbers of genetic markers discovered via family and twins studies [3, 4]. Since then many epidemiologic and molecular studies have emerged to test the hypothesis that variations between individuals may be associated with a predisposition to this heterogeneous disease.

Inflammation plays a pivotal role in the formation and progression of ischemic stroke and cardiovascular disease that represents an important causative factor for ischemic stroke [5-7]. The human *PTX1* gene with a spanning size of 2,880 bps is mapped at chromosome 1 at q21-q23. The acute-phase protein belongs to the pentraxin protein family and functions through Fc γ receptors to defend against

inflammatory responses and autoimmune diseases [8], and the genes in the inflammatory cytokine pathways in turn control the expression of *PTX1* [9]. There is mounting evidence of serum *PTX1* levels as a predictor of ischemic stroke [10-12]. The magnitude of *PTX1* expression is genetically determined, and almost half of the alternation depends on host factors [13, 14]. As *PTX1* gene variations involves in the regulation of the protein levels [15], investigating the association of the single nucleotide polymorphisms (SNPs) and ischemic stroke susceptibility may have diagnostic and prognostic implications.

The promoter SNPs in *PTX1* gene have been proposed as possible biomarkers and can be used to predict ischemic stroke [16]. The existing literature also suggests that the development of ischemic stroke should not ascribe to *PTX1* SNPs [17-19]. In view of the inconsistent reports, we undertook a meta-analysis to assess the risk of ischemic stroke associated with *PTX1*-717A>G and -286C>T>A.

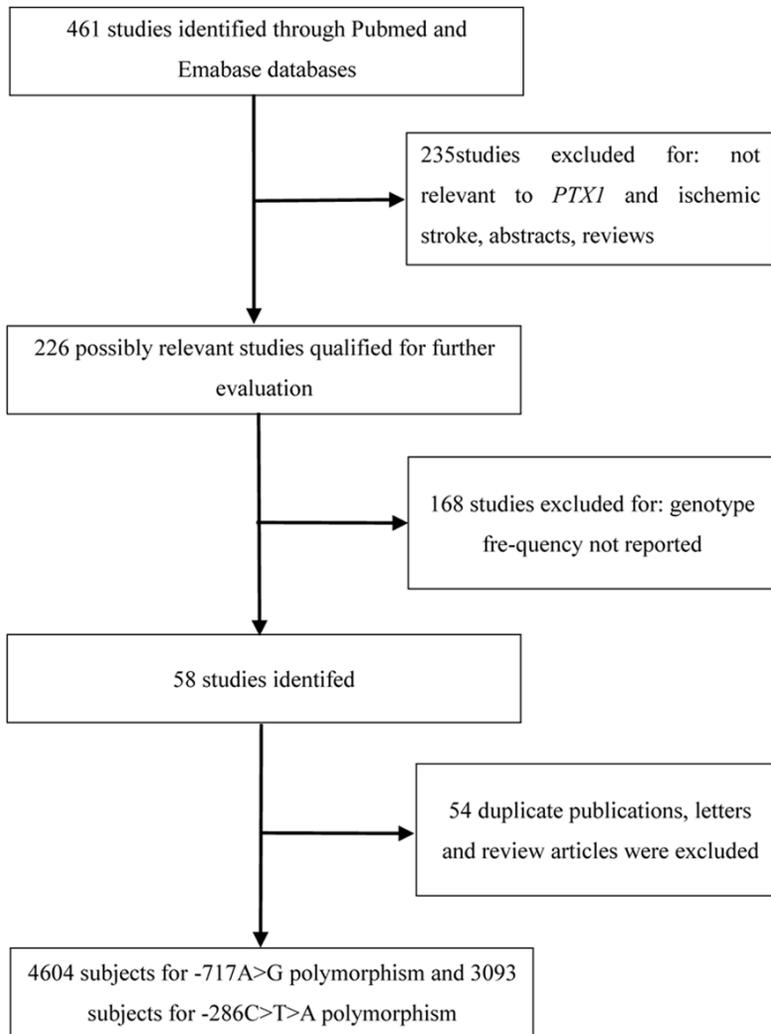


Figure 1. Flow diagram detailing procedures of selecting eligible studies.

Materials and methods

Identification of eligible studies

We first used “PTX1”, “C-reactive protein”, “polymorphism”, “polymorphisms”, “genetic variants”, and “ischemic stroke” to retrieve the potentially relevant papers collected in the PubMed (website: <http://www.ncbi.nlm.nih.gov/pubmed>). The latest search was undertaken on January 10, 2014. We then manually searched the bibliographies of studies that remained after excluding those obviously irrelevant to the genetic polymorphisms being investigated and ischemic stroke. The studies were included unless satisfying the following criteria: 1) patients with ischemic stroke and well-matched healthy controls were investigated; 2) they should be case-control studies; 3)

the study contained complete data that helped to calculate an odds ratio and 95% confidence intervals (OR and 95% CI); 4) genotype distribution in controls was in Hardy-Weinberg equilibrium (HWE). Accordingly, the following exclusion criteria were also used: 1) not relevant to PTX1 and ischemic stroke, abstracts, reviews; 2) genotype frequency not reported; 3) duplicate publications, letters and review articles were excluded.

Data abstraction

To facilitate a general understanding of the eligible studies, in addition to allele and genotype data, two investigators also extracted first author’s surname, publication year, samples’ ethnicity, matching, control sources, DNA source, number of cases and controls, *P* value for HWE wherever possible, and measurement methods in duplicate. Discussion among all investigators was the solution to disagreements.

Statistical analysis

Based on the genotype data in controls, we tested HWE for all studies using the goodness-of-fit chi-square test. The association between PTX1 SNPs and ischemic stroke risk was evaluated using crude OR with 95% CI. Summary ORs were calculated assuming homozygote, dominant and recessive contrast models for both SNPs.

Inconsistency across studies (heterogeneity) was determined with the chi-square-based Q-test and the I^2 metric [20]. *P*-values above 0.10 or I^2 more than 50% was representative of significant heterogeneity, we therefore appropriately selected the fixed-effects model (Mantel and Haenszel method) to calculate the effects size; otherwise, the random-effects model (DerSimonian and Laird method) was performed [21, 22].

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Table 1. Characteristics and summary ORs of case-control studies included in the meta-analysis

Author	Year	Population	cases/controls	DNA source	Genetic variant	Homozygote ^a	Dominant ^a	Recessive ^{a,b}
						OR (95% CI)	OR (95% CI)	OR (95% CI)
Ladenvall [17]	2006	Caucasian	599/600	Venous blood	-717A>G	1.00 (0.65, 1.56)	1.00 (0.82, 1.22)	1.00 (0.65, 1.54)
Ladenvall [17]	2006	Caucasian	79/79	Venous blood	-286C>T>A	4.14 (0.46, 37.27)	1.12 (0.72, 1.73)	4.10 (0.46, 36.91)
Ben-Assayag [18]	2007	Caucasian	217/520	Venous blood	-717A>G	1.33 (0.61, 2.92)	1.27 (0.93, 1.72)	1.20 (0.55, 2.60)
Wang [16]	2009	Asian	564/564	Venous blood	-717A>G	2.09 (1.01, 4.35)	1.07 (0.84, 1.37)	2.09 (1.01, 4.33)
Wang [16]	2009	Asian	31/30	Venous blood	-286C>T>A	0.76 (0.38, 1.52)	0.86 (0.67, 1.11)	0.81 (0.40, 1.60)
Shen [19]	2013	Asian	546/994	Blood	-717A>G	0.77 (0.41, 1.46)	0.97 (0.78, 1.20)	0.77 (0.41, 1.46)
Shen [19]	2013	Asian	542/994	Blood	-286C>T>A	0.82 (0.52, 1.31)	0.92 (0.76, 1.12)	0.86 (0.54, 1.36)
					-717A>G (Total)	1.12 (0.83, 1.50)	1.04 (0.93, 1.17)	1.10 (0.82, 1.47)
					-286C>T>A (Total)	0.86 (0.59, 1.25)	0.92 (0.80, 1.06)	0.89 (0.62, 1.30)

^aGG vs. AA, AA vs. TT; ^aGG + GA vs. AA, AA + AT vs. TT; ^bGG vs. GA + AA, AA vs. AT + TT for -717A>G and -286T>A, respectively.

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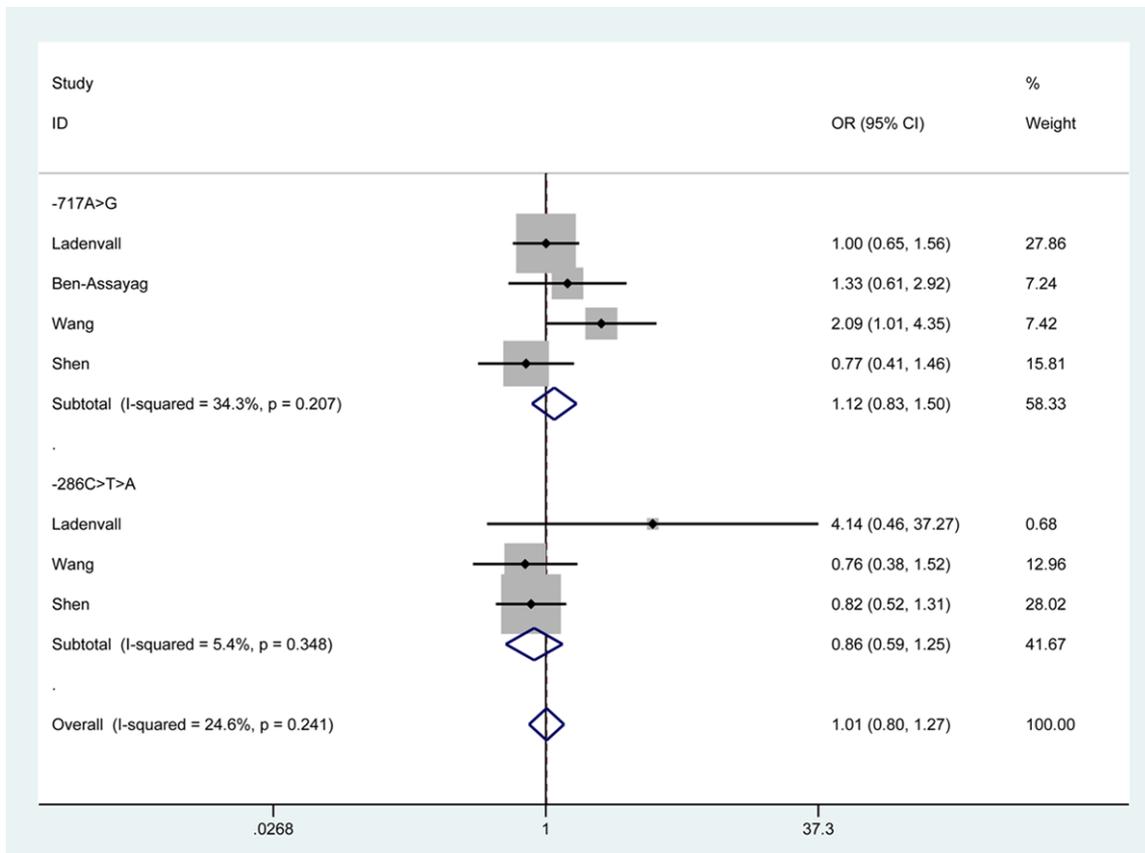


Figure 2. Overall estimates of PTX1 gene polymorphisms examined for ischemic stroke under the homozygote model. The summary odds ratio (OR) is shown by the middle of a solid diamond whose left and right extremes represent the corresponding 95% confidence interval (95% CI). Horizontal axis represents ORs, which are calculated against controls for each study.

Sensitivity analysis, Egger's test and Begg's funnel plot were performed respectively to examine the stability and reliability of the combined effects in this meta-analysis [23-25]. All statistical data were analyzed with STATA software (version 12.0, STATA Corp., College Station, TX). All two-tailed *P* values were considered significant at 0.10 unless specially stated.

Results

Selection of studies

As detailed in **Figure 1**, a total of 461 records matching the search terms were obtained from the PubMed database. We referred to the above-mentioned criteria and found three publications eligible for this analysis [16-19]. Another study was identified through manual search, thus the total pooling dataset combined data from four different studies, providing 4,604 subjects for -717A>G polymorphism

and 3,093 subjects for -286C>T>A polymorphism.

Characteristics of studies

For the studies finally considered in this meta-analysis, there was an equal distribution with respect to ethnicity. However, they varied in control source from Clinic to hospital, and in genotyping measurements from TaqMan to PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism). Inconsistency with HWE was not seen in any control group ($P > 0.10$). More information can be captured in **Table 1**.

Meta-analysis results

As shown in **Table 1**, only one single study demonstrated that the -717GG genotype was associated with over 2-fold greater risk of ischemic stroke compared to the -717AA genotype or the AA and GA genotypes (GG vs. AA, OR = 2.09,

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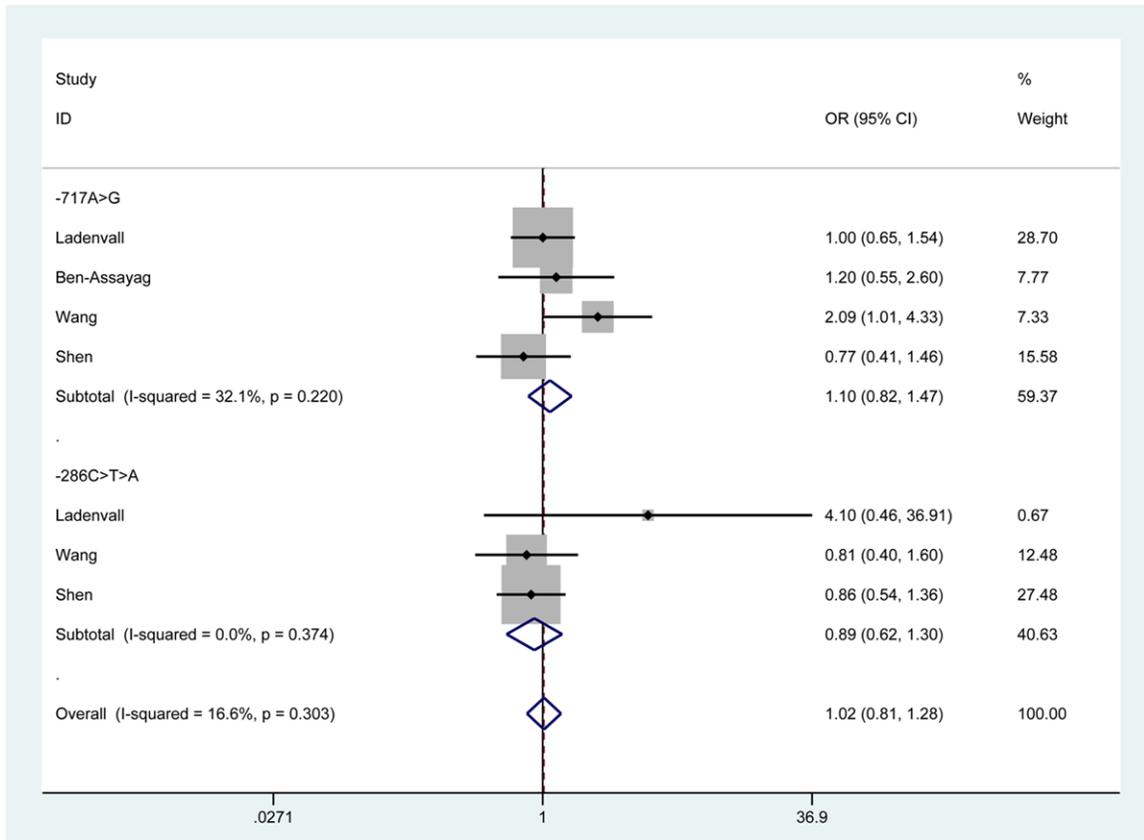


Figure 3. Overall estimates of PTX1 gene polymorphisms examined for ischemic stroke under the recessive model. The summary odds ratio (OR) is shown by the middle of a solid diamond whose left and right extremes represent the corresponding 95% confidence interval (95% CI). Horizontal axis represents ORs, which are calculated against controls for each study.

95% CI = 1.01-4.35; GG vs. GA + AA, OR = 2.09, 95% CI = 1.01-4.33). Such a significant association was lost when all studies were pooled into a meta-analysis (GG vs. AA, OR = 1.12, 95% CI = 0.83-1.50, $P_{\text{Het}} = 0.207$; GG + GA vs. AA, OR = 1.04, 95% CI = 0.93-1.17, $P_{\text{Het}} = 0.533$; GG vs. GA + AA, OR = 1.10, 95% CI = 0.82-1.47, $P_{\text{Het}} = 0.220$) (**Figure 3, Table 1**).

In terms of the analysis of -286C>T>A polymorphism in total subjects, we did not observe any statistical significance either in each single or the combined dataset (AA vs. CC, OR = 0.86, 95% CI = 0.59-1.25, $P_{\text{Het}} = 0.348$; AA vs. CC, OR = 0.92, 95% CI = 0.80-1.06, $P_{\text{Het}} = 0.609$; AA vs. CC, OR = 0.89, 95% CI = 0.62-1.30, $P_{\text{Het}} = 0.374$) (**Figure 2, Table 1**).

Sensitivity analysis

We sequentially excluded the single studies from the overall pooled analysis to check whether the summary ORs were materially changed. The recalculated ORs were found sta-

ble, indicating our results are valuable (data not shown).

Publication bias

Using Begg's funnel plot and Egger's test, statistically significant publication bias was not revealed in this meta-analysis, as funnel plots showed a symmetrical distribution of the studies and Egger's test exhibited no statistical evidence of publication bias ($P = 0.355$, $t = 1.19$ for GG vs. GA + AA; $P = 0.932$, $t = -0.11$ for AA vs. CC) (**Figures 4, 5**).

Discussion

In the current meta-analysis, consisting of 4,604 subjects for -717A>G polymorphism and 3,093 subjects for -286C>T>A polymorphism, we clarified whether there exists an association between the two PTX1 genetic variants and ischemic stroke risk. Overall, the -717A>G was found to not play a major role in the development of ischemic stroke. We also found that both of

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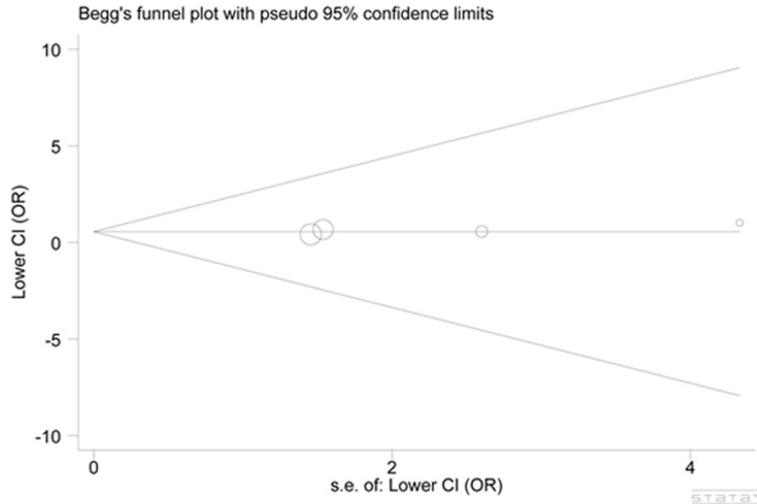


Figure 4. Funnel plots for association studies between SNP -717A>G and ischemic stroke (the recessive model).

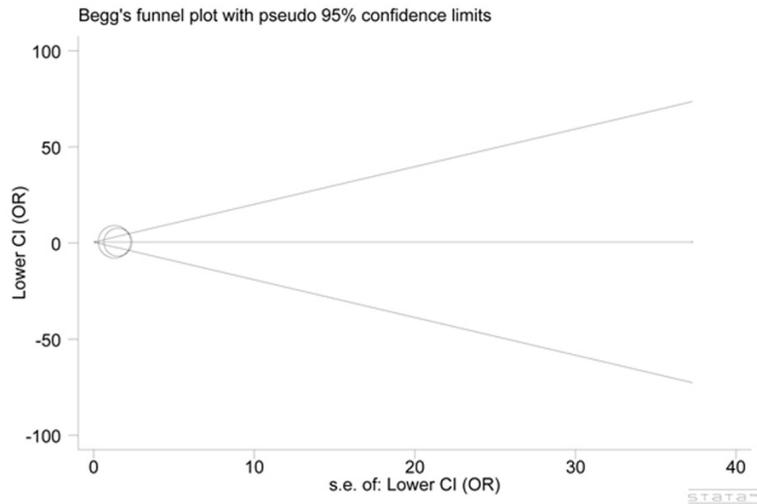


Figure 5. Funnel plots for association studies between SNP -286C>T>A and ischemic stroke (the homozygote model).

the C allele and the A allele of SNP -286C>T>A were not associated with a significantly increased risk of ischemic stroke in general population.

The identification of *PTX1* gene as a marker of inflammation degrees leads to great interest in the ischemic stroke genetic community [26]. An increasing body of epidemiologic and molecular evidence over the past ten years has validated the *PTX1* as an ischemic stroke susceptibility locus [27-31]. The involvement of the *PTX1* gene in ischemic stroke predisposition as evidenced in previous reports, however, has

come under question due to the inconsistency of the reported associations between the *PTX1* polymorphisms and ischemic stroke susceptibility. The majority of studies in recent years have reported not finding a role of these polymorphisms in the disease [17-19], but replication of this finding has been mixed [16]. The scantiness of replicable associations plausibly due to methodological limitations including selection of inappropriate controls, utility of different platforms in genotype determination, inadequate sample size, and poor study design.

A number of prospective studies have reported a marked impact of high *PTX1* levels on risk of cardiovascular events and all-cause mortality, even in apparently healthy subjects [32, 33]. The significant association between *PTX1* concentration and ischemic stroke susceptibility is further confirmed by a meta-analysis in which individuals without a history of vascular disease are analyzed [34]. Herein, we could infer that a minor change in the protein level of *PTX1*, which has been shown to be affected by the promoter polymorphisms, may lead to a significantly increased likelihood of predisposing to ischemic stroke.

The failure to provide supportive evidence of such a theoretically positive association in our study is most likely because of the inadequate sample size. Therefore, explorations should be continued to determine the role of *PTX1* gene and the genetic polymorphisms in ischemic stroke.

During the malignant progression of ischemic stroke, complex interactions between genetic and environmental factors are reportedly to play an important role. Individuals of different ethnicities have different lifestyles, dietary patterns, and residential environments; an unfa-

avorable combination of these factors may constitute a decisive factor for ischemic stroke. The incidence rate of this disease in Asians, especially those from east Asian countries, is obviously lower compared to the Caucasians and blacks [35], suggesting Asian populations may be less susceptible to ischemic stroke than Caucasian and black populations.

In this study, we are not allowed to identify whether the *PTX1* gene polymorphisms predispose to ischemic stroke in an ethnicity-dependent manner or not, due to the inaccessibility of raw data. This is the first limitation that future studies should overcome. Second, genetic predisposition may differ depending on the age, gender, and other confounders, hence interplay of genetic and environmental factors should be considered on condition that original articles report sufficient data. Third, as there may be unpublished data beyond our reach, we cannot assure inclusion of all studies that meet all selection criteria in this analysis, although we have put equal emphasis on publication during literature search. However, meta-analysis has stronger statistical power than any individual study in detecting genetic associations, so the results revealed in our analysis are trustworthy.

In conclusion, this meta-analysis demonstrated little evidence to support a role of *PTX1* gene polymorphisms in ischemic stroke predisposition. Further larger studies are needed to confirm these findings and to validate the association between *PTX1* gene polymorphisms and ischemic stroke in various ethnic groups.

Disclosure of conflict of interest

None.

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