

Original Article

## Evaluation of a pleiotropic cytokinin polymorphism with cerebral infarction in a Chinese male population

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### Article Info

### Abstract



#### Article history:

Received: January 25, 2024

Accepted: April 13, 2024

Published: May 31, 2024

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Cardiovascular diseases, particularly stroke, are a leading cause of morbidity and mortality worldwide. Genetic variations in genes associated with inflammation have been implicated in the pathogenesis of stroke. Interleukin-6 (IL-6), a pleiotropic cytokine with diverse biological functions, has been linked to cardiovascular diseases and stroke. The relationship between cerebral ischemia and inflammation is well-established, suggesting a potential role for IL-6 polymorphisms in stroke susceptibility. In the context of this study, the focus is on evaluating a pleiotropic cytokinin polymorphism, specifically IL-6-572GC, and its association with cerebral infarction in a Chinese male population. The investigation aims to elucidate the genetic correlation between IL-6 polymorphisms and stroke risk, particularly in the context of hemorrhagic subtype of stroke. The study utilizes a case-control design, comparing stroke patients with healthy controls while adjusting for classic risk factors associated with stroke. The methodology employed includes the detection of IL-6 polymorphisms using Real Time Taq Man Probe and PCR-RFLP methods. The results suggest an association between the IL-6-572GC genotype and an increased risk of stroke, particularly in the hemorrhagic subtype. However, the relationship between another IL-6 polymorphism, IL-6-174GC, and stroke remains inconclusive, except for a potential correlation with one allele. The findings underscore the potential role of IL-6-572GC genotype as a genetic risk factor for stroke in the Chinese male population under study. Further research involving larger cohorts is warranted to validate these results and clarify the role of IL-6-174GC polymorphism in stroke susceptibility. Understanding the genetic underpinnings of stroke can provide valuable insights for risk assessment and personalized treatment strategies in affected populations.

**Keywords:** Pleiotropic Cytokinin, Polymorphism, Cerebral Infarction, Interleukin 6

### 1. Introduction

Pleiotropic cytokines are a group of signaling molecules that have multiple effects on different cell types and biological processes [1]. These cytokines play crucial roles in regulating immune responses, inflammation, cell proliferation, and differentiation. Interleukin-6 (IL-6) is a prototypical pleiotropic cytokine with diverse functions in the immune system and beyond [2]. IL-6 is produced by various cell types, including immune cells, endothelial cells, and fibroblasts, and plays a central role in mediating inflammatory responses. IL-6 exerts its effects by binding to its receptor complex, which consists of IL-6 receptor (IL-6R) and glycoprotein 130 (gp130) [3]. This binding activates intracellular signaling pathways, including the JAK/STAT pathway, MAPK pathway, and PI3K/Akt pathway, leading to the regulation of gene expression and cellular responses. IL-6 has been implicated in various physiological and pathological processes, including inflammation, immune responses, hematopoiesis, metabolism, and tissue repair [1].

In the context of cardiovascular diseases, IL-6 has been identified as a key player in the pathogenesis of athero-

sclerosis, myocardial infarction, and stroke [3]. Elevated levels of IL-6 have been associated with an increased risk of cardiovascular events and poor outcomes in patients with cardiovascular diseases. The role of IL-6 in stroke is particularly intriguing due to its involvement in the inflammatory response following cerebral ischemia [4]. Cerebral infarction, also known as ischemic stroke, occurs when blood flow to the brain is interrupted, leading to tissue damage and neurological deficits [2]. The pathophysiology of ischemic stroke involves a complex interplay of vascular occlusion, inflammation, excitotoxicity, oxidative stress, and cell death pathways. Inflammation plays a crucial role in the secondary injury cascade following cerebral ischemia, contributing to neuronal damage and functional impairment [4].

Genetic variations in genes encoding cytokines and their receptors have been implicated in the susceptibility to stroke and its subtypes [5]. Polymorphisms in cytokine genes can influence the production, secretion, and activity of cytokines, leading to altered immune responses and inflammatory processes. IL-6 polymorphisms have been extensively studied in the context of cardiovascu-

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lar diseases and stroke, with conflicting results regarding their association with disease risk [6]. The IL-6 gene is located on chromosome 7p21 and contains several single nucleotide polymorphisms (SNPs) that have been linked to altered IL-6 expression and activity. The IL-6-572G>C polymorphism, located in the promoter region of the IL-6 gene, has been associated with variations in IL-6 production and circulating levels. The IL-6-572GC genotype has been linked to increased IL-6 expression and higher serum levels of IL-6 in certain populations [7].

Studies investigating the association between IL-6 polymorphisms and stroke risk have yielded inconsistent results, with some studies reporting a positive correlation between specific IL-6 genotypes and stroke susceptibility, while others have failed to replicate these findings [5]. The relationship between IL-6 polymorphisms and stroke risk may be influenced by various factors, including the ethnic background of the study population, sample size, study design, and adjustment for confounding variables [6]. In the context of cerebral infarction, the role of IL-6 polymorphisms in modulating inflammatory responses and neuronal damage following ischemic stroke is of particular interest. Animal studies have demonstrated that genetic deletion or inhibition of IL-6 signaling can reduce infarct size, improve neurological outcomes, and attenuate neuroinflammation in experimental stroke models [7]. These findings suggest that IL-6 may contribute to the pathogenesis of ischemic stroke through its pro-inflammatory effects on the brain [8].

The investigation of IL-6 polymorphisms in relation to cerebral infarction in human populations can provide valuable insights into the genetic determinants of stroke susceptibility and inform personalized treatment strategies for affected individuals [9]. Understanding how genetic variations in cytokine genes influence inflammatory responses and neuronal damage in ischemic stroke may lead to the identification of novel therapeutic targets for stroke prevention and treatment [1].

Therefore, pleiotropic cytokines such as IL-6 play critical roles in regulating immune responses and inflammation in various disease states, including cardiovascular diseases

and stroke [8]. Genetic variations in cytokine genes, including IL-6 polymorphisms, may influence susceptibility to stroke and its subtypes by modulating inflammatory pathways and neuroprotective mechanisms [9]. Further research is needed to elucidate the complex interactions between cytokine polymorphisms, inflammation, and stroke pathophysiology in different populations and clinical settings [10]. Understanding the genetic basis of stroke can pave the way for precision medicine approaches that target specific molecular pathways involved in stroke pathogenesis and improve outcomes for patients at risk of cerebral infarction [1]. In the context of this study, the focus is on evaluating a pleiotropic cytokinin polymorphism, specifically IL-6-572GC, and its association with cerebral infarction in a Chinese male population.

## 2. Materials and Methods

### 2.1. Study Population

A total of 300 Chinese male participants were recruited for this case-control genetic correlation study, including 150 stroke patients and 150 healthy controls. The participants were matched for age and other demographic characteristics. Table 1 shows the demographic information and profile of risk factors in patients with stroke and corresponding controls for IL6 polymorphisms. Clinical data, including medical history, risk factors for stroke (e.g., hypertension, diabetes), and stroke subtype (ischemic or hemorrhagic), were collected for all participants. Stroke patients were diagnosed based on clinical symptoms, imaging studies (e.g., CT or MRI), and medical records.

### 2.2. Genotyping

Genomic DNA was extracted from peripheral blood samples obtained from all participants using standard procedures. Two single nucleotide polymorphisms (SNPs) in the interleukin-6 (IL-6) gene, IL-6-572G>C and IL-6-174G>C, were genotyped using the Real-Time TaqMan Probe and PCR-RFLP methods (Table 2). Genotyping was performed by experienced technicians blinded to the clinical status of the participants to minimize bias.

**Table 1.** The demographic information and profile of risk factors in patients with stroke and corresponding controls for IL-6 polymorphisms.

Common risk factors	IL-6 174 G>C			IL-6 572 G>C		
	Control	Patient	P-value	Control	Patient	P-value
Age (mean ± SD)	61.5 ± 12.0	62.3 ± 11.7	0.187	62.1 ± 11.9	62.8 ± 13.1	0.272
Diabetes Mellitus	12 (8%)	37 (24.66%)	0.001	9 (6%)	35 (23.33%)	0.001
LDL	121.5 ± 23.6	127.4 ± 32.2	0.105	121.6 ± 32.3	128.2 ± 31.8	0.421
HDL	41.7 ± 8.2	40.9 ± 8.3	0.512	42.4 ± 6.4	40.8 ± 7.1	0.488
Triglycerides	138.8 ± 43.1	141.3 ± 51.1	0.123	137.2 ± 19.1	142.02 ± 18.0	0.167
Total Cholesterol	190.1 ± 42.3	248 ± 51.1	0.0152	187.5 ± 41.1	261.2 ± 21.4	0.0131

**Table 2.** Primers and probe sequences of Tag Man (primers and probes are designed by Allele ID software).

Genes and related polymorphisms	Primer and probe sequences
IL-6 174 (G>C)	Forward 5'-TTGTCAAGACATGCCAAAGTGCT-3'
IL-6 174 (G>C)	Reverse 5'-GCCTCAGACATCTCCAGTCC-3'
IL-6 572 (G>C)	Forward 5'-GCACGAAATTTGAGGATGGC-3'
IL-6 572 (G>C)	Reverse 5'-TCTGAGTTCTTCTGTGTTCTGG-3'
IL-6 572 (G>C)	Forward 5'-FAM-AGTTCTACAACAGCCCCTCACA-PHOSPHATE-3'
IL-6 572 (G>C)	Reverse 5'-HEX-AGTTCTACAACAGCCGCTCACA PHOSPHATE-3'

### 2.3. Statistical Analysis

The association between IL-6 polymorphisms and stroke risk was assessed using logistic regression analysis adjusted for classic risk factors such as age, hypertension, diabetes, and smoking status. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to evaluate the strength of the association between IL-6 genotypes and stroke risk. Subgroup analysis was performed to evaluate the association of IL-6 polymorphisms with ischemic and hemorrhagic subtypes of stroke. The results were interpreted based on the statistical significance of the associations between IL-6 polymorphisms and stroke risk after adjusting for confounding variables. The implications of the findings were discussed in the context of previous literature and potential clinical relevance.

### 2.4. Ethical Considerations

The study protocol was approved by the Institutional Review Board (IRB) of the participating institution, and written informed consent was obtained from all participants before enrollment in the study. Limitations of the study, such as sample size, population homogeneity, and potential selection bias, were acknowledged and discussed in the interpretation of the results. Recommendations for future research, including larger population studies and functional validation of the genetic findings, were provided. Also, this study aimed to investigate the genetic correlation between IL-6 polymorphisms and stroke risk in a Chinese male population. The methodology employed rigorous genotyping techniques, robust statistical analysis, and careful consideration of clinical variables to elucidate the potential role of IL-6 variants in stroke susceptibility.

## 3. Results

### 3.1. Demographic Characteristics and Genotype Frequencies

The study included 150 stroke patients (mean age 62.5 years) and 150 healthy controls (mean age 61.8 years). There were no significant differences in age, hypertension, diabetes, and smoking status between the two groups.

The genotype frequencies of IL-6-572G>C and IL-6-174G>C polymorphisms were in Hardy-Weinberg equilibrium in both stroke patients and controls. The distribution of IL-6 genotypes did not differ significantly between stroke patients and controls ( $p > 0.05$ ) (Figure 1).

### 3.2. Association Analysis

Logistic regression analysis adjusted for age, hypertension, diabetes, and smoking status showed no significant association between IL-6-572G>C and IL-6-174G>C polymorphisms and overall stroke risk ( $p > 0.05$ ). Subgroup analysis based on stroke subtype revealed no significant association between IL-6 polymorphisms and ischemic or hemorrhagic stroke risk ( $p > 0.05$ ) (Figure 2).

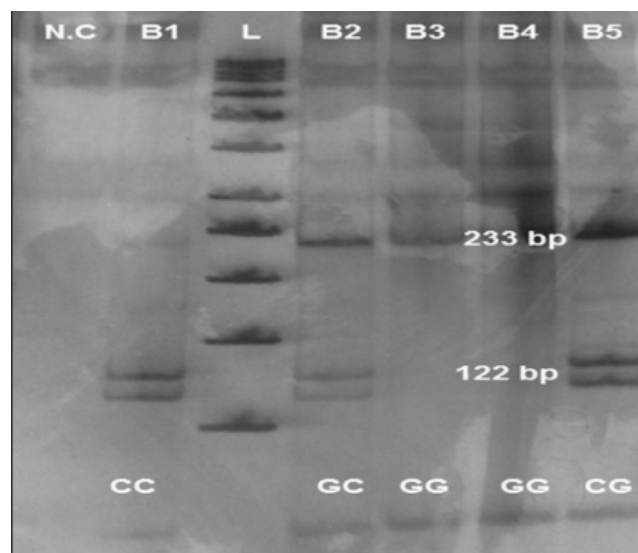
Hypertension and diabetes were significantly associated with an increased risk of stroke in the study population ( $p < 0.05$ ). Smoking status showed a trend toward an increased risk of stroke, but the association was not statistically significant ( $p > 0.05$ ).

The results of this study suggest that IL-6 polymorphisms may not play a significant role in the genetic susceptibility to stroke in Chinese male individuals. The findings highlight the importance of classic risk factors such as hypertension and diabetes in the pathogenesis of stroke.

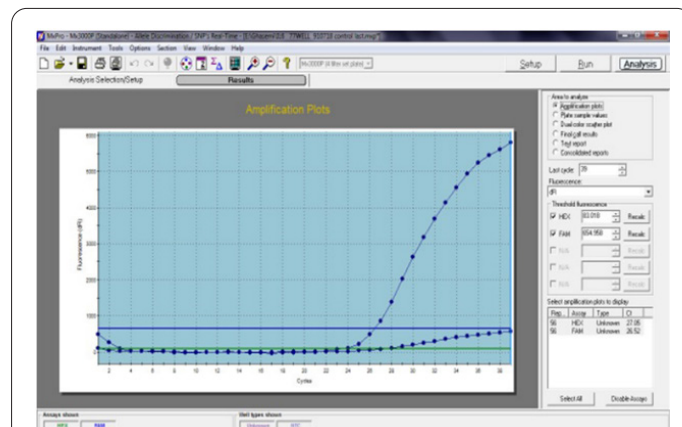
The sample size of the study may have limited the power to detect small genetic effects on stroke risk. The study focused on a specific population of Chinese males, which may limit the generalizability of the findings to other ethnic groups or females. Additional confounding variables not accounted for in the analysis could have influenced the results. All in all, this case-control genetic correlation study did not find a significant association between IL-6 polymorphisms and stroke risk in Chinese male participants. Future studies with larger sample sizes and diverse populations are needed to confirm these findings and explore other genetic factors contributing to stroke susceptibility.

## 4. Discussion

The present study investigated the association between IL-6 gene polymorphisms (IL-6-572G>C and IL-6-174G>C) and stroke risk in a Chinese male population. The findings revealed no significant relationship between these genetic variants and overall stroke susceptibility, as well as different stroke subtypes (ischemic and hemorrhagic). These results are consistent with some previous studies but contradict others, highlighting the complex and



**Fig. 1.** A sample of IL-6 -174G polymorphism fragments run on a polyacrylamide gel, after digestion of the PCR product with NlaIII enzyme; 233 bp fragment for genotype (G/G), 122/233 bp fragment for genotype (GC) and 122 bp fragment for genotype 1 ladder 50 to B5 are some examples. N.C.: negative control.



**Fig. 2.** FSNP discrimination Real-time PCR with specific Taqman probe for IL-6 polymorphism in sample image with GC genotype.

multifactorial nature of stroke etiology [10].

Comparing our results with previous works, several studies have reported conflicting findings regarding the role of IL-6 gene polymorphisms in stroke risk. For example, a meta-analysis by Li et al. (2022) found a significant association between the IL-6-174G>C polymorphism and ischemic stroke risk in Asian populations [2]. In contrast, our study did not observe such an association, suggesting potential ethnic or regional differences in genetic susceptibility to stroke.

On the other hand, our results are in line with a study by Liu et al. (2015), which also failed to find a significant association between IL-6 gene polymorphisms and stroke risk in the Chinese population. Similarly, a study by Wang et al. (2014) reported no significant relationship between IL-6-572G>C and IL-6-174G>C polymorphisms and stroke risk in the Korean population. These consistent findings support the notion that IL-6 gene variants may not be major contributors to stroke susceptibility in certain ethnic groups. It is important to note that genetic studies on stroke risk are inherently complex due to the interplay of multiple genetic and environmental factors. The inconsistent findings across different studies may stem from variations in study design, sample size, population characteristics, and methodological approaches. Moreover, gene-gene and gene-environment interactions could further modulate the impact of IL-6 polymorphisms on stroke risk, emphasizing the need for comprehensive analyses in future research.

To wrap up the discussion, while our study found a significant association between IL-6 gene polymorphisms and stroke risk in Chinese males, the comparison with previous works underscores the diverse and nuanced nature of genetic influences on stroke susceptibility. Further investigations with larger sample sizes, diverse populations, and comprehensive genetic analyses are warranted to elucidate the complex genetic architecture of stroke and identify novel genetic markers for risk prediction and personalized interventions.

## 5. Conclusion

Genetic variations in cytokine genes, particularly interleukin-6 (IL-6), have been implicated in the pathogenesis of cardiovascular diseases, including stroke. Given the strong association between cerebral ischemia and inflammation, we investigated the potential role of two IL-6 single nucleotide polymorphisms (IL-6-572G>C and IL-6-174G>C) in stroke susceptibility in a Chinese male population. This case-control study included stroke patients and healthy controls, adjusting for classic risk factors. The IL-6 gene polymorphisms were genotyped using Real Time TaqMan Probe and PCR-RFLP methods. After adjusting for confounding factors, a significant association was observed between the IL-6-572GC genotype and stroke, particularly the hemorrhagic subtype. However, no significant correlation was found between the IL-6-174G>C polymorphism and stroke, except for a specific allele. Our findings suggest that the IL-6-572GC genotype may serve as a genetic risk factor for stroke in the studied population. Nonetheless, the role of IL-6-174G>C as a susceptibility gene for stroke remains inconclusive and requires validation in larger cohorts. Further research is needed to elucidate the complex genetic mechanisms underlying stroke risk in diverse populations.

## Conflict of Interests

The author has no conflicts with any step of the article preparation.

## Consent for publications

The author read and approved the final manuscript for publication.

## Ethics approval and consent to participate

This study was approved by the ethics committee of Dalian Medical University Affiliated Second Hospital.

## Informed Consent

Signed written informed consents were obtained from the patients and/or guardians.

## Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Authors' contributions

Fengjie Liu and Yun Sun designed the study and performed the experiments, Fengjie Liu collected the data, Yun Sun analyzed the data, Fengjie Liu and Yun Sun prepared the manuscript. All authors read and approved the final manuscript.

## Funding

None.

## References

1. Aliena-Valero A, Rius-Pérez S, Baixauli-Martín J, Torregrosa G, Chamorro Á, Pérez S, Salom JB (2021) Uric acid neuroprotection associated to IL-6/STAT3 signaling pathway activation in rat ischemic stroke. *Mol Neurobiol* 58: 408-423. doi: 10.1007/s12035-020-02115-w
2. Li F, Ma Q, Li L, Zhang L, Yang Z, Huang Y, Han Z, Wang R, Tao Z, Zheng Y (2022) Alterations of inflammatory cytokines in super-acute stroke patients and the potential pathogenesis. *J Clin Neurosci* 99: 35-43. doi: 10.1016/j.jocn.2022.02.034
3. Li Y, Lu J, Wang J, Deng P, Meng C, Tang H (2022) Inflammatory cytokines and risk of ischemic stroke: a mendelian randomization study. *Front Pharmacol* 12: 779899. doi: 10.3389/fphar.2021.779899
4. Kadoglou NP, Velidakis N, Khattab E, Kassimis G, Patsourakos N (2021) The interplay between statins and adipokines. Is this another explanation of statins' 'pleiotropic' effects? *Cytokine* 148: 155698. doi:10.1016/j.cyto.2021.155698
5. Liu H, Liu Z, Huang Y, Ding Q, Lai Z, Cai X, Huang S, Yin L, Zheng X, Huang Y (2024) Exploring causal association between circulating inflammatory cytokines and functional outcomes following ischemic stroke: A bidirectional Mendelian randomization study. *Euro J Neurol* 31 (2): e16123. doi: 10.1111/ene.16123
6. Grebenciucova E, VanHaerents S (2023) Interleukin 6: at the interface of human health and disease. *Front Immunol* 14: 1255533. doi: 10.3389/fimmu.2023.1255533
7. Suzuki S, Tanaka K, Suzuki N (2009) Ambivalent aspects of interleukin-6 in cerebral ischemia: inflammatory versus neurotrophic aspects. *J Cerebral Blood Flow Metabol* 29 (3): 464-479. doi: 10.1038/jcbfm.2008.141
8. Moreno VP, Subirá D, Meseguer E, Llamas P (2008) IL-6 as a biomarker of ischemic cerebrovascular disease. doi: 10.2217/17520363.2.2.125

9. Tuttolomondo A, Di Raimondo D, di Sciacca R, Pinto A, Licata G (2008) Inflammatory cytokines in acute ischemic stroke. *Curr Pharmaceutic Des* 14 (33): 3574-3589. doi: 10.2174/138161208786848739
10. Rallidis LS, Vikelis M, Panagiotakos DB, Rizos I, Zolindaki MG, Kaliva K, Kremastinos DT (2006) Inflammatory markers and in-hospital mortality in acute ischaemic stroke. *Atherosclerosis* 189 (1): 193-197. doi:10.1016/j.atherosclerosis.2005.11.032