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RESEARCH PAPER

Association analysis of rs1800470 (T869C) variation in TGFB1 gene with the risk of ischemic stroke in Asian population

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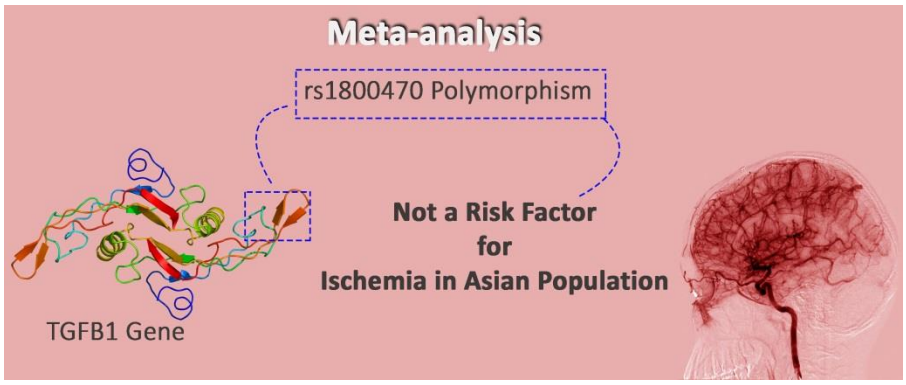
Highlights

- TGFB1 gene could be involved in the pathogenesis of stroke risk.
- The rs1800470 is a common polymorphism on TGFB1 gene.
- The rs1800470 polymorphism is not a risk factor for ischemia in Asian population.

Graphical Abstract



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Abstract

Stroke is a disease that can lead to death or long-term disability. Genetic polymorphisms in key genes can alter the risk of developing the disease. The aim of this study was to investigate the association of rs1800470 polymorphism in the TGFB1 gene with the risk of stroke in the Asian population by a meta-analysis approach. In this study, six eligible studies were included using a systematic search. The strength of the association of the polymorphism with the risk of stroke was assessed by estimating the odds ratio (OR) and 95% confidence interval (CI). The results of our study showed that rs1800470 with risk of stroke in seven genetic models' allelic model, recessive model, dominant model, overdominant model, CC vs. TT, CC vs. CT, and CT vs. TT is not associated. Of course, there was significant heterogeneity among the included studies and there was no publication bias in the meta-analysis. Based on these results, rs1800470 polymorphism cannot be considered a suitable biomarker for stroke risk in the Asian population, and further studies are needed to achieve more accurate results.

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Introduction

One of the main causes of long-term disability and even mortality is stroke, the etiology of which is still unknown, and appropriate treatments are not known for it. So, it's a huge burden on health around the world. Most strokes (about 87%) are of ischemic origin, with the involvement of many genetic and environmental factors (1). There is ample evidence of the important role of inflammation in the pathogenesis of this disease. One of the components of the inflammatory system is cytokines that are expressed even in neuronal and glial cells. The mechanisms involved in altering the expression of cytokines in people with ischemic stroke are unclear. Further research has shown that genetic polymorphisms in the cytokine family can be considered genetic risk factors for this disease (2, 3).

One member of the cytokine family is the transforming growth factor-beta (TGF-β), which contains three isoforms of TGF-β-1-3 with similar physiological functions. This cytokine is involved in important biological processes including lipid metabolism, cell apoptosis, immune regulation, inflammation control, blood pressure, and the development of atherosclerotic plaques. This immunological factor is altered by genetic factors and under different physiological conditions. After the stroke, this factor can reduce the efficiency and expression of some other cytokines, reduce glial activity, increase angiogenesis in the penumbra, reduce cerebral edema, inhibit dangerous oxygen and nitrogen products, and etc. (4-6).

The chromosomal locus of the TGF-β1 gene is 19q13.2, which covers seven exons and six introns with a length of 23.56 kbp. Numerous regions for single nucleotide polymorphisms (SNPs) have been identified in this gene (7). Analysis of polymorphisms in the regulatory regions of this gene showed that this gene contains three key polymorphisms common in its upstream. These SNPs include rs1800468, rs1800469, and rs1800470, which can affect the expression of the TGF-β1 gene at the transcriptional level. The rs1800468 and rs1800469 polymorphisms are located in the gene promoter and it has been shown that the rs1800470 variety is located on the signal sequence and increases the expression of the TGF-β1 gene (8-10).

In recent years, the relationship between TGF-β1 varieties and the risk of ischemic stroke has been investigated in several studies. One study found that the T869C and C-509T gene variants of the gene may be associated with a higher risk of stroke in the Chinese population (11). Another study found that TGFβ1 polymorphisms and haplotypes could serve as molecular biomarkers of cerebral infarction in the Chinese population (12). In another study by Kumar et al. in the Indian population, it was found that some polymorphisms of this gene may be associated with the risk of ischemic stroke in the Indian population (4). Given that some association of TGF-β1 polymorphisms has been investigated in Asian populations, the results cannot be conclusive. Therefore, the aim of this study was to investigate the association of rs1800470-T869C polymorphism of TGF-β1 gene with the risk of ischemic stroke in Asian population with a meta-analysis approach.

Materials and Methods

Reliable electronic databases such as EMBASE, MEDLINE, PubMed, and Google Scholar using the keywords "ischemia", "ischemic stroke", “stroke”, "TGF-β1 gene", "transforming growth factor-β1", "polymorphism", "Variety" and "Mutation" were independently researched by two researchers till May 2022. There was no time or language limitation for the search. Two researchers consulted in cases where there was an inconsistency in the search, and ambiguities were resolved. References to found articles were also reviewed to find missing qualified articles.

The studies selected for the meta-analysis approach should meet the following criteria: 1) Case studies that examined the association of the rs1800470 polymorphism of the transforming growth factor-β1 gene with the risk of stroke; 2) They had adequate genotypic frequency to calculate odds ratio (OR) and 95% confidence interval (CI). 3) The studies had to be of human origin. In addition, the criteria for excluding articles from the meta-analysis were as follows: 1) Genotypic frequency was not reported adequately. 2) Studies were of animal origin. 3) Review articles, meta-analyses, case reports, and similar cases were also excluded from the study. In

cases where there are several articles by one author and it is possible to overlap, a newer article with a larger sample size should be used.

Data extraction

After selecting the eligible articles, two authors extracted different data from the articles and consulted with each other in case of disagreement and resolved the discrepancies. Information was extracted from qualified articles including the name of the first author, year of publication, country, genotyping method and genotypic frequency.

Statistical analysis

The association between the rs1800470 genetic variant and the risk of ischemic stroke was calculated using ORs and the corresponding 95%CIs. Pooled ORs were evaluated in Allele contrast, Recessive model, Dominant model, Overdominant model, CC vs. TT, CC vs. CT, and CT vs. TT genetic models. The existence of heterogeneity was assessed using the Chi-square-based Q-test. If there was heterogeneity (P> 0.1), the random-effect model was used, otherwise the fixed-effect model was used. Publication bias was assessed using the Egger test and the funnel plot (13, 14). All calculations were performed using MetaGenyo online software.

Results

Included studies

We performed an initial search on reputable databases and found 154 articles, of which 126 articles were removed from the study after the titles and abstracts were assessed. The remaining 28 articles were carefully evaluated, some of which were reviews, meta-analyzes, or worked on a hemorrhagic stroke. Other papers also examined other polymorphisms in the TGFB1 gene. Of course, it is worth noting that we included articles on Asian populations, and therefore excluded articles on other populations, such as Caucasian. Finally, 6 articles were included in our meta-analysis (4, 11, 15-18), the characteristics of which are listed in Table 1. Hardy-Weinberg equilibrium was present in the control group of all 6 articles. Of these projects, two were conducted in China, two in Korea, one in Japan and one in India. The sample size and genotyping method for each study are summarized in Table 1.

Table 1. Characteristics of included studies in meta-analysis rs1800470.

Author, Year	Country	Sample size	Genotyping method	Genotype frequencies						*P* HWE
				Case			Control			
				TT	TC	CC	TT	TC	CC	
Kim (2006)	Korea	478	TaqMan	79	123	69	42	110	55	0.334
Tao (2010)	China	900	(AS-PCR)	152	193	105	111	217	122	0.458
Katakami (2011)	Japan	3793	Allele-specific	68	170	93	935	1689	838	0.166
Peng (2011)	China	331	RFLP-PCR	34	70	60	46	86	35	0.656
Kumar (2017)	India	500	SNaPshot	164	79	7	215	33	2	0.560
Kim (2020)	Korea	896	TaqMan	124	223	94	103	226	104	0.361

AS-PCR, Allele specific PCR; HWE, Hardy-Weinberg equilibrium; PCR, Polymerase chain reaction; RFLP, Restriction fragment length polymorphism.

Association of rs1800470 genetic variation with risk of stroke

We analyzed the association of rs1800470 polymorphism with the risk of ischemia in seven genetic models. We found that the frequency of rs1800470 polymorphism is different between case and control groups in overall but this difference was not statistically significant. As summarized in Table 2, after analysis, we found that there is not significant association between the mentioned polymorphism and risk of ischemic stroke in any seven

genetic models (Allele: OR= 1.1653, 95%CI= 0.87-1.57, P= 0.314; Recessive: OR= 1.1280, 95%CI= 0.84-1.51, P= 0.423; Dominant: OR= 1.1201, 95%CI= 0.70-1.78, P= 0.631; Overdominant: OR= 1.0276, 95%CI= 0.74-1.42, P= 0.869; CC vs. TT: OR= 1.1039, 95%CI= 0.69-1.76, P= 0.679; CC vs. CT: OR= 1.0941, 95%CI= 0.94-1.28, P= 0.255; CT vs. TT: OR= 1.0636, 95%CI= 0.68-1.67, P= 0.788). The forest plots for Allele, Recessive, Dominant, and Overdominant genetic models are depicted in Figure 1.

Table 2. Association results of meta-analysis**.**

Genetic Model	Number of studies	Association test		
		OR	95% CI	p-value
Allele	6	1.1653	[0.87-1.57]	0.314
Recessive	6	1.1280	[0.84-1.51]	0.423
Dominant	6	1.1201	[0.70-1.78]	0.631
Overdominant	6	1.0276	[0.74-1.42]	0.869
CC vs. TT	6	1.1039	[0.69-1.76]	0.679
CC vs. CT	6	1.0941	[0.94-1.28]	0.255
CT vs. TT	6	1.0636	[0.68-1.67]	0.788

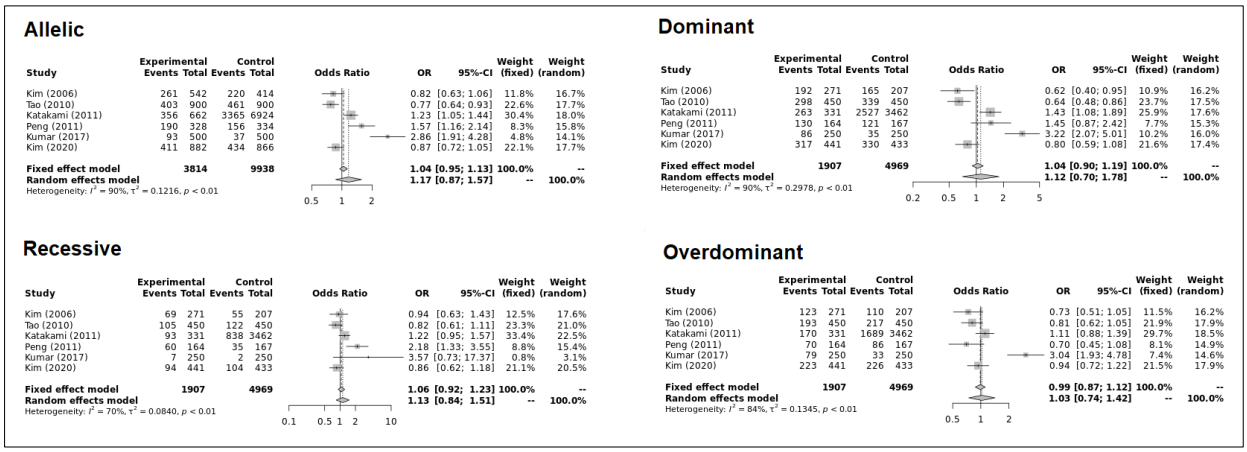

Figure 1. Forest plots for allelic, dominant, recessive, and over dominant models.

Heterogeneity and publication bias outcomes

The results of heterogeneity and publication bias are summarized in Table 3. After analysis, we found that there is true heterogeneity in all seven Allele, Recessive, Dominant, Overdominant, CC vs. TT, and CT vs. TT genetic models except CC vs. CT (P= 0.168, I^2= 0.3587) comparison. Moreover, we analyzed the publication bias by Egger's linear regression test and Funnel plot and the outcomes are summarized in Table 3. Our data revealed that there is no publication bias an any seven genetic models (P> 0.05). The funnel plots for Allele, Recessive, Dominant, and Overdominant genetic models are depicted in Figure 2.

Table 3. Results of heterogeneity and publication bias

Genetic Model	Heterogeneity			Publication bias
	Model	p-value	I^2	Egger's test p-value
Allele	Random	0	0.904	0.244
Recessive	Random	0.005	0.6998	0.320
Dominant	Random	0	0.9021	0.504
Overdominant	Random	0	0.8441	0.654
CC vs. TT	Random	0	0.8235	0.458
CC vs. CT	Fixed	0.168	0.3587	0.361
CT vs. TT	Random	0	0.8838	0.691

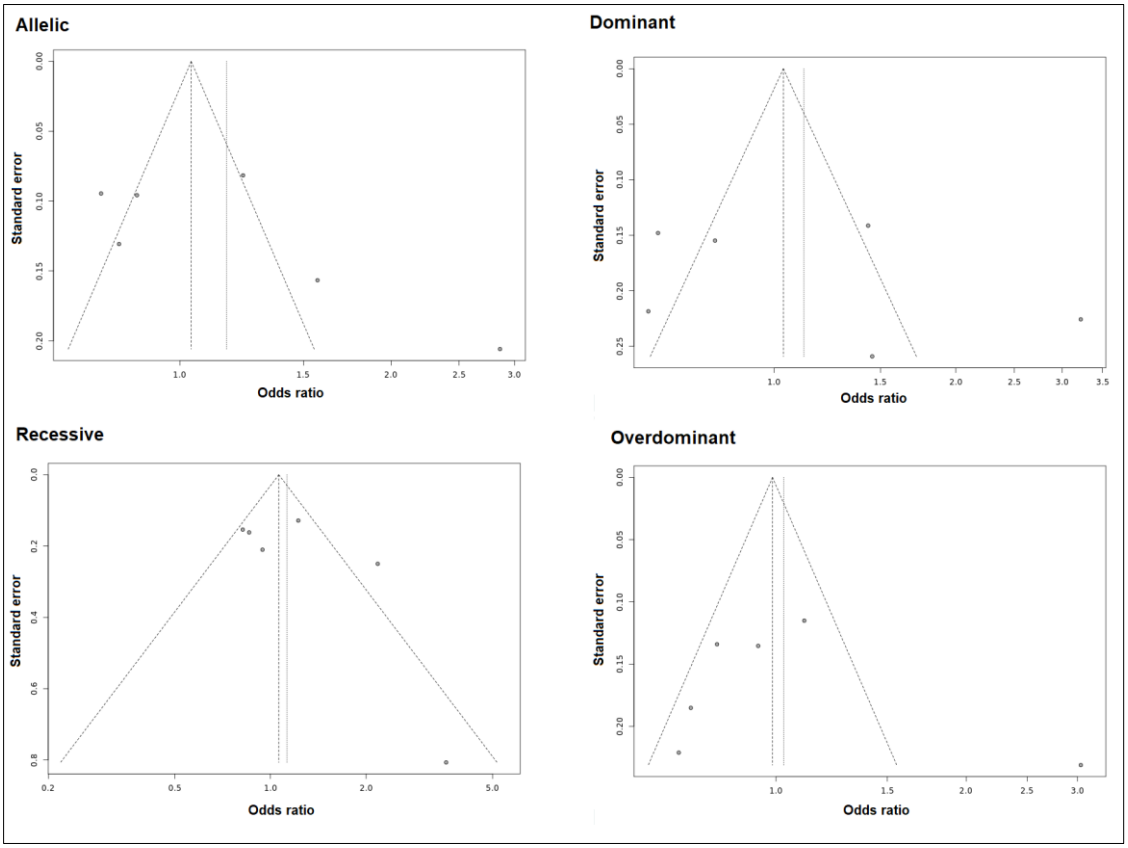

Figure 2. Funnel plots for allelic, dominant, recessive, and over dominant models.

Discussion

In this study, we analyzed the association of rs1800470 (T869C) variation in TGFB1 gene with the risk of ischemic stroke in Asian population. After search procedure, we found seven eligible articles investigating the association of the mentioned genetic polymorphism TGFB1 gene and the risk of ischemic stroke in Asian population. It is worth noting that articles from other populations, such as Caucasian, were excluded from our meta-analysis and our study was limited to the Asian population. Our data revealed that there is no significant association between rs1800470 and ischemia in Asian population in any genetic models. Results of heterogeneity showed that there is significant heterogeneity among included studies in meta-analysis in six genetic models. Also, there was no significant publication bias in any genetic models. Therefore, the result of meta-analysis could be reliable and robust. The different results between studies may be due to environmental and geographical factors. However, this polymorphism cannot be considered a suitable risk factor or biomarker to predict the risk of stroke.

The TGF-β family is a group of proteins that are involved in many cellular and molecular processes including apoptosis, cell proliferation, cell differentiation, and cell growth. Three TGF-β1 to 3 members have been identified that have their own receptors. This protein was first discovered in platelets in human blood. This protein is initially made as a precursor containing 390 amino acids, much of which are cleaved by proteolytic activity, and the final protein is made up of 112 residues (19). This protein can be involved in many biological processes and is associated with many diseases including hypertension, cancer, cardiovascular disease, and so on. For example, it was found that the expression level of TGF-β1 in apoplexy and in the serum of patients decreases, which indicates the key role of this molecule in ischemia (20). Studies indicate an important role for TGF-β1 in controlling cell activity, but the mechanisms involved are not well understood. One of the cellular pathways that activate TGF-β1 is the TGF-β1/Smads cellular pathway (21).

Of course, the TGF-β1 gene also has other key polymorphisms, one of which is -509C/T, which is located on the promoter of this gene and can affect gene expression. A meta-analysis study was conducted in 2016 and showed that this polymorphism is not associated with the risk of stroke. But this meta-analysis was performed

on two studies that included 614 cases and 617 controls. This could be one of the important limitations of this article because the sample size was very low. The authors of the article also suggested that further studies are needed to achieve more accurate results. Another important and common polymorphism of TGF-β1 gene is rs1800468 polymorphism, which is also located on the promoter. A study was conducted in the North Indian population that examined the rs1800468 polymorphism with two previous polymorphisms in stroke patients. The results of their study showed that these polymorphisms as well as their haplotypes are associated with the risk of stroke (4). Given the presence of three common polymorphisms in the TGF-β1 gene, we also suggest that the association of all three polymorphisms with the risk of stroke be investigated by the meta-analysis approach. Genetic polymorphisms can exist in different regions of a gene, and their presence anywhere can cause a specific molecular effect. For example, promoter polymorphisms affect gene expression, while exon polymorphisms affect protein structure and function. Intron polymorphisms can also affect the splicing process. Examining the exact mechanism of the effect of polymorphisms on the function of a gene through experimental studies is very costly and time-consuming. But examining the role of these polymorphisms using computational studies makes the project much easier. Therefore, it is suggested that the effect of the studied polymorphism in this meta-analysis be investigated by bioinformatics tools.

Conclusion

Our study showed that the rs1800470 polymorphism (T869C) in the TGFB1 gene could not affect the risk of stroke in the Asian population. Of course, subsequent studies with larger sample sizes and in different countries can provide more accurate results. Of course, this study had other limitations. That our study was limited to the Asian population and that other populations, such as blacks and whites, had no role in it. We also did not have access to key data such as age, gender, body mass index, etc. so that we could not adjust our data with them. Also, as mentioned, studying the role of this polymorphism on gene function by bioinformatics tools can be a suitable approach to identify its pathophysiological role.

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