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Possible Effect of Genetic Polymorphism with Thrombophilia on Brain White Matter Lesions: A Pilot Study

Hakan Şilek[1], Rana Çağla Akduman[1], Onur Tuncer[2], Zeynep Fırat[2]

[1]Department of Neurology, Yeditepe University, Faculty of Medicine, İstanbul, Turkey
[2]Department of Radiology, Yeditepe University, Faculty of Medicine, İstanbul, Turkey

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Abstract

Objective: The aim of this study was to determine the relationship between coincidental white matter lesions in the brain and genetic mutation in young adults without any chronic neurological disease and without risk of vascular atherosclerosis and to examine the characteristics of the lesions in brain magnetic resonance images of patients with genetic mutations.

Methods: The morphology of subcortical and periventricular hyperintense white matter lesion regions were measured from high-resolution, 3-dimensional fluid-attenuated inversion recovery images acquired for 15 active participants. Subjects ranged in age from 30 to 55 years and were divided into 2 groups, the first was completely asymptomatic and the other was not diagnosed with a definite rheumatic disease but was divided into arthritis or serologically positive patients. Brain magnetic resonance images of the participants were measured both on a visual scale and volumetric measurements of detected lesions using active contour segmentation with the Insight Segmentation and Registration Toolkit (ITK)- Simple Anatomic Parsing (SNAP) viewer technique.

Results: In patients who coincidentally found multiple brain white matter lesions, the most frequent gene mutations were plasminogen activator inhibitor type 1 4G/5G and methylenetetrahydrofolate reductase A1298C heterozygous. When evaluated radiologically concerning the lesion depth and configuration, they were found compatible with ischemic lesions. No statistically significant difference was demonstrated between the 2 groups in the mean volume of lesions using the semi-automated method.

Conclusion: The plasminogen activator inhibitor type 1 4G/5G and methylenetetrahydrofolate reductase AC1298 C gene mutations were perceived to be one of the most common polymorphism combinations in the healthy young population that were coincidentally attested to have hyperintense white matter lesions in the brain at an early stage in an asymptomatic young population.

Keywords: White matter lesions, hyperintense, thrombophilia, mutations, polymorphism

Introduction

Hyperintense white matter lesions (HWMLs) are commonly observed radiological findings in T2-weighted images. The HWMLs are characterized by axonal loss, demyelination, and gliosis mostly as the result of chronic cerebral ischemia.[1] Although the presence of HWMLs has been reported in various cerebrovascular, neurodegenerative, and neuropsychiatric disorders, the definite etiology of these lesions remains unclear.

The HWMLs are frequently encountered in patients with a history of cerebrovascular disorders and vasculopathic risk factors such as hypertension, hypercholesterolemia, diabetes, and smoking history. The prevalence and the extent of HWMLs increase with age.[1,2] As magnetic resonance image (MRI) scanning has become widely available, the frequency of these lesions has also significantly increased in the younger populations. Studies showed marked variability in hyperintense white matter lesions (HWML) prevalence among young individuals and depending on the inclusion criteria, the prevalence ranges between 5.3% and 99%.[3]

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Corresponding author: Hakan Şilek, Department of Neurology, Yeditepe University Faculty of Medicine, İstanbul, Turkey
e-mail: hsilekyahoo.com
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Among younger populations, HWMLs are commonly observed in adults with comorbidities and atherosclerotic risk factors.[3] However, in young and healthy individuals without a significant medical history, assessing the etiology and pathophysiology of these incidental findings patients results in extensive research. In young populations, acquired or inherited hypercoagulable states, collectively known as thrombophilia, carry a high risk of arteriovenous thrombotic events, leading to an increased risk of cerebral ischemia and small vessel disease. Recently, it has been hypothesized that these mutations could lead to HWMLs in otherwise healthy patients, hence providing a possible explanation for these incidental white matter findings.[4,5]

Factor V Leiden, Factor II, and methylenetetrahydrofolate reductase (MTHFR) are among the common thrombophilia mutations detected in these patients. Recent studies focused on plasminogen activator inhibitor type 1 (PAI-1) polymorphisms, highlighting a possible relationship between PAI-1 and ischemic stroke.

Plasminogen Activator Inhibitor-1 Polymorphisms

The serine protease PAI-1 plays a key role in the fibrinolytic system and is responsible for impeding fibrinolysis by inhibiting tissue and urokinase-type plasminogen activators.[6] Various polymorphisms are reported in PAI-1 gene expression, hence affecting the overall PAI concentration. It has been demonstrated that PAI levels are directly related to the degree of fibrinolysis, higher plasma levels of PAI are associated with thrombosis whereas lower levels

indicate enhanced fibrinolysis.[7,8] In addition, higher PAI-1 concentration has been linked with increased cytokine and acute phase protein levels, hypertriglyceridemia, and insulin resistance.[8,9]

One of the widely studied polymorphisms is PAI 4G/5G, 4/5 guanosine in the promoter region, and several in vitro studies have reported that the 4G allele has higher transcriptional activity, resulting in increased PAI-1 levels.[10] Conversely, homozygotes for the 5G allele exhibit decreased PAI-1 levels and heterozygotes present with intermediate levels. The relationship between these polymorphisms and ischemic stroke is still unclear and results are controversial. Recent studies highlight that 4G polymorphisms are associated with a higher risk of ischemic stroke, whereas few other studies show that the same genotype can be considered a protective factor.[11,12]

In this study, we aim to investigate the relationship between WMHLs in healthy individuals and certain thrombophilia mutations. We retrospectively analyzed the cranial MRI scans of young patients without any vascular risk factors and comorbidities who had multiple incidentally discovered HWMLs. The burden of these white matter lesions regarding volume, shape, and configuration was assessed. Meanwhile, thrombophilia panels including Factor V Leiden, Factor II, MTHFR C677, A1298C, and PAI-1 4G/5G, 4G/4G polymorphisms were studied. These patients were also further investigated regarding the presence of possible rheumatological and autoimmune disorders.

Methods

The study protocol was approved by the Yeditepe University Non-intervational Research Ethics Committee (Date: April 12, 2022, Number: 4). All patients were informed about the objectives of the study and gave written informed consent for participation.

Patients

The mean age of the 15 patients (10 female, 5 male) was (40.86 ± 6.16 years). All participants underwent a cranial MRI examination between January 2020 and December 2021. Patients' initial presentation and demographics are presented in Table 1.

Patients with known demyelinating or cerebrovascular disorders, metabolic diseases, history of substance abuse, neoplasms, traumatic brain injury with loss of consciousness, and psychiatric disorders were excluded. Patients' vasculopathic risk factors were evaluated; individuals with previously recognized or newly detected hyperlipidemia, hypertension, and diabetes were also excluded from the study. Patients went through an extensive vasculitis panel work-up to assess the presence of any concomitant rheumatic disease. Symptoms suggestive of rheumatic diseases such as chronic joint pain were also evaluated.

Neuroradiological Findings

All patients underwent a cranial MRI using a combination of axial and coronal spin-echo T1, axial T2, coronal cube fluid-attenuated inversion recovery, axial diffusion, axial susceptibility-weigh ted imaging, and coronal T2 fast multiplanar inversion recovery sequences acquired at 3 Tesla. The MRI scans were evaluated both by a senior neuroradiologist and a neurologist.

The HWML severity was assessed by using the Fazekas scale.[13] Periventricular white matter (PWM) and deep white matter (DWM) were evaluated separately and scored between 0 and 3 depending on the size and extent of the HWM. Regarding DWM lesions (DWML), MRI images without any hyperintensities were assigned a rating of 0, and the presence of multiple punctate lesions, lesions beginning to confluence, and large confluent hyperintensities were assigned as 1, 2, and 3, respectively. In addition, Insight Segmentation and Registration Toolkit (ITK)- Simple Anatomic Parsing (SNAP), a semi-automated segmentation program, was used to assess the volumetric analysis HWML.[14] Standards for ReportIng Vascular changes on neuroimaging (STRIVE) consortium consensus was used in terms of intelligibility and feasibility when describing the lesion.[1]

Table 1. Patient Demographics and Clinical Findings

Patient	Age	Sex	Initial Complaint/ Presenting Symptoms	Rheumatic or Autoimmune Findings/Symptoms
1	47	F	Cervical pain	-
2	41	M	Headache	-
3	44	F	Memory impairment	-
4	38	M	Headache	-
5	48	M	Tinnitus	-
6	45	M	Bell's palsy	Chronic arthralgia ANA +
7	35	F	Headache	-
8	40	F	Headache	-
9	38	F	Dizziness	-
10	41	F	Headache	Chronic arthralgia Leukopenia
11	38	F	Upper and lower extremity numbness	-
12	30	F	Postpartum seizure	-
13	37	F	Headache	-
14	36	F	Headache	Chronic arthralgia
15	55	M	Dizziness	-

ANA, antinuclear antibodies.

Genetic Evaluation

For the thrombophilia panel, 3 mL of blood was obtained via venipuncture and collected in an Ethylenediaminetetraacetic acid (EDTA)-containing tube. Samples were sent to Yeditepe University Department of Medical Genetics. Factor V G1691A (Leiden), Factor V H1299R, Prothrombin G20210A, MTHFR C677T, MTHFR A1298C, and PAI-1 4G/5G polymorphism have been assessed in each individual by using CVD StripAssay Kit (ViennaLab, Austria).[15]

Statistical Analysis

Data were analyzed using Statistical Package for the Social Sciences 26.0 (IBM SPSS Corp.; Armonk, NY, USA). Kruskal–Wallis and Mann–Whitney *U* test were used to compare gene multiplicity, HWMLs lesion volume, and patient symptoms. $P < .05$ was considered statistically significant.

Results

Patients

The most common complaint among patients was headache, observed in 8 out of 15 individuals. Two patients presented to the

clinic with dizziness. Three out of 15 participants had chronic arthralgia suggestive of rheumatic or autoimmune disease. Among these, Antinuclear Antibodies (ANA) positivity and leukopenia were detected in 2 individuals. Based on these findings, patients were further divided into 2 groups. The first group consisted of 12 participants without any risk factors or findings suggestive of autoimmunity. The second group consisted of the remaining 3 individuals who exhibited positive autoimmune or rheumatic findings, hence named as symptomatic where the former group as asymptomatic.

Mutation Analysis

Samples were collected from 15 individuals, consisting of 10 females and 5 males. Regarding the genes studied, the most common mutation in patients with HWMLs is found to be PAI-1 4G/5G polymorphism (n = 13/15) followed by MTHFR A1298C (n = 8/15) and MTHFR C677T (n = 6/15). Factor V mutation was observed in 3 individuals, whereas mutation in Factor II was reported in 2 samples (Tables 2 and 3).

Multiplicity was detected in 14 individuals, the majority of them (n = 8/15) carrying 2 mutations. The coexistence of MTHFR and PAI polymorphisms was reported in 12 samples, which is in turn the most commonly detected combination (Table 4).

Radiological Findings

Major radiological findings are summarized in Table 5. Based on visual assessment, lesions' morphological characteristics differed among symptomatic and asymptomatic groups. In asymptomatic cases, lesions were mostly punctuating and less than 1 mm in size. Conversely, HWMLs encountered in symptomatic individuals demonstrated demyelinating features such as smooth halo and periventricular caps. In addition to the visual scale evaluations, volumetric analysis was performed by ITK-SNAP and reported in cubic millimeter.

Hyperintense White Matter Lesions Volume and Number

The mean volume and standard deviation of patients with a single mutation is 26.70 ± 7.91 mm^3, while the mean lesion volume in patients with 2 genetic mutations is found to be 69.08 ± 84.11 mm^3. The mean volume is calculated as 26.85 ± 10.01 mm^3 for individuals with more than 2 mutations. While the mean and standard deviation of the HWML volume in asymptomatic patients is 27.17 ± 11.02 mm^3, the mean volume in the symptomatic group is calculated as 76.99 ± 90.96 mm^3 (Table 6). According to the Kruskal–Wallis analysis, the number of genetic mutations did not have a significant effect on the volume of HWMLs ($P > .05$), and based on Mann–Whitney *U* statistical analysis, there was no statistically significant difference in the mean lesion volume of symptomatic and asymptomatic groups ($P > .05$).

The mean HWML number in patients with a single genetic mutation is 21.67 ± 5.86, whereas the mean number is calculated

Table 2. Genetic Mutations of the Patients

Genetic Mutations	Number of Patients (n = 15)
PAI-1 4G/5G	11
MTHFR A1298C	8
MTHFR C677T	6
FACTOR V	3
PAI-1 4G/4G	2
FACTOR II	2

MTHRF, Methylenetetrahydrofolate reductase; PAI, plasminogen activator inhibitor 1.

Table 3. Thrombophilia Mutations

Patient	Number of Mutations	Detected Thrombophilia Mutations	Allele Zygosity
1	3	MTHFR C 677 T	Heterozygous
		MTHFR A1298C	Heterozygous
		PAI-1 4G/5G	Heterozygous
2	2	**MTHFR A1298C**	**Homozygous**
		PAI-1 (4G/5G)	Heterozygous
3	2	MTHFR A1298C	Heterozygous
		PAI-1 (4G/4G)	**Homozygous**
4	1	PAI-1 (4G/5G)	Heterozygous
5	2	**MTHFR A1298C**	**Homozygous**
		PAI-1 (4G/5G)	Heterozygous
6	2	MTHFR C677T	Heterozygous
		PAI-1 (4G/5G)	Heterozygous
7	2	MTHFR C 677 T	Heterozygous
		PAI-1 (4G/5G)	Heterozygous
8	3	MTHFR C 677 T	Heterozygous
		PAI-1 4G/4G	**Homozygous**
		FACTOR V R2	Heterozygous
9	1	**PAI-1 (4G/4G)**	**Homozygous**
10	1	**MTHFR A1298C**	**Homozygous**
11	4	FACTOR V	Heterozygous
		FACTOR II	Heterozygous
		MTHFR A1298C	Heterozygous
		PAI-1 4G/5G	**Homozygous**
12	2	MTHFR A1298C	Heterozygous
		PAI-1 4G/5G	Heterozygous
13	2	**MTHFR A1298C**	**Homozygous**
		PAI-1 4G/5G	Heterozygous
14	2	MTHFR C677T	
		PAI-1 4G/5G	
15	4	FACTOR V	Heterozygous
		FACTOR II	Heterozygous
		MTHFR C677T	Heterozygous
		PAI-1 4G/5G	Heterozygous

Table 4. Multiplicity of Mutations

Number of Co-Occurring Mutations	Number of Individuals
1	3
2	8
3	2
4	2

as 23.50 ± 18.74 for patients with 2 genetic mutations. In patients with 3 or more mutations, the mean number of HWML is estimated to be 6.67 ± 8.96. Kruskal–Wallis test demonstrated a *P*-value of .328, indicating that there is no statistical significance between the number of HWMLs and the multiplicity of genetic mutations (Table 7).

Discussion

WMHLs are divided into 2 major groups: PMW lesion (PWML) and DWML. Fazekas et al[13] defined PWML as white matter hyperintensities that were adjacent to the ventricle whereas DWMLs were separate from the ventricle margins.

Visual scales, especially the Fazecas system, are commonly used to radiologically assess HWML burden. Although they provide a practical and expeditious approach, visual scales are largely user-dependent and the comparison of different scales might yield controversial results.

Table 5. Radiological Findings of HWMLs

	Radiological Findings					
	Lesion Type	**Morphology**	**Location**	**Total Number of HWMLs**	**Maximum Volume (mm³)**	**Fazekas Scale**
1		Punctuate	Deep	3		1
2		Punctuate	Deep	8	105	1
3		Punctuate	Deep	12	199	2
4		Punctuate	Deep	26	60.6	1
5		Punctuate	Deep	41	289	1
6		**Punctuate + beginning to confluence**	**Deep**	**41**	**775**	**3**
7		Punctuate	Deep		46	1
8		Punctuate	**Deep**	17	239.2	2
9		Punctuate	**Deep**	15	123	1
10		**Punctuate + small caps**	**Deep**	**23**	**182**	**2**
11		Punctuate	**Deep**	1	23	1
12		Punctuate	**Deep**	39	331.3	2
13		Punctuate	**Deep**	11	77	1
14		Punctuate	**Deep**	**1**	**239**	**1**
15		Punctuate	**Deep**	2	26	1

HWMLs, hyperintense white matter lesions.

Table 6. Number of Genetic Mutations and the Volume of the Lesions, Comparison of HWML Volumes in Asymptomatic and Symptomatic Groups

	Volume (mm³)						
Genetic Mutation Multiplicity	**Mean**	**SD**	**Median**	**Min**	**Max**	**Test Used**	***P***
1	26.70	7.91	24.14	20.39	35.58	KW (χ^2) 1.654	.437
2	69.08	84.11	40.74	16.79	239.00		
≥3	26.85	10.01	23.21	19.18	38.17		
Groups						Z −1.868	.073
Asymptomatic	27.17	11.02	23.21	16.79	48.48		
Symptomatic	76.99	90.96	38.17	24.14	239.00		

SD, Standard Deviation; KW, Kruskall Wallis Test; Z, Mann Whitney *U* Test; Min, Minimum; Max, Maximum.

Table 7. The Correlation Between the Number of HWMLs and the Number of Genetic Mutations

	HWML Count (in Number)						
Genetic Mutation Multiplicity	**Mean**	**SD**	**Median**	**Min**	**Max**	**Test Used**	***P***
1	21.67	5.86	24.00	15.00	26.00	KW (χ^2) 2.320	.328
2	23.50	18.74	25.00	1.00	41.00		
≥ 3	6.67	8.96	2.00	1.00	17.00		

HWMLs, hyperintense white matter lesion; KW, Kruskal–Wallis test; SD, standard deviation; Z, Mann–Whitney *U* test.

It is challenging to distinguish the border between PWMLs and DWMLs since spatial analysis is partially arbitrary. Hence, an arbitrary distance of 10 mm from the ventricle surface was applied to assess the localization of the lesions.[16] In our study, we applied the same rule to evaluate the localization. In our study, we aimed to combine both qualitative and quantitative scales to provide better results since quantitative scales could provide more objective and solid data through machine learning-based algorithms.[17,18]

The irregular nature of these WMHLs poses a significant challenge regarding further quantification and classification; however, recent studies suggest that the shape of these lesions might carry etiologic and prognostic value. Smooth halo and periventricular caps are associated with non-ischemic causes such as demyelination and associated with disrupted ependymal lining.[16] Conversely, irregular PWMLs and DWHLs are related to ischemic changes. Punctate and confluent lesions were mostly attributed to this chronic hemodynamic insufficiency, especially confluent lesions indicating extensive ischemic damage.[16,19]

In addition to lesion configuration, we believe lesion volume might carry diagnostic significance. We measured the volume of each lesion by semi-automated assessment, and the maximum lesion volume was found to be significantly different in asymptomatic groups compared to symptomatic groups. In our study, we observed that 3 out of 15 individuals who had chronic arthralgia and positive autoimmune findings had high WMHL volume, the maximum volume documented as 700 mm^3, whereas in the asymptomatic group, the maximum was calculated as 180 mm^3 Majority of these lesions had features suggestive of non-ischemic in origin.

As the neuroimaging techniques advance, the number of HWMLs that are incidentally found has significantly increased. However, studies highlight a great variability regarding the presence of HWMLs in individuals. In a recent study by Hopkins et al,[3] the prevalence of total HWMLs in healthy asymptomatic subjects was found to be 5.3%, whereas another study reported a prevalence of 0.5% in 1000 asymptomatic patients aged between 3 and 83 years.[20,21] Greater rates of HWMLs have been also reported: ranging from 30% to 99%. This high discrepancy particularly depends on the designated inclusion criteria of each study. Studies conducted in 'elderly populations' would yield a higher incidence of HWMLs since these hyperintensities significantly increase with older age. In fact, it has been demonstrated that individuals who are 55 years or older experience a 10-fold increase in the prevalence of HWMLs; hence, age cut-off values carry a major significance.[3] On the other hand, even in younger cohorts, the presence of certain comorbidities such as atherosclerotic risk factors act as a major contributor to the HWML prevalence, hence further causing this discrepancy among the studies. There are only a few studies that assess the HWML burden in young individuals.[3,20] Although various genetic mutations have been studied, we prefer to focus on 5 mutations: Factor V Leiden, Factor II, MTHFR C677, A1298C, and PAI-1 4G/5G, 4G/4G polymorphisms due to their prevalence.[22-25] To our knowledge, this is the first study to compare the presence of thrombophilia mutations and HWML burden in young healthy individuals.

Undoubtedly, initially, differentiating between demyelinating and ischemic lesions might possess challenges since both types of hyperintensities could be observed as millimetric periventricular lesions at the beginning. However, as the number of studies regarding the volume and configuration of HWMLs increases, it would be easier to identify the nature of these hyperdensity. Although the mechanisms involved in HWML have not been fully elucidated, we believe genetic mutations could act as a precipitating factor. Therefore, assessing the presence of major thrombophilia mutations in otherwise healthy young patients could prevent further expensive large-scale investigations and aid clinicians to provide a definite diagnosis.

Limitations

The small sample size created a major limitation. Hence, we believe this study can be multiplicated with a larger population. We also observed that various studies used different distancing rules; hence, it was not possible to reach a consensus in terms of radiological measurements. Also, the difficulty in differentiating advanced and contiguous WMHLs created further challenges to establish a widely used algorithm for lesion localization and configuration.

Conclusion

The HWMLs in young individuals without any comorbidities pose further diagnostic challenges in the healthcare setting. Thrombophilia mutations can be an etiological factor that needs to be assessed; however, further research is required to fully understand the relationship between HWML burden and thrombophilia mutations.

Data Availability: Data used in this study can be provided on reasonable request.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of University of Yeditepe (Date: April 12, 2022, Number: 4).

Informed Consent: Written informed consent was obtained from the patients/patient who agreed to take part in the study.

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