Correlation of P352L, R538G Col4A1 Gene, RS951733 Col4A2 Gene Locus Polymorphisms, IFN-□, IL-17, and VE-Cadherin Levels with the Event of Non-Lobar Spontaneous Intracerebral Hemorrhage in Hypertension Patients: Research Protocol

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Abstract

Background: Stroke is an acute and focal neurological deficit syndrome. By definition, it can be interpreted as a clinical syndrome resulting from vascular injury (infarction, bleeding) in the central nervous system. Hypertension is the main risk factor of non-lobar intracerebral hemorrhage (ICH), in which around 85% of ICH patients are hypertensive. Hypertension is often found to damage the basement membrane of blood vessel walls. It can also induce tissue injury by increasing the inflammatory cytokines IL 17 and IFN-? and reducing the expression of VE-Cadherin. Genetically, mutation of the genes Col4A1 and Col4A2 has been correlated with the incidence of non-lobar intracerebral hemorrhage.

Objective: The aim is to investigate the correlation between the P352L, R538G Col4A1 gene, RS9521733 Col4A2 gene locus polymorphisms, levels of IFN-?, IL-17, and VE-Cadherin with the event of non-lobar spontaneous intracerebral hemorrhage in hypertensive patients.

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Research Protocol

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ABSTRACT

Background: Stroke is an acute and focal neurological deficit syndrome. By definition, it can be interpreted as a clinical syndrome resulting from vascular injury (infarction, bleeding) in the central nervous system. Hypertension is the main risk factor of non-lobar intracerebral hemorrhage (ICH), in which around 85% of ICH patients are hypertensive. Hypertension is often found to damage the basement membrane of blood vessel walls. It can also induce tissue injury by increasing the inflammatory cytokines IL 17 and IFN-γ and reducing the expression of VE-Cadherin. Genetically, mutation of the genes Col4A1 and Col4A2 has been correlated with the incidence of non-lobar intracerebral hemorrhage.

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Conclusion: A study of the relationship between Col4A1, Col4A2 gene polymorphisms, levels of IFN-γ, IL-17, and VE-Cadherin on the incidence of non-lobar intracerebral hemorrhage in hypertension patients is expected to increase understanding and expand knowledge of the pathophysiology of the risk of non-lobar intracerebral hemorrhage in hypertension patients.

Keywords: Non-Lobar Intracerebral Haemorrhage, Hypertension, Col4A1, Col4A2, Interleukin-17, Interveron-γ, VE-Cadherin, Elisa, polymerase chain reaction.

Introduction

Stroke is an acute and focal neurological deficit syndrome. By definition, it can be interpreted as a clinical syndrome resulting from vascular injury (infarction, bleeding) in the central nervous system. Stroke is the second leading cause of death and disability in the world. Stroke is not a single disease but can be caused by various risk factors, disease processes, and mechanisms. Intracerebral hemorrhage is a very severe form of stroke, which is defined as brain damage associated with the acute release of blood and blood components into the brain parenchyma due to the rupture of cerebral blood vessels. Intracerebral hemorrhage in America accounts for 10% of all 795,000 stroke cases [1].

Non-lobar intracerebral hemorrhage (ICH) is often associated with hypertension as the

highest risk factor for intracerebral hemorrhage, namely around 85% [2]. Hypertension is often found to damage the basement membrane of blood vessel walls. The basement membrane in the brain has the main component Collagen IV. Collagen is the main component of balance by maintaining the integrity and stability of blood vessel walls. Unbalanced collagen turnover by uncontrolled formation and/or degradation can lead to pathological conditions such as fibrosis. Thinning of the walls due to collagen degradation or deficiency can cause rupture of the blood vessel wall or aneurysm. Type IV collagen, together with laminin, forms a strong extracellular matrix network stabilized by nidogen and perlecan bridges [3].

The Col4A1 mutation was found to be a missense mutation retaining a glycine residue in the triple helix domain of Col4A1 (p.G627W). The advantage of proximity and line of sight in regulating the Col4A1 and Col4A2 genes, a targeted mutation generation approach was used to deactivate the function of these genes simultaneously. The mutation target is to delete exon 1 of Col4A1 and exons 1-3 of Col4A2 so that these two genes do not have alleles [4]. Col4A2 is significantly associated with the deep ICH phenotype, namely RS9521732 [5], while Col4A1, namely p.P352L and p.R538G, is sporadic (non-familial) and late-onset intracerebral hemorrhage [6].

Apart from hypertension being caused by abnormalities in the basement membrane of the blood vessel walls, hypertension can also increase inflammatory processes. Chronic hypertension can induce tissue injury, which together with oxidative stress caused by vasoactive peptides such as Angiotensin-II (Ang-II) or endothelin-1 creates favorable conditions for the development of damage-associated molecular patterns (DAMPs) and neoantigens, such as additional isoskeletal protein products. DAMP will activate innate immunity through toll-like receptors (TLR) on type 1 macrophages, type 1 dendritic cells, natural killer-β (NK-β) cells, and IL-23, causing T cell proliferation and production of IL-17A, IFN-γ, and TNF-α [7]. IFN-γ affects tight junctions and causes endothelial dysfunction at the blood-brain barrier by damaging pericyte function and increasing Rho kinase activity [8]. Meanwhile, IL-17 will cause damage to the thigh junction in the blood-brain barrier by increasing the permeability of brain endothelial cells and reducing the expression of the thigh junction molecules occludin and zooccludin-1 (ZO-1) [9].

Another factor is that hypertension induces hypertrophy of the endothelial monolayer and increases the ratio of tight junctions relative to the lateral membrane surface, which are associated with a continuous increase in blood pressure. Stressed cells show an elongated and tortuous shape along with a multilayered structure and less VE-cadherin expression. Vascular endothelial cadherin (VE-Cadherin) is an endothelium-specific adhesion molecule located at the junctions between

endothelial cells. VE-Cadherin has a major function in maintaining vascular integrity through vascular permeability and inhibiting uncontrolled vascular growth. The mechanism of action of VE-Cadherin is very complex and includes remodeling and organization of the endothelial cell cytoskeleton and modulation of gene transcription [10]. VE-Cadherin dysfunction can be caused by disturbances in its receptors, resulting in disruption of the maintenance of the physical integrity of the endothelial junctions of blood vessels, and regulation of the opening and closing of endothelial junctions [11].

Research on hypertension, Col4A1, Col4A2, IFN-γ, IL-17, and VE-Cadherin has been widely carried out. However, a better understanding of the genes, immunology, and endothelial factors that influence the occurrence of intracerebral hemorrhage can provide insight into the dominant factors in the occurrence of intracerebral hemorrhage in hypertensive patients. Therefore, this protocol was designed to investigate the relationship between the P352L, R538G Col4A1 Gene, and RS9521733 Col4A2 Gene Locus Polymorphisms, Levels of IFN- γ, IL-17, and VE-Cadherin with the Event of Non-Lobar Spontaneous Intracerebral Hemorrhage in Hypertension Patients.

Methods

Research design

This research will be an observational study with a comparative cross-sectional approach where the dependent and independent variables will be examined at the same time. This research was designed to investigate the relationship between the P352L, R538G Col4A1 Gene, and RS9521733 Col4A2 Gene Locus Polymorphisms, IFN- γ, IL-17 Levels, and VE-Cadherin with the Event of Spontaneous Non-Lobar Intracerebral Hemorrhage in Hypertension Patients.

Ethics Approval

Approval for this research protocol was granted by the Medical and Health Research Ethics Unit, Faculty of Medicine, Riau University with number: No: B/ 159 /UN19.5.1.1.8/UEPKK/2023.

Consent to Participate and Publication

Consent for data collection and publication will be obtained from the patient (control) and the patient's immediate family (sample). Consent will be requested after the patient and immediate family have received an explanation from the principal investigator.

The data collected will be stored and uploaded via the internet without any identification.

This protocol was prepared in accordance with the 2013 Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) checklist for reporting protocol studies.

Population and Sample

Research Population

1. The population in this study will be all patients with hypertension who experienced intracerebral hemorrhage and came to the emergency room. Hypertensive patients who do without intracerebral hemorrhage will serve as controls.
2. Research Sample

   The sample will be part of the population that meets the inclusion and exclusion criteria, using the formula for nominal sample size:

   a. Sample size, Sampling will be carried out by consecutive sampling. To fulfill adequate statistical calculations, a sample is needed according to the formula Unpaired Categorical Comparative Analysis, namely :

$$n1 = n2 = \left[\frac{Z_\alpha \sqrt{2PQ} + Z_\beta \sqrt{P_1Q_1 + P_2Q_2}}{P_1 - P_2} \right]^2$$

   so that the minimum number of samples for each group will be 34 people (n1=n2), rounded to 40 samples for each group so that the total required number of samples will be 80 people.

   b. Sample Criteria

   1. Sample Inclusion Criteria:
      b. Agree to take part in the research by filling out informed consent
      c. Hypertensive patients diagnosed with intracerebral hemorrhage by a head CT-Scan
      d. Age > 18 years
   2. Sample Exclusion Criteria:
      a. Patients with a diagnosis of coagulation factor disorders
      b. Patients with intracerebral hemorrhage related to trauma
      c. Use of anti-platelet drugs, thrombolytics, anticoagulants, or traditional medicines before inclusion.
      d. Use of corticosteroid drugs for a long time.

c. Control Criteria :

1. Control Inclusion Criteria:
   a. Agree to take part in the research by filling out informed consent.
   b. Hypertensive patients without intracranial bleeding.
   c. Age > 18 years.
2. Control Exclusion Criteria:
   a. Patients with a diagnosis of coagulation factor disorders
   b. Using corticosteroid drugs for a long time
   c. Use of anti-platelet drugs, thrombolytics, anticoagulants, or traditional medicines before inclusion.
   d. Age < 18 years

Participants in this study will be registered using a sequential recruitment method. The immediate family of sufferers of intracerebral hemorrhage stroke and sufferers of essential hypertension who meet the requirements will receive information about the research procedures and will be allowed to decide whether they want to take part in the study for the duration of the study.

Research purposes

We can divide the objectives of this research into:

1. General Objectives

   Proving that there is a relationship between the P352L Locus Polymorphism, R538G Col4A1 Gene, and RS9521733 Col4A2 Gene, Levels of IFN- γ, IL-17, and VE-Cadherin with the Event of Non-Lobar Spontaneous Intracerebral Hemorrhage in Hypertension Patients.

2. Specific Objectives
   a. Analyzing the relationship between the P352L, R538G Col4A1 gene, and RS9521733 Col4A2 gene polymorphisms on the incidence of non-lobar spontaneous intracerebral hemorrhage in hypertension sufferers and control sufferers.
   b. Analyzing IL-17 levels in the incidence of spontaneous non-lobar intracerebral hemorrhage in hypertension sufferers and control sufferers.
   c. Analyzing IFN-γ levels in the incidence of spontaneous non-lobar intracerebral hemorrhage in hypertensive patients and control patients.
   d. Analyzing VE-Cadherin levels in the incidence of spontaneous non-lobar intracerebral hemorrhage in hypertensive patients and control patients.

e. Analyzing the most dominant variables in the incidence of non-lobar spontaneous intracerebral hemorrhage in hypertension sufferers and control sufferers.

Nature of Research and Sample Technique

This will be a quantitative research with comparative methods. The sampling technique used in this study will be consecutive sampling, that is, sampling will be carried out on all subjects who met the selection criteria and included in the study until the number of subjects was met. The blood samples will undergo similar handling for examination at the molecular laboratory at the Faculty of Medicine, Andalas University.

Research Process and Data Collection

The research process will be as follows:

1. Specimen collection

   In this study, 3 tubes of EDTA blood samples will be collected for each sample. The samples will be taken to the FK-UNRI Biomolecular Laboratory, centrifuged, and separated into red blood cells, serum, and whole blood in micro tubes. The separated specimens will then be stored in a freezer (-400 C). Next, it will be sent to the Biomolecular Laboratory of FK Unand for PCR and ELISA.

2. Total DNA isolation

   The working procedure for blood DNA isolation using the GeneJet Thermo Fisher kit will be as follows:

   a. Prepare blood/whole blood in an EDTA Vacutainer
   b. Take a 200 µL blood sample into a sterile 1.5 mL tube, add 400 µL of lysis solution and 20 µL of proteinase K, and mix gently with Virtex or pipetting.
   c. Incubate at 56°C for 10 minutes. While vortexing several times during the incubation period.
   d. Add 200 µL EtOH absolute and mix with a vortex or pipette up and down.
   e. Transfer the lysate (liquid mixture in the previous step) into a spin column that has been paired with a collection tube.
   f. Centrifuge (Thermo Scientific) 6,000 x g for 1 minute. Then discard the collection tube containing the liquid that fell during centrifugation
   g. Move the spin column to the new collection tube
   h. Add 500 µL Washing Buffer I (EtOH added first) to the spin column. And centrifuge (Thermo Scientific) 8,000 x g for 1 minute.

i. Add 500 µL Washing Buffer II (EtOH has been added first) to the spin column. And centrifuge (Thermo Scientific) at maximum speed (> 12,000 x g) for 3 minutes.
j. Empty the collection tube and re-centrifuge again at maximum speed for 1 minute
k. Discard the collection tube and place the spin column into a sterile 1.5 mL microcentrifuge tube.
l. Add 50-100 µL Elution Buffer to the center of the spin columns. Incubate RT for 2 minutes and centrifuge at 8,000 x g for 1 minute (Discard the spin column, and in the microtube there is DNA isolate.
m. DNA can be used directly or stored in the freezer for subsequent PCR use.

3. The PCR working procedures will be as follows:
   - Into the PCR tube (0.25ml tube) completely insert the following components :

No.	PCR Component	Volume (µL)
1.	Bioline Mytaq PCR mix,	12,5
2.	Primer -F	1
3.	Primer -R	1
4.	ddH_2O	8.5
5.	Sample DNA	2
	Total	25

   - Centrifuge for a few seconds so that all components are at the bottom of the tube
   - Insert it into the PCR machine and set the PCR program as shown in the following table :

No.	Tahap PCR	Suhu (°C)	Durasi	Siklus
1.	Initial denaturation	95	2 minute	
2.	Further denaturation	95	15 minute	35 times
3.	Annealing	60	15 minute	
4.	Elongation	72	10 minute	
5.	Final Elongation	72	5 minute	

   - After the PCR process is complete, carry out electrophoresis to see the PCR results
   - If after electrophoresis a bright DNA band is obtained and the size matches the target, the sample can be sent for sequencing.

4. The ELISA working procedure will be as follows:
   a. Prepare all reagents, standard solutions, and samples at room temperature before use
   b. Determine the number of wells to be used according to the number of samples to be examined. Store remaining wells that are not used at a temperature of 2-8°C.
   c. Add 50µl of standard solution into the standard well.

d. Add 40µl sample into sample wells and add 10µl biotylated antibody into sample wells, and add 50µl streptavidin-HRP into sample wells and standard wells. Mix well. Cover with sealer. Incubate 60 minutes at 37°C.
e. Rinse the plate 5 times with wash buffer
f. Add 50µl of substrate solution A into each well and 50µl of substrate solution B into each well. Incubate the plate for 10 minutes at 37°C in a dark room.
g. Add 50µl Stop Solution into each well, the color of the liquid will change from blue to yellow.
h. Determine the absorbance value (OD value) of each well using a 450 nm microplate reader immediately after adding the stop solution.

Data Analysis

After data collection, the editing, coding, entry, and cleaning processes will be done manually using a computer. Next, data analysis will be carried out.

1. Descriptive analysis

   This descriptive analysis will be used to determine the characteristics of the research samples. Data analysis to see an overview of the proportions of each variable will be presented using a table.

2. Univariate analysis

   Univariate analysis will be used to see the distribution of data for each variable and then presented in the form of a table or diagram. The data will consist of characteristics of Interferon-γ levels, interleukin-17 levels, and expression of the Col4A2-Col4A1 polymorphism.

3. Bivariate Analysis

   Bivariate analysis using chi-square will be used to see differences in Interferon- γ levels, interleukin-17 levels, VE-Cadherin levels, and the expression of the Col4A2-Col4A1 polymorphism between the two groups. Statistical significance will be set at 0.05.

Expected results

This research has been ongoing since May 2023. The results of this research are expected to be available by the end of 2024.

Discussion

Intracerebral hemorrhage (ICH) is a type of stroke characterized by bleeding within the brain tissue. ICH is a life-threatening disease. This disease accounts for 15-30% of all strokes and is associated with high morbidity and mortality. ICH is most often caused by hypertension, arteriovenous malformation, or head trauma. Symptoms of ICH can include headache, nausea, vomiting, lethargy, confusion, sudden weakness or numbness, loss of consciousness, and seizures. ICH treatment focuses on stopping bleeding, removing blood clots (hematomas), and reducing pressure on the brain. Medical or surgical treatment is performed depending on the cause, location, and size of the hematoma.

Non-lobar intracerebral hemorrhage (ICH) refers to bleeding that occurs in areas of the brain other than the lobes, such as the basal ganglia, thalamus, cerebellum, brain stem, and internal capsule. Lobar ICH, on the other hand, occurs in the cerebral cortex and subcortical regions. Non-lobar ICH is more frequently associated with small vessel disease, such as hypertensive vasculopathy, and is classified as primary intracerebral hemorrhage. Risk factors for non-lobar ICH are similar to lobar ICH, however the underlying vascular disease may differ in etiology [12].

Cerebral vascular damage associated with hypertension involves multiple pathophysiological mechanisms that contribute to the development of stroke, cerebral small vessel disease, and cognitive decline. Some important aspects include [12] [13] :

1. Endothelial dysfunction: impaired endothelium-dependent vasodilation leads to decreased regulation of cerebral blood flow and increased susceptibility to cerebral ischemia.
2. Arterial stiffness: as hypertension progresses, the arteries become stiffer, resulting in increased pulse wave velocity and increased transmission of aortic pulsatility to the brain, causing increased pulsatility in the large blood vessels of the brain.
3. Autonomic dysfunction: changes in the balance between the sympathetic and parasympathetic nervous systems lead to changes in blood pressure variability, which contributes to the risk of ischemic and intracerebral hemorrhage.
4. Oxidative stress: increased production of free radicals damages blood vessel endothelium and other tissue structures in the brain.
5. Inflammation: activation of immune cells and release of proinflammatory molecules exacerbate cerebral vascular injury.
6. Altered cerebral autoregulation: cerebral autoregulation that maintains constant cerebral blood flow during blood pressure fluctuation becomes less effective in conditions of prolonged hypertension.
7. Extracellular matrix remodeling: changes in the composition of the extracellular matrix affect the

structural integrity of cerebral blood vessels.

8. Renin-angiotensin-aldosterone system (RAAS): excessive RAAS activation plays an important role in increasing hypertension and cerebral vascular damage [14].

These mechanisms interact in a complex manner to produce chronic cerebrovascular injury, which manifests clinically as stroke, subclinical cerebrovascular abnormalities, and cognitive decline.

Intracerebral hemorrhage is a multifactorial disease caused by several interacting and overlapping risk factors and etiologies. The etiology of intracerebral hemorrhage is complex and varies according to a person's age and predisposing factors. Risk factors for intracerebral hemorrhage include hypertension, cerebral amyloid angiopathy, use of anticoagulants, and structural vascular abnormalities. The causes of stroke recurrence are also multifactorial, and the subtypes of index stroke and recurrent stroke are often not the same [15]. The presentation and evaluation of intracranial hemorrhage on MRI depends primarily on the onset of the hemorrhage and other factors such as hematoma location, local partial pressure of oxygen in the tissue, local pH, patient hematocrit, local glucose concentration, and blood glucose levels, hemoglobin concentration, blood-brain-barrier integrity, and patient’s body temperature [16]. Overall, the etiologies of intracerebral hemorrhage are multifactorial and complex, with multiple risk factors contributing to its development.

COL4A1 and COL4A2 are genes that encode the alpha chain of type IV collagen, which is a major component of the basement membrane (BM). BM mainly consists of extracellular matrix (ECM) proteins including collagen IV, fibronectin, laminin, nidogen, and heparin sulfate proteoglycans such as perlecan and agrin [17]. COL4A1 and COL4A2 are genes that code for collagen type IV alpha-1 and alpha-2 chains, respectively. Variants or mutations in this gene have been associated with cerebral small vessel disease, which can cause intracerebral hemorrhage (ICH) [18]. Rare functional missense variants in COL4A1 and COL4A2 have been identified to be associated with sporadic ICH, and annotation and simulation studies suggest that these variants are highly functional and may represent treatment targets [19].

VE-cadherin is a transmembrane adhesion protein that connects with proteins on adjacent cells and closes the spaces between cells. VE-cadherin is a transmembrane adhesion protein that connects with proteins on adjacent cells and closes the intercellular space [17]. VE-cadherin plays an important role in intracerebral hemorrhage (ICH)-related complications due to its involvement in blood-brain barrier (BBB) integrity and maintenance of endothelial cell junctions. Studies have shown that VE-cadherin is particularly relevant in the context of intraventricular hemorrhage (IVH), which often accompanies ICH and can lead to hydrocephalus. In a mouse model of IVH, VE-

cadherin levels are decreased in the choroid plexus, contributing to the development of hydrocephalus. Metformin has been identified as a potential protective agent because it maintains VE-cadherin expression in the choroid plexus, which is dependent on the suppression of the VEGF/ VEGFR2/p-Src pathway [20]. In addition, the interaction of VE-cadherin with other proteins such as p120-catenin and β-catenin is critical for maintaining vascular integrity. Loss of VE-cadherin function can disrupt junctional complexes and alter the localization of other junctional proteins, leading to increased vascular permeability and impaired BBB function [20]. These findings suggest that VE-cadherin is a key player in the pathophysiology of ICH and associated complications, making it a promising target for developing therapies aimed at protecting the BBB and improving patient outcomes after intracranial hemorrhage incidents.

The role of IL-17 and interferon-gamma (IFN-γ) in intracerebral hemorrhage (ICH) has been the subject of research. A study found a significant negative correlation between serum IL-17 levels and neurological recovery status in ICH patients, suggesting a neurotoxic role of IL-17 in neural stem cell differentiation after ICH [21]. Another study stated that IL-17 and IFN-γ mRNA expression increased in the brain and systemically after ICH, indicating their potential involvement in the inflammatory response after ICH [22]. Furthermore, interactions between IFN-γ-secreting Th1 cells and IL-17-secreting Th17 cells may contribute to the inflammatory cascade in ICH [23]. However, the specific mechanisms and therapeutic implications of IL-17 and IFN-γ in ICH require further investigation. These findings suggest that IL-17 and IFN-γ may play a role in the inflammatory response and neurological damage after ICH. Further research is needed to fully understand its specific contribution and potential as a therapeutic target.

Thank you note

The authors would like to thank the family and medical staff for the support provided in conducting this research.

Abbreviation

ICH : Intracerebral haemorhage

Col4A1 : Collagen IV Alpha-1

Col4A2 : Collagen IV Alpha-2

IL-17 : Interleukin 17

IFN-γ : Interferon Gamma
VE-Cadherin : Vascular Endothelial-Cadherin
Elisa : Enzyme-linked immunosorbent assay
PCR : Polymerase chain reaction
DAMPs : Damage-associated molecular patterns
TLRs : Toll-like receptors
ZO-1 : Zonula occludens-1
DNA : Deoxyribonucleic acid
RAAS : Renin angiotensin aldosteron system
BM : Base membrane
BBB : Blood brain barier
IVH : Intraventricular haemorhage
VEGF : Vascular endothelial growth Factor

Conflict of interest

The authors have no conflict of interest.

Reference

[1] S. M. Greenberg *et al.*, *2022 Guideline for the Management of Patients With Spontaneous Intracerebral Hemorrhage: A Guideline From the American Heart Association/American Stroke Association*, vol. 53, no. 7. 2022.

[2] S. J. Murphy and D. J. Werring, “Stroke: causes and clinical features,” *Med. (United Kingdom)*, vol. 48, no. 9, pp. 561–566, 2020, doi: 10.1016/j.mpmed.2020.06.002.

[3] L. Xu, A. Nirwane, and Y. Yao, “Basement membrane and blood-brain barrier,” *Stroke Vasc. Neurol.*, vol. 4, no. 2, pp. 78–82, 2019, doi: 10.1136/svn-2018-000198.

[4] M. Jeanne, J. Jorgensen, and D. B. Gould, “Molecular and genetic analyses of collagen type IV mutant mouse models of spontaneous intracerebral hemorrhage identify mechanisms for stroke prevention,” *Circulation*, vol. 131, no. 18, pp. 1555–1565, 2015, doi: 10.1161/CIRCULATIONAHA.114.013395.

[5] K. Rannikmäe *et al.*, “Common variation in COL4A1/COL4A2 is associated with sporadic cerebral small vessel disease,” *Neurology*, vol. 84, no. 9, pp. 918–926, 2015, doi: 10.1212/WNL.0000000000001309.

[6] Y. C. Weng *et al.*, “COL4A1 mutations in patients with sporadic late-onset intracerebral

hemorrhage," *Ann. Neurol.*, vol. 71, no. 4, pp. 470–477, 2012, doi: 10.1002/ana.22682.

[7] A. Caillon, P. Paradis, and E. L. Schiffrin, "Role of immune cells in hypertension," *Br. J. Pharmacol.*, vol. 176, no. 12, pp. 1818–1828, 2019, doi: 10.1111/bph.14427.

[8] D. Jansson *et al.*, "Interferon-γ blocks signalling through PDGFRβ in human brain pericytes," *J. Neuroinflammation*, vol. 13, no. 1, pp. 1–19, 2016, doi: 10.1186/s12974-016-0722-4.

[9] C. Sierra, A. Coca, and E. L. Schiffrin, "Vascular mechanisms in the pathogenesis of stroke," *Curr. Hypertens. Rep.*, 2011, doi: 10.1007/s11906-011-0195-x.

[10] M. G. Lampugnani, E. Dejana, and C. Giampietro, "Vascular endothelial (VE)-cadherin, endothelial adherens junctions, and vascular disease," *Cold Spring Harb. Perspect. Biol.*, vol. 10, no. 10, 2018, doi: 10.1101/cshperspect.a029322.

[11] X. Cong and W. Kong, "Endothelial tight junctions and their regulatory signaling pathways in vascular homeostasis and disease," *Cell. Signal.*, vol. 66, p. 109485, 2020, doi: 10.1016/j.cellsig.2019.109485.

[12] P. H. C. Kremer *et al.*, "Risk factors for lobar and non-lobar intracerebral hemorrhage in patients with vascular disease," *PLoS One*, vol. 10, no. 11, pp. 1–10, 2015, doi: 10.1371/journal.pone.0142338.

[13] T. Di Chiara *et al.*, "Pathogenetic Mechanisms of Hypertension–Brain-Induced Complications: Focus on Molecular Mediators," *Int. J. Mol. Sci.*, vol. 23, no. 5, 2022, doi: 10.3390/ijms23052445.

[14] G. Rossi, A. Rossi, A. Sacchetto, E. Pavan, and A. C. Pessina, "Hypertensive Cerebrovascular Disease and the Renin-Angiotensin System," *Stroke*, vol. 26, no. 9, pp. 1700–1706, Sep. 1995, doi: 10.1161/01.STR.26.9.1700.

[15] M. A. Ikram, R. G. Wieberdink, and P. J. Koudstaal, "International Epidemiology of Intracerebral Hemorrhage," *Curr. Atheroscler. Rep.*, vol. 14, no. 4, pp. 300–306, Aug. 2012, doi: 10.1007/s11883-012-0252-1.

[16] T. Hillen, C. Coshall, K. Tilling, A. G. Rudd, R. McGovern, and C. D. A. Wolfe, "Cause of Stroke Recurrence Is Multifactorial," *Stroke*, vol. 34, no. 6, pp. 1457–1463, Jun. 2003, doi: 10.1161/01.STR.0000072985.24967.7F.

[17] T. Okada, H. Suzuki, Z. D. Travis, and J. H. Zhang, "The Stroke-Induced Blood-Brain Barrier Disruption: Current Progress of Inspection Technique, Mechanism, and Therapeutic Target," *Curr. Neuropharmacol.*, vol. 18, no. 12, pp. 1187–1212, Dec. 2020, doi: 10.2174/1570159X18666200528143301.

[18] S. Guey and D. Hervé, "Main features of COL4A1-COL4A2 related cerebral

microangiopathies," *Cereb. Circ. - Cogn. Behav.*, vol. 3, p. 100140, 2022, doi: 10.1016/j.cccb.2022.100140.

[19] J. Chung *et al.*, "Rare Missense Functional Variants at COL4A1 and COL4A2 in Sporadic Intracerebral Hemorrhage," *Neurology*, vol. 97, no. 3, pp. E236–E247, 2021, doi: 10.1212/WNL.0000000000012227.

[20] D. Shen *et al.*, "Metformin Preserves VE–Cadherin in Choroid Plexus and Attenuates Hydrocephalus via VEGF/VEGFR2/p-Src in an Intraventricular Hemorrhage Rat Model," *Int. J. Mol. Sci.*, vol. 23, no. 15, 2022, doi: 10.3390/ijms23158552.

[21] L. Gao *et al.*, "Neurotoxic role of interleukin-17 in neural stem cell differentiation after intracerebral hemorrhage," *Neural Regen. Res.*, vol. 15, no. 7, p. 1350, 2020, doi: 10.4103/1673-5374.272614.

[22] Q. Zhang *et al.*, "Interleukin-17 and ischaemic stroke," *Immunology*, vol. 162, no. 2, pp. 179–193, Feb. 2021, doi: 10.1111/imm.13265.

[23] C. Tschoe, C. D. Bushnell, P. W. Duncan, M. A. Alexander-Miller, and S. Q. Wolfe, "Neuroinflammation after Intracerebral Hemorrhage and Potential Therapeutic Targets," *J. Stroke*, vol. 22, no. 1, pp. 29–46, Jan. 2020, doi: 10.5853/jos.2019.02236.

Supplementary Files