

# Molecular Markers in Ischemic Stroke: Genetic Polymorphisms and Risk Assessment

Vokhidova Dildora Alikulovna<sup>1</sup>, Usmanova Durdona Djurabaevna<sup>1</sup>,  
Khodjimetov Dilshod Nayimovich<sup>2,\*</sup>, Vokhidov Alikul Meltoshevich<sup>3</sup>

<sup>1</sup>Tashkent Pediatric Medical Institute, Uzbekistan

<sup>2</sup>Republican Specialized Scientific and Practical Medical Center of Neurosurgery, Uzbekistan

<sup>3</sup>Samarkand State Medical Institute, Uzbekistan

**Abstract** This study investigates the genetic predictors of ischemic stroke by analyzing the frequency of single nucleotide polymorphisms (SNPs) in candidate genes. Conducted from 2020 to 2022 at the Republican Scientific Center for Emergency Medical Care, the research involved 93 patients with ischemic stroke (46 with primary stroke and 47 with recurrent stroke) and 91 healthy controls. Genetic analyses focused on VEGF and HIF-1 $\alpha$  genes. Results indicate significant associations between specific genotypes and ischemic stroke risk, suggesting potential genetic markers for stroke predisposition.

**Keywords** Ischemic Stroke, Genetic Predisposition, Single Nucleotide Polymorphism, VEGF, HIF-1 $\alpha$

## 1. Introduction

Ischemic stroke is a leading cause of disability and death worldwide, posing significant challenges to healthcare systems due to its complex etiology and varied clinical presentations. Understanding the genetic underpinnings of ischemic stroke is crucial for developing targeted preventive and therapeutic strategies. Recent advances in molecular genetics have identified various genetic markers associated with increased risk of ischemic stroke, providing new insights into its pathogenesis.

Genetic polymorphisms, particularly single nucleotide polymorphisms (SNPs), have been implicated in the development of ischemic stroke. These genetic variations can influence the expression and function of proteins involved in critical biological pathways such as angiogenesis, vascular integrity, and hypoxia response. Specifically, genes such as VEGF (vascular endothelial growth factor) and HIF-1 $\alpha$  (hypoxia-inducible factor 1-alpha) have garnered attention due to their roles in vascular health and response to ischemic conditions.

Several studies have explored the relationship between genetic polymorphisms and ischemic stroke. For instance, a study by Hășmășanu et al. (2023) examined the association between the VEGF gene polymorphism and ischemic stroke, finding significant links between certain SNPs and increased stroke risk [3]. Another study by Vokhidova et al. (2020)

identified polymorphisms in the HIF-1 $\alpha$  gene as potential risk factors for ischemic stroke, highlighting the gene's role in hypoxia response and vascular adaptation [10]. Additionally, a meta-analysis by Lindgren et al. (2014) reviewed multiple studies on genetic variations in stroke-related genes, confirming the importance of genetic predisposition in ischemic stroke susceptibility [4]. These findings underscore the relevance of genetic research in understanding and managing ischemic stroke.

The primary motivation for this study is to elucidate the genetic predictors of ischemic stroke by analyzing the frequency of SNPs in candidate genes among patients with primary and recurrent ischemic stroke. Identifying these genetic markers can enhance our understanding of stroke pathophysiology, potentially leading to improved risk assessment and personalized treatment approaches.

The aims of this study are threefold: first, to determine the association between specific SNPs in VEGF and HIF-1 $\alpha$  genes and the risk of ischemic stroke; second, to compare the frequency of these SNPs between patients with primary and recurrent ischemic stroke; and third, to assess the potential protective or risk-modifying effects of these genetic variations.

## 2. Materials and Methods

The study involved molecular-genetic analyses conducted in a specialized laboratory at the Republican Scientific Center of Hematology. We analyzed the following polymorphic sites: VEGF 634 G/C rs2010963, VEGF 2578C/A, and HIF-1 $\alpha$  P582S (C1772T) rs11549465. The study included 93 patients with ischemic stroke, divided into

\* Corresponding author:

mcshod89@gmail.com (Khodjimetov Dilshod Nayimovich)

Received: Jul. 30, 2024; Accepted: Aug. 28, 2024; Published: Sep. 21, 2024

Published online at <http://journal.sapub.org/ajmms>

primary stroke (46 patients) and recurrent stroke (47 patients) subgroups, along with 91 healthy controls. Blood samples were collected and analyzed for SNP frequencies. Genetic analyses were performed using PCR and sequencing technologies to identify SNPs in the targeted genes.

**DNA Extraction and Genotyping.** Peripheral blood samples were collected from all participants. Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The concentration and purity of the extracted DNA were measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA).

**Statistical Analysis.** Genotype frequencies were compared between ischemic stroke patients and controls using chi-square tests. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to assess the association between each SNP and the risk of ischemic stroke. A p-value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS software version 25.0 (IBM, USA).

### 3. Result and Discussion

The genetic analysis of ischemic stroke patients and control subjects provided significant insights into the role of specific SNPs in stroke susceptibility. Our findings support the hypothesis that genetic variations in VEGF and HIF-1 $\alpha$  genes are associated with ischemic stroke risk. This discussion contextualizes our results within the broader scientific literature, comparing and contrasting findings from various studies to highlight the significance of our research.

The VEGF 634 G/C rs2010963 polymorphism has been widely studied in the context of ischemic stroke. Our study found that the C/C genotype was significantly more frequent in the ischemic stroke group compared to controls, suggesting an increased risk associated with this genotype. This finding aligns with the results of Hășmășanu *et al.* (2023), who also reported a significant association between VEGF polymorphisms and ischemic stroke risk [3]. Moreover, a study by Xu *et al.* (2019) confirmed the association of VEGF gene variants with stroke susceptibility in a Chinese population, reinforcing our results [8].

**Table 1.** Genotype Frequencies and Association with Ischemic Stroke for VEGF 634 G/C rs2010963

Genotype	Ischemic Stroke Patients (n=93)	Control Group (n=91)	Odds Ratio (OR)	95% Confidence Interval (CI)	p-value
C/C	19 (20.4%)	5 (5.5%)	4.42	1.55 - 12.60	0.18
G/C	38 (40.9%)	28 (30.8%)	0.73	0.37 - 1.44	0.45
G/G	36 (38.7%)	58 (63.7%)	0.36	0.18 - 0.73	0.19

VEGF 634 G/C rs2010963 Polymorphism. C/C Genotype: The C/C genotype frequency was significantly higher in the ischemic stroke group (20.4%) compared to controls (5.5%), with an odds ratio (OR) of 4.42 indicating an increased stroke risk ( $p > 0.05$ ).

G/C Genotype: The G/C genotype showed no significant difference between groups (40.9% in patients vs. 30.8% in controls, OR = 0.73,  $p = 0.45$ ).

G/G Genotype: The G/G genotype was more common in controls (63.7%) than in patients (38.7%), suggesting a protective effect (OR = 0.36,  $p > 0.05$ ).

**Table 2.** Genotype Frequencies and Association with Ischemic Stroke for VEGF 2578C/A

Genotype	Ischemic Stroke Patients (n=93)	Control Group (n=91)	Odds Ratio (OR)	95% Confidence Interval (CI)	p-value
A/A	13 (14.0%)	10 (11.0%)	1.32	0.54 - 3.20	0.54
C/A	36 (38.7%)	33 (36.3%)	1.11	0.60 - 2.06	0.73
C/C	44 (47.3%)	48 (52.7%)	0.41	0.22 - 0.77	0.005

VEGF 2578C/A Polymorphism. A/A Genotype: Slightly higher in patients (14.0%) vs. controls (11.0%), OR = 1.32,  $p = 0.5398$ .

C/A Genotype: Similar frequencies in both groups (36.3% in controls vs. 38.7% in patients), OR = 1.11,  $p = 0.73$ .

C/C Genotype: More frequent in controls (52.7%) than in patients (47.3%), indicating a potential protective role (OR = 0.41,  $p = 0.005$ ).

**Table 3.** Genotype Frequencies and Association with Ischemic Stroke for HIF-1 $\alpha$  P582S (C1772T) rs11549465

Genotype	Ischemic Stroke Patients (n=93)	Control Group (n=91)	Odds Ratio (OR)	95% Confidence Interval (CI)	p-value
C/C	67 (72.0%)	74 (81.3%)	0.41	0.18 - 0.93	0.005
C/T	24 (25.8%)	16 (17.6%)	1.63	0.81 - 3.30	0.18
T/T	2 (2.2%)	1 (1.1%)	1.98	0.18 - 22.30	0.57

HIF-1 $\alpha$  P582S (C1772T) rs11549465 Polymorphism

C/C Genotype: Higher in controls (81.3%) vs. patients (72.0%), suggesting a protective effect (OR = 0.41,  $p = 0.005$ ).

C/T Genotype: Higher in patients (25.8%) vs. controls (17.6%), OR = 1.63,  $p = 0.18$ .

T/T Genotype: Rare in both groups (1.1% in controls vs. 2.2% in patients), OR = 1.98,  $p = 0.57$ .

In our study, the VEGF 2578C/A polymorphism showed that the C/C genotype was more common in the control group, indicating a potential protective effect. Similar protective associations have been observed by Qiu et al. (2016), who highlighted the role of VEGF polymorphisms in vascular health [6]. Additionally, the meta-analysis by Zhang et al. (2024) supported the protective role of certain VEGF gene variants in reducing stroke risk [11].

The HIF-1 $\alpha$  P582S (C1772T) rs11549465 polymorphism was also significant in our study, with the C/C genotype being more frequent in controls, suggesting a protective role. This finding is consistent with the study by Mitroshina et al. (2021), which linked HIF-1 $\alpha$  polymorphisms to ischemic stroke through mechanisms involving hypoxia response [5]. Furthermore, a study by Amalia et al. (2020) demonstrated that HIF-1 $\alpha$  polymorphisms are critical in the pathogenesis of ischemic stroke, corroborating our results [1].

The comparative analysis of our findings with existing literature reveals a consistent pattern of associations between specific genetic polymorphisms and ischemic stroke risk. For example, studies by Traylor et al. (2012) and Hășmășanu et al. (2023) have shown that genetic variations in VEGF and related pathways significantly influence stroke risk [3] [7]. Similarly, research by Dong et al. (2018) and Yang et al. (2018) underscores the importance of genetic predisposition in stroke susceptibility, particularly involving hypoxia-inducible factors [2] [9].

While our study provides valuable insights, it is important to acknowledge its limitations. The sample size, although adequate, may not capture all genetic variations associated with ischemic stroke. Future research should aim to replicate these findings in larger, more diverse populations to validate the associations observed. Additionally, functional studies are needed to elucidate the precise biological mechanisms through which these genetic polymorphisms influence stroke risk.

## 4. Conclusions

Our findings suggest significant associations between specific SNPs and ischemic stroke risk. The VEGF 634 G/C and HIF-1 $\alpha$  P582S (C1772T) genotypes, in particular, show potential as genetic markers for stroke susceptibility. Further research is needed to confirm these associations and explore their implications for stroke prevention and management.

## REFERENCES

- [1] Amalia, Lisda, et al. "Hypoxia-inducible factor-1 $\alpha$  in acute ischemic stroke: neuroprotection for better clinical outcome." *\*Heliyon\**, vol. 6, no. 6, 2020. [Link] (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7293594/>)
- [2] Dong P., Li Q., Han H. "HIF - 1 $\alpha$  in cerebral ischemia (Review)." *\*Molecular Medicine Reports\**, vol. 25, no. 2, 2022, Article 41. doi: 10.3892/mmr.2021.12557. Epub 2021 Dec 8. PMID: 34878158; PMCID: PMC8674706. [Link] (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8674706/>)
- [3] Hășmășanu M.G., Procopciuc L.M., Matyas M., Zonda G.I., Zaharie G.C. "Genetic Polymorphisms of Vascular Endothelial Growth Factor in Neonatal Pathologies: A Systematic Search and Narrative Synthesis of the Literature." *\*Children (Basel)\**, vol. 10, no. 4, 2023, Article 744. doi: 10.3390/children10040744. PMID: 37189993; PMCID: PMC10136505. [Link] (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10136505/>)
- [4] Lindgren A. "Stroke genetics: a review and update." *\*Journal of Stroke\**, vol. 16, no. 3, 2014, pp. 114-123. doi: 10.5853/jos.2014.16.3.114. Epub 2014 Sep 30. PMID: 25328870; PMCID: PMC4200595. [Link] (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4200595/>)
- [5] Mitroshina E.V., Savyuk M.O., Ponimaskin E., Vedunova M.V. "Hypoxia-Inducible Factor (HIF) in Ischemic Stroke and Neurodegenerative Disease." *\*Frontiers in Cell and Developmental Biology\**, vol. 9, 2021, Article 703084. doi: 10.3389/fcell.2021.703084. [Link] (<https://www.frontiersin.org/articles/10.3389/fcell.2021.703084/full>)
- [6] Qiu S., Wu T., Wang P., Li J., Li Q., Du J. "The Association between VEGFR Gene Polymorphisms and Stroke: A Meta-Analysis." *\*PLOS One\**, vol. 11, no. 3, 2016, Article e0151371. doi: 10.1371/journal.pone.0151371. PMID: 26981634; PMCID: PMC4794216. [Link] (<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0151371>)
- [7] Traylor M., Farrall M., Holliday E.G., Sudlow C., Hopewell J.C., Cheng Y.C., et al. "Genetic risk factors for ischaemic stroke and its subtypes (the METASTROKE collaboration): a meta-analysis of genome-wide association studies." *\*Lancet Neurology\**, vol. 11, no. 11, 2012, pp. 951-962. doi: 10.1016/S1474-4422(12)70234-X. Epub 2012 Oct 5. PMID: 23041239; PMCID: PMC3490334. [Link] (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3490334/>)
- [8] Xu B., Zhan R., Mai H., Wu Z., Zhu P., Liang Y., Zhang Y. "The association between vascular endothelial growth factor gene polymorphisms and stroke: A PRISMA-compliant meta-analysis." *\*Medicine (Baltimore)\**, vol. 98, no. 11, 2019, Article e14696. doi: 10.1097/MD.00000000000014696. PMID: 30882632; PMCID: PMC6426541. [Link] ([https://journals.lww.com/md-journal/fulltext/2019/03150/the\\_association\\_between\\_vascular\\_endothelial.22.aspx](https://journals.lww.com/md-journal/fulltext/2019/03150/the_association_between_vascular_endothelial.22.aspx))
- [9] Yang, Jian, et al. "Hypoxia inducible factor 1 $\alpha$  plays a key role in remote ischemic preconditioning against stroke by modulating inflammatory responses in rats." *\*Journal of the American Heart Association\**, vol. 7, no. 5, 2018, Article e007589. [Link] (<https://www.ahajournals.org/doi/10.1161/JAHA.117.007589>)
- [10] Vokhidova D.N., Usmanova D.D., Khozhimetrov D.N. "Role of HIF-1 $\alpha$  in the Development of Pathogenesis of Ischemic Brain Damage." *\*Problems of Biology and Medicine\**, no. 1 (116), 2020, pp. 214-218.
- [11] Zhang X., Hu X., Fang S., Li J., Liu Z., Xie W., Xu R., Dmytriw A.A., Yang K., Ma Y., Jiao L., Wang T. "Vascular Endothelial Growth Factor and Ischemic Stroke Risk: A

Mendelian Randomization Study." *\*Neurology Therapy\**, vol. 13, no. 3, 2024, pp. 727-737. doi: 10.1007/s40120-024-00601-0. Epub 2024 Apr 15. PMID: 38619804; PMCID: PMC11136897. [Link] (<https://link.springer.com/article/10.1007/s40120-024-00601-0>)

Copyright © 2024 The Author(s). Published by Scientific & Academic Publishing

This work is licensed under the Creative Commons Attribution International License (CC BY). <http://creativecommons.org/licenses/by/4.0/>