Journal of the American Heart Association

ORIGINAL RESEARCH

Heme Oxygenase-1 Gene (GT)n Polymorphism Linked to Deep White Matter Hyperintensities, Not Periventricular Hyperintensities

Junting Chen, MD*; Jinrui Li, MD*; Xiaomian Wang, MD; Xiaoli Fu, MD; Jianxia Ke, MD; Jintao Li, MD; Jia Wen, MD; Kailin Cheng, MD; Shuen Li, MD*; Zhu Shi, PhD*

BACKGROUND: Oxidative stress plays a principal role in the pathogenesis of white matter hyperintensities (WMHs). The induction of heme oxygenase-1 (*HO-1*) gene in the brain represents 1 of the pivotal mechanisms to counteract the noxious effects of reactive oxygen species, and the transcriptional modulation of *HO-1* induction depends on the length of a GT-repeat (GT)n in the promoter region. We investigated whether the *HO-1* gene (GT)n polymorphism is associated with the risk of WMHs.

METHODS AND RESULTS: A total of 849 subjects from the memory clinic were consecutively enrolled, and the *HO-1* (GT)n genotype was determined. WMHs were assessed with the Fazekas scale and further divided into periventricular WMHs and deep WMHs (DWMHs). Allelic *HO-1* (GT)n polymorphisms were classified as short (≤24 (GT)n), median (25≤[GT]n<31), or long (31≤[GT]n). Multivariate logistic regression analysis was used to evaluate the effect of the *HO-1* (GT)n variants on WMHs. The number of repetitions of the *HO-1* gene (GT)n ranged from 15 to 39 with a bimodal distribution at lengths 23 and 30. The proportion of *S/S* genotypes was higher for moderate/severe DWMHs than none/mild DWMHs (22.22% versus 12.44%; *P*=0.001), but the association for periventricular WMHs was not statistically significant. Logistic regression suggested that the *S/S* genotype was significantly associated with moderate/severe DWMHs (*S/S* versus non-*S/S*: odds ratio, 2.001 [95% CI, 1.323–3.027]; *P*<0.001). The *HO-1* gene (GT)n *S/S* genotype and aging synergistically contributed to the progression of DWMHs (relative excess risk attributable to interaction, 6.032 [95% CI, 0.149–11.915]).

CONCLUSIONS: Short (GT)n variants in the *HO-1* gene may confer susceptibility to rather than protection from DWMHs, but not periventricular WMHs.

REGISTRATION: URL: https://www.chictr.org.cn; Unique identifier: ChiCTR2100045869.

Key Words: cognitive impairment ■ *HO-1* gene ■ oxidative stress ■ polymorphism ■ white matter hyperintensity

White matter hyperintensities (WMHs) are frequently observed on brain magnetic resonance imaging (MRI) scans in the elderly population.[1–3] WMHs are often regarded as an indicative sign of cerebral small-vessel disease and are linked to an increased risk of cognitive impairment and dementia, including Alzheimer disease (AD).[4–7] With increasing age, there is a substantial increase in the occurrence of WMHs. In individuals aged 60 to 70 years, the occurrence of WMHs ranges between 68% and 87%, whereas in

Correspondence to: Zhu Shi, PhD, Department of Neurology and Stroke Center, Affiliate Dongguan People's Hospital, Southern Medical University, Dongguan 523018, China. Email: sound_shi@126.com

*J. Chen, JR. Li, S. Li, and Z. Shi contributed equally.

This manuscript was sent to Neel S. Singhal, MD, PhD, Associate Editor, for review by expert referees, editorial decision, and final disposition.

Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.123.033981

For Sources of Funding and Disclosures, see page 10.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- In a study of 849 patients from a memory clinic, our results support the presence of genetic heterogeneity in deep white matter hyperintensities (DWMHs) in brain magnetic resonance imaging, compared with periventricular white matter hyperintensities.
- Short (GT)n repeat sequence of the heme oxygenase-1 (*HO-1*) gene was associated with DWMHs but not periventricular white matter hyperintensities.
- Short (GT)n repeat sequence of *HO-1* gene synergizes with aging on the development of DWMHs.

What Are the Clinical Implications?

- Our findings provide evidence for variation in the pathophysiology of white matter hyperintensities in distinct brain regions, specifically in periventricular white matter hyperintensities compared with DWMHs, highlighting the need for customized interventions targeting each of these areas.
- HO-1, an essential regulator in the cellular antioxidant defense, also exerts significant relevant control over the iron-mediated neuronal and glial cell death pathway. HO-1 may act synergistically with aging and participate in the development of DWMHs; further investigations are warranted to investigate HO-1 as a promising therapeutic target with potential to delay or even reverse DWMHs.

Nonstandard Abbreviations and Acronyms

DWMH	deep white matter hyperintensity
PVH	periventricular white matter hyperintensity
WMH	white matter hyperintensity

those aged 80 to 90 years, the prevalence even surges to 95% to 100%.[8–10] WMHs represent a multifaceted and heterogeneous condition that is intricately linked to the process of aging and also influenced by multiple genetic and environmental factors.[11,12] Although the precise mechanisms responsible for the formation of WMHs remain incompletely understood, accumulating evidence suggests that cellular oxidative stress, which is perturbed as a consequence of ischemia, hypoxia, and inflammation, plays a pivotal role in the pathogenesis of WMHs,[13–15] particularly in patients with deep white matter hyperintensities (DWMHs).[16,17]

Heme oxygenase-1 (HO-1), encoded by the *HO-1* gene at 22q12, is an inducible heme oxygenase that is expressed at low levels under normal conditions in the brain; however, its activity is elevated in response to oxidative stress factors, such as ischemia, hypoxia, and inflammatory response, and it plays a pivotal role in exerting anti-inflammatory and antioxidant cytoprotective effects.[18–20] The major regulator of *HO-1* gene expression is the (*GT*)n repeat polymorphism site located at the 5′ end of the *HO-1* promoter region. The shorter the (*GT*)n repeat sequence, the higher the level of *HO-1* promotor activity.[21,22] HO-1 degrades the free heme, which has pro-oxidative, proinflammatory, and cytotoxic effects, and breaks it down into carbon monoxide, Fe^{2+}, and biliverdin, the latter of which is subsequently converted to bilirubin by biliverdin reductase. Previous studies have reported that HO-1 exerted anti-inflammatory, antioxidative stress, and antiapoptotic protective effects in a variety of disease processes.[23–29] Recent investigations, however, have shed light on the potential detrimental effects of *HO-1* gene overexpression. It has been found that excessive accumulation of Fe^{2+} and CO can impair mitochondrial and cellular integrity through the activation of the iron death pathway.[30,31] For instance, the upregulation of the neuroglial HO-1 gene has been linked to the abnormal accumulation of iron in the brain, intracellular damage caused by oxidative stress, and impaired bioenergetics in disorders like AD.[32] One potential hypothesis is that polymorphisms at the upstream promotor of the *HO-1* gene might be implicated in the onset and progression of WMHs by the aforementioned regulatory pathways. However, there is currently no clinical evidence to corroborate this assertion.

The aim of this study was to investigate the association between length of GT(n) repeat sequence of the *HO-1* gene and severity of WMHs, and further to evaluate the synergistic effects of *HO-1* (GT)n with other risk factors associated with WMHs.

METHODS

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Study Population

This was a perspective cross-sectional study, and the study protocol was approved by the institutional review board (KYKT2021-013), and informed written consent was obtained from all patients. We included consecutive patients attending the memory clinic service from January 2020 to January 2023 at the 10th Affiliate Hospital, Southern Medical University.

The inclusion criteria were as follows: (1) age >18 years; (2) no metal implants, claustrophobia, or other contraindications to perform MRI scans; and (3)

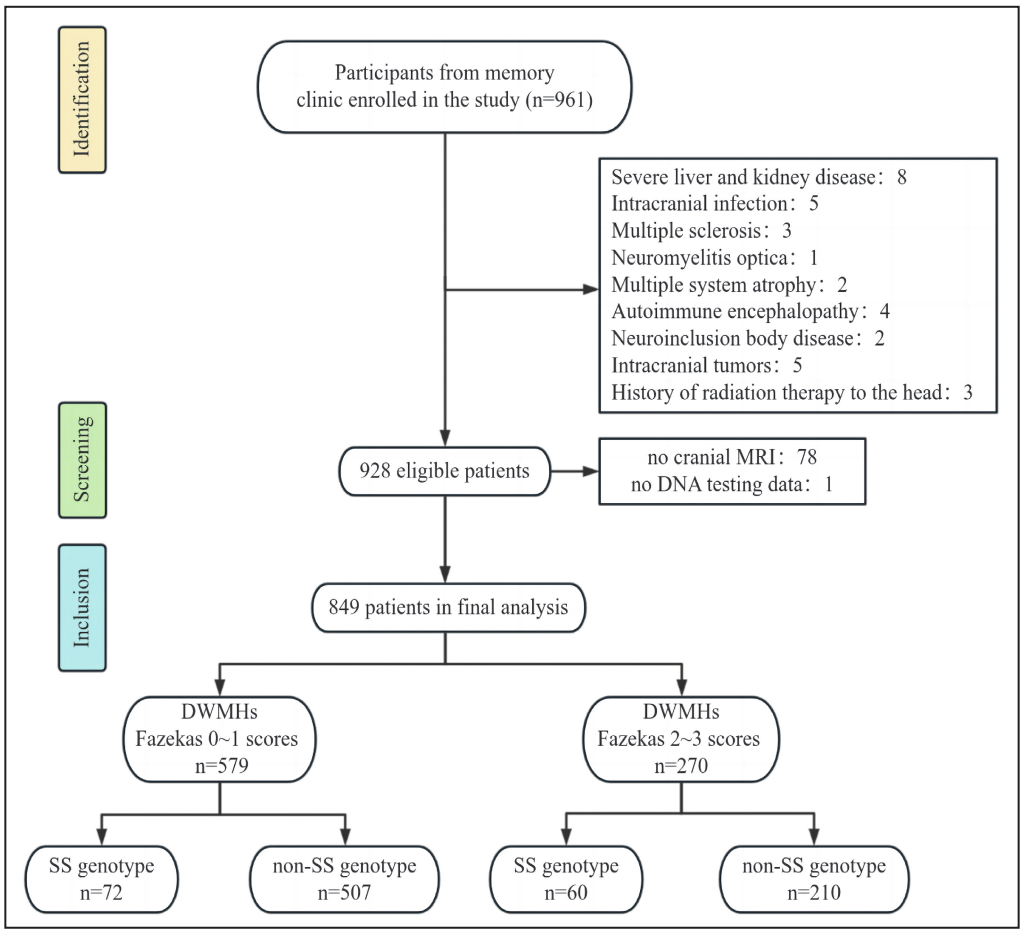

Figure 1. Flowchart of the study population.
DMHW indicates deep white matter hyperintensity; and MRI, magnetic resonance imaging.

patient or proxy signed informed consent. Exclusion criteria were as follows: (1) severe hepatic and renal insufficiency, hematological disorders, previous bone marrow transplantation, and radiotherapy; (2) WMHs attributable to other definite causes (eg, intracranial infections, inflammatory demyelinating diseases, autoimmune encephalitis, tumors, or previous radiotherapy); (3) poor MRI quality for analysis; and (4) genetic test results not completed or not available.

Clinical information, including age, sex, and atherosclerotic risk factors, was recorded using a standardized table by experienced neurologists. Laboratory tests performed 6 hours after admission on a fasting basis included measurements of total cholesterol (TC), low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, homocysteine, uric acid, total bilirubin, direct bilirubin, and indirect bilirubin.

Magnetic Resonance Protocol

All participants in our memory clinic service underwent multimodal brain MRI as part of the protocol, including T1-weighted imaging, T2-weighted imaging, magnetic resonance angiography, and fluid-attenuated inversion recovery. Magnetic resonance images were acquired using a 3T Siemens Prisma MR scanner (Siemens Healthcare, Germany) with a 32-channel head coil for reception. WMHs were defined as hyperintense signal abnormalities in the white matter tracts on fluid-attenuated inversion recovery or T2-weighted images. According to the Fazekas scale, both deep and periventricular WMHs were coded from 0 to 3, for a total score of 0 to 6.[33] Lesions displaying high signal intensity within 10 mm of the ventricle were classified as periventricular white matter hyperintensities (PVHs), whereas WMHs surrounded by brain tissue were designated as DWMHs.[34,35] The Fazakas scale was assessed by 2 senior neurologists (X.L.F. and J.T.L.) and neuroimaging specialists (Z.S.) who were blinded to the clinical and genetic information. Any disagreements were resolved through negotiation.

Genetic Testing

The assessment of (GT)n repeat sequence in the promoter region of the *HO-1* gene was conducted

Table 1. Characteristics of 849 Patients According to PVHs and DWMHs

Characteristic	PVHs Fazekas 0 to 2 (n=729)	PVHs Fazekas 3 (n=120)	*P* value	DWMHs Fazekas 0 to 1 (n=579)	DWMHs Fazekas 2 to 3 (n=270)	*P* value
Age, y	65 (54–74)	74 (69–79)	<0.001	64 (53–72)	73 (67–78)	<0.001
Male sex, n (%)	409 (56.1)	76 (63.3)	0.138	323 (55.8)	162 (60.0)	0.248
Education, y	7.0±3.3	6.5±2.8	0.079	7.0±3.6	6.7±2.7	0.223
MMSE score	26.3±2.7	26.0±4.0	0.297	26.3±2.9	26.0±3.3	0.180
Risk factors, n (%)						
Hypertension	479 (65.7)	107 (89.2)	<0.001	355 (61.3)	231 (85.6)	<0.001
Diabetes	213 (29.2)	44 (36.7)	0.100	163 (28.2)	94 (34.8)	0.049
Hyperlipidemia	228 (31.3)	33 (27.5)	0.406	188 (32.5)	73 (27.0)	0.110
Smoking	199 (27.3)	35 (29.2)	0.671	159 (27.5)	75 (27.8)	0.923
Stroke	184 (25.2)	63 (52.5)	<0.001	123 (21.2)	124 (45.9)	<0.001
Laboratory tests						
TC, mmol/L	4.60 (3.8–5.5)	4.40 (3.6–5.1)	0.022	4.60 (3.9–5.5)	4.40 (3.6–5.2)	0.006
HDL-C, mmol/L	1.11 (0.9–1.3)	1.09 (0.9–1.3)	0.406	1.11 (0.9–1.3)	1.11 (0.9–1.3)	0.562
LDL-C, mmol/L	2.92 (2.2–3.7)	2.77 (1.9–3.4)	0.094	2.94 (2.3–3.7)	2.80 (2.0–3.5)	0.043
Homocysteine, μmol/L	10.98 (9.0–13.6)	12.79 (11.0–16.1)	<0.001	10.61 (8.8–13.3)	12.29 (10.6–14.8)	<0.001
T-bil, μmol/L	12.50 (9.6–16.9)	12.80 (9.7–18.0)	0.641	12.60 (9.6–16.6)	12.00 (9.5–17.5)	0.961
D-bil, μmol/L	3.70 (2.3–5.2)	3.95 (2.5–6.0)	0.083	3.80 (2.4–5.1)	3.70 (2.2–6.0)	0.474
I-bil, μmol/L	9.10 (6.8–12.0)	8.65 (6.3–12.8)	0.586	9.20 (6.9–11.9)	8.70 (6.3–12.4)	0.585
Uric acid, μmol/L	364.40 (301.1–439.0)	374.10 (288.7–451.6)	0.765	365.95 (299.6–440.1)	364.40 (300.0–446.5)	0.700
HO-1 (GT)n genotype, n (%)			0.219			0.005
SS	108 (14.8)	24 (20.0)		72 (12.4)	60 (22.2)	
SM	241 (33.1)	35 (29.2)		199 (34.4)	77 (28.5)	
SL	111 (15.2)	17 (14.2)		83 (14.3)	45 (16.7)	
MM	122 (16.7)	22 (18.3)		107 (18.5)	37 (13.7)	
ML	110 (15.1)	21 (17.5)		92 (15.9)	39 (14.4)	
LL	37 (5.1)	1 (0.8)		26 (4.5)	12 (4.4)	
Non-*SS*	621 (85.2)	96 (80.0)	0.146	507 (87.6)	210 (77.8)	<0.001

Data are presented as mean ±SD, median (quartile 1–quartile 3), or number (percentage) and analyzed using the Student *t* test, Mann-Whitney *U* test, or the χ^2 test as appropriate. D-bil indicates direct bilirubin; DWMH, deep white matter hyperintensity; HDL-C, high-density lipoprotein cholesterol; *HO-1*, heme oxygenase-1; I-bil, indirect bilirubin; LDL-C, low-density lipoprotein cholesterol; MMSE, Mini-Mental State Examination; PVH, periventricular white matter hyperintensity; T-bil, total bilirubin; and TC, total cholesterol.

following previously reported techniques.[26] Genomic DNA was isolated from peripheral blood (DNA Extraction Kit, TIANGEN Biochemical Technology). Specific primers were designed according to the conserved sequence of the variable number tandem repeat of the promoter region (GT) of the *HO-1* gene (*HO-1*-F: 5′-GAA GGT GAC CAA GTT CAT GCT AGA GCC TGG GGT TGC TAA GT; *HO-1*-R: 5′-ACA GCT GAT GCC CAC TTT CT). The polymerase chain reaction products were labeled with a 6-fluorescein amidites forward primer (5′-GAA GGT GAC CAA GTT CAT GCT), and the fragment size was determined on a capillary sequencer (ABI 3730xl DNA Analyzer). To ensure accurate and reproducible sizing, all samples underwent a minimum of 2 independent polymerase chain reactions, with the obtained results validated to be accurate within ±1 GT repeats. Genotyping was performed by laboratory personnel who were blind to the clinical and imaging data.

Consistent with prior investigations in Asian populations, we observed that 23 and 30 (GT)n were the dominant alleles within our study cohort. We therefore selected 25 (GT)n and 30 (GT)n as cutoffs to classify study subjects in the genetic analysis. GT repeat of ≤24 was designated as the short allele, the repeats between 25 and 30 as the median allele, and the repeats of ≥31 as the long allele.

Statistical Analysis

Statistical analysis was performed using SPSS (IBM SPSS 26.0 Statistics, Chicago, IL) and R4.2.3

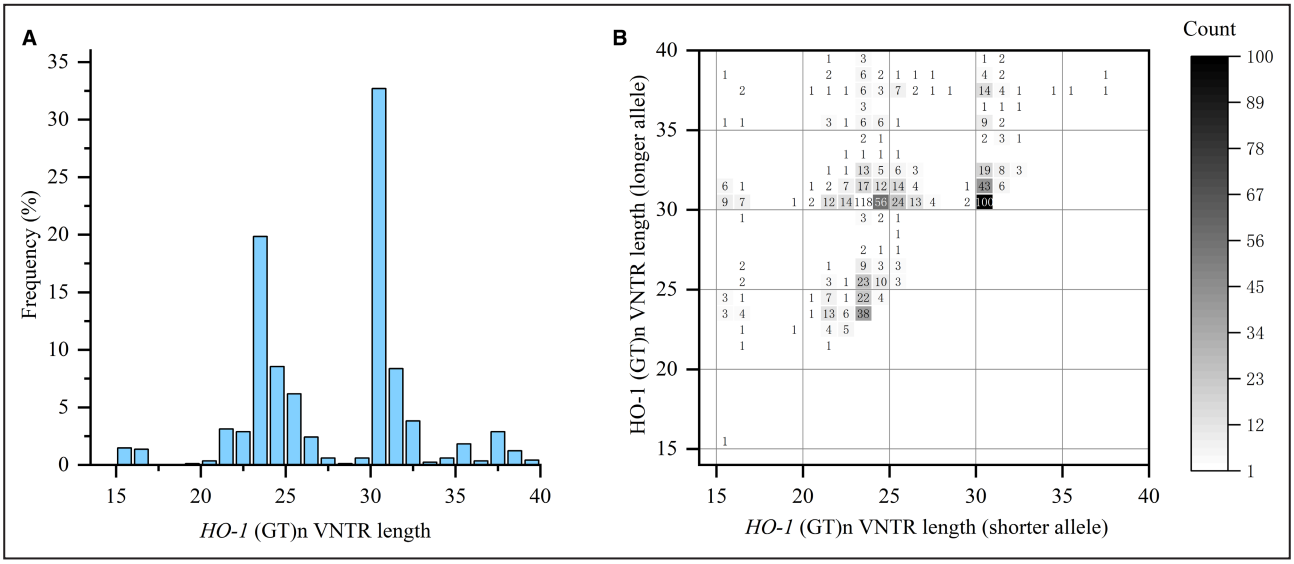

Figure 2. *HO-1* (GT)n genotype distribution of study population.
A, Histogram of allele frequency distribution for all subjects. **B**, Joint distribution of *HO-1* VNTR length on each allele. The numbers represent the number of subjects, and the darker the color, the greater the number of subjects. HO-1 indicates heme oxygenase-1; and VNTR, variable number tandem repeat.

(R Foundation for Statistical Computing, Vienna, Austria). Continuous variables were presented as the mean±SD if normally distributed; otherwise, they were presented as the median (interquartile range). Categorical variables were presented as frequency and percentage. Comparisons between groups were performed using Welch *t* test, the χ^2 test, or Fisher exact test. In the multivariate logistic regression analysis, variables demonstrating statistical significance with $P<0.05$ were included in model 2, whereas those with $P<0.1$ were considered for inclusion in model 3. The Kaplan-Meier curve of the cumulative probability of moderate/severe DWMHs with age according to *SS* versus non-*SS* genotypes was presented. All tests were 2 tailed, and $P<0.05$ was considered significant.

RESULTS

Baseline Characteristics and *HO-1* (GT)n Repeat Sequence Genotypes

A total of 961 consecutive subjects at the memory clinic were enrolled during the study period. Among them, 8 had comorbid serious liver and renal dysfunction, 25 were identified with other definite cause of WMHs, 1 did not yield successful gene sequencing results because of unsuccessful assay, and 78 lacked good quality MRI for accurate analysis. A total of 849 eligible patients were included in the final analysis. The flowchart is presented in Figure 1.

The mean±SD age of the study population was 68.8±13.5 years, and 57.1% (485/849) were men. Among them, 69.0% (586 cases) presented with hypertension, 30.3% (257 cases) with diabetes, 30.7% (261 cases) with hyperlipidemia, 27.6% (234 cases) with smoking, and 29.1% (247 cases) with a history of stroke (Table 1).

The number of (GT)n repeats of the *HO-1* gene ranged from 15 to 39, showing a bimodal distribution with peaks at lengths 23 and 30, which accounted for 19.8% and 32.7% of the alleles, respectively (Figure 2). The predominant genotypes were $(GT)_{23}/(GT)_{30}$ (n=56, 6.6%) and $(GT)_{30}/(GT)_{30}$ (n=100, 11.8%). The alleles were divided according to the number of (GT)n repeats of the *HO-1* gene (*S*≤24, *M*=25–30, and *L*≥31), and the genotypes were subsequently classified into 6 subtypes: *S/S* (n=132, 15.5%), *S/M* (n=276, 32.5%), *S/L* (n=128, 15.1%), *M/M* (n=144, 17.0%), *M/L* (n=131, 15.4%), and *L/L* (n=38, 4.5%).

Association Between WMHs and (GT)n Repeat Sequence of the *HO-1* Gene

On the basis of the distinctive characteristics of PVH and DWHM in terms of pathologic mechanisms and imaging assessment,[36–38] subjects were divided into 4 groups according to the Fazekas scores for PVHs and DWMHs: 729 cases of none/mild PVHs with Fazekas 0 to 2, 120 cases of severe PVHs with Fazekas 3, 579 cases of none/mild DWMHs with Fazekas 0 to 1, and 270 cases of

Table 2. Baseline Characteristics of 849 Patients According to *HO-1* (GT)n Genotypes

Characteristic	*SS* (n=132)	*SM* (n=276)	*SL* (n=128)	*MM* (n=144)	*ML* (n=131)	*LL* (n=38)	Non-*SS* (n=717)	*P* value*	*P* value#
Age, y	69.0 (58.5–76.0)	67.0 (55.0–74.0)	66.5 (55.3–75.8)	68.0 (55.0–74.0)	68.0 (56.3–74.0)	68.0 (56.5–74.0)	67.0 (56.0–74.0)	0.654	0.164
Male sex, n (%)	76 (57.6)	152 (55.1)	83 (64.8)	82 (56.9)	74 (56.5)	18 (47.4)	409 (57.0)	0.404	0.910
Risk factors, n (%)									
Hypertension	88 (66.7)	184 (66.7)	95 (74.2)	101 (70.1)	90 (68.7)	28 (73.7)	498 (69.5)	0.677	0.524
Diabetes	43 (32.6)	91 (33.0)	33 (25. 8)	40 (27.8)	40 (30.5)	10 (26.3)	214 (30.0)	0.666	0.531
Hyperlipidemia	37 (28.0)	81 (29.4)	40 (31.3)	47 (32.6)	47 (35.9)	9 (23.7)	224 (31.2)	0.627	0.463
Stroke	34 (25.8)	90 (32.6)	31 (24.2)	41 (28.5)	37 (28.2)	14 (36.8)	213 (29.7)	0.414	0.359
Smoking	34 (25.8)	76 (27.5)	39 (30.5)	42 (29.2)	34 (26.0)	9 (23.7)	200 (27.9)	0.924	0.614
Laboratory tests									
TC, mmol/L	4.5 (3.9–5.3)	4.4 (3.7–5.4)	4.6 (3.7–5.5)	4.4 (3.8–5.2)	4.8 (3.7–5.7)	4.7 (3.7–5.8)	4.6 (3.7–5.5)	0.813	0.526
HDL, mmol/L	1.1 (1.0–1.3)	1.1 (0.9–1.4)	1.1 (0.9–1.3)	1.1 (0.9–1.4)	1.1 (0.9–1.3)	1.1 (0.9–1.2)	1.1 (0.9–1.3)	0.665	0.375
LDL, mmol/L	2.9 (2.3–3.7)	2.9 (2.2–3.5)	2.9 (2.0–3.6)	2.9 (2.4–3.4)	3.0 (2.1–4.0)	3.0 (2.1–4.0)	2.9 (2.2–3.6)	0.891	0.668
Homocysteine, μmol/L	11.2 (9.3–14.1)	11.1 (9.0–13.6)	11.5 (9.2–14.6)	11.1 (9.0–13.5)	11.8 (9.1–14.3)	10.9 (9.4–12.5)	11.2 (9.1–13.9)	0.307	0.457
T-bil, μmol/L	13.0 (9.9–17.6)	12.4 (9.5–16.9)	12.0 (9.9–16.5)	12.0 (9.1–17.1)	13.1 (9.6–16.9)	13.4 (8.8–16.4)	12.4 (9.5–16.7)	0.804	0.155
D-bil, μmol/L	3.8 (2.4–5.3)	3.6 (2.2–5.5)	3.4 (2.1–4.7)	3.8 (2.2–5.4)	3.9 (2.5–5.7)	3.8 (2.6–6.0)	3.7 (2.3–5.4)	0.566	0.485
I-bil, μmol/L	9.5 (6.9–12.8)	8.9 (6.5–12.0)	8.7 (7.3–11.5)	8.6 (6.3–11.9)	9.4 (6.6–12.0)	9.4 (6.8–11.8)	8.8 (6.6–11.9)	0.869	0.226
UA, μmol/L	376.1 (308.0–431.6)	349.2 (284.4–435.4)	373.9 (328.8–457.5)	364.2 (314.3–428.5)	376.4 (280.7–456.3)	327.8 (266.8–407.9)	362.9 (297.6–444.)	0.175	0.751
PVHs, n (%)								0.219	0.146
Fazekas 0–2	108 (81.8)	241 (87.3)	111 (86.7)	122 (84.7)	110 (84.0)	37 (97.4)	621 (86.6)		
Fazekas 3	24 (18.2)	35 (12.7)	17 (13.3)	22 (15.3)	21 (16.0)	1 (2.6)	96 (13.4)		
DWMHs, n (%)								0.005	<0.001
Fazekas 0–1	72 (54.6)	199 (72.1)	83 (64.8)	107 (74.3)	92 (70.2)	26 (68.4)	507 (70.7)		
Fazekas 2 to 3	60 (45.4)	77 (27.9)	45 (35.2)	37 (25.7)	39 (29.8)	12 (31.6)	210 (29.3)		

Data are given as median (quartile 1–quartile 3) or number (percentage). DWMH indicates deep white matter hyperintensity; HDL, high-density lipoprotein; *HO-1*, heme oxygenase-1; LDL, low-density lipoprotein; PVH, periventricular white matter hyperintensity; TC, total cholesterol; and UA, uric acid.

**P* value indicated 1-way ANOVA test for 6 *HO-1* (GT) genotypes.

#P value indicated Welch *t* test or χ^2 test for *SS* vs non-*SS* genotypes.

Table 3. Association of *HO-1* (GT)n Genotypes With DWMHs

Variable	OR (95% CI)	*P* value
SS	Reference	
SM	0.466 (0.302–0.717)	0.001
SL	0.643 (0.390–1.059)	0.083
MM	0.415 (0.250–0.689)	0.001
ML	0.509 (0.306–0.846)	0.009
LL	0.563 (0.262–1.210)	0.141
Non-*SS* vs *SS*	0.497 (0.340–0.726)	<0.001

Adjusted for age and sex. DWMH indicates deep white matter hyperintensity; *HO-1*, heme oxygenase-1; and OR, odds ratio.

moderate/severe DWMHs with Fazekas 2 to 3. The (GT)n repeat sequence in the *HO-1* gene was subsequently examined and compared. The proportion of *S/S* genotypes was higher in the moderate/severe DWMH group compared with the none/mild DWMHs group (22.2% versus 12.4%; *P*=0.001), but no further discrepancies of genotypes were detected between PVH groups (Tables 1 and 2).

The clinical characteristics of patients with moderate/severe DWMHs were examined using univariate comparisons. Compared with the subjects with none/mild DWMHs, the subjects with moderate/severe DWMHs were older (72.1±10.3 versus 60.6±13.3 years; *P*<0.001), more likely to have hypertension (85.6% versus 61.3%; *P*<0.001), diabetes (34.8% versus 28.2%; *P*=0.049), and stroke (45.9% versus 21.2%; *P*<0.001), had higher levels of homocysteine (12.3 versus 10.6 μmol/L; *P*<0.001), but lower levels of total cholesterol (4.4 versus 4. 6 mmol/L; *P*=0.006) and low-density lipoprotein cholesterol (2.8 versus 2.9 mmol/L; *P*=0.043) (Table 1).

Adjusting for age and sex, the multivariate logistic model revealed that subjects with the *S/S* homozygote genotype were significantly associated with an increased risk of moderate/severe DWMHs. The non-*S/S* subjects had a significantly lower risk of moderate/severe DWMHs (odds ratio [OR], 0.497 [95% CI, 0.340–0.726]; *P*<0.001). Additionally, specific genotypes showing statistical significance in terms of moderate/severe DWMH risk included the following: *S/M* genotype (OR, 0.466 [95% CI, 0.303–0.717]; *P*=0.001), *M/M* genotype (OR, 0.415 [95% CI, 0.250–0.689]; *P*=0.001), and *M/L* genotype (OR, 0.509 [95% CI, 0.306–0.846]; *P*=0.009) (Table 3). However, no significant associations were detected between the *HO-1* genotype and PVHs (Table S1).

Sensitivity analysis was performed using restricted cubic splines to evaluate the exact nature of the correlation between the *HO-1* gene (GT)n repeat length of the shorter allele and the presence of moderate/severe DWMHs, without consideration of predefined cutoff criteria. Remarkably, the analysis yielded significant findings for the shorter allele (*P*=0.0085) and provided a post hoc confirmation of the predefined cutoff values of 24 and 31 (Figure 3).

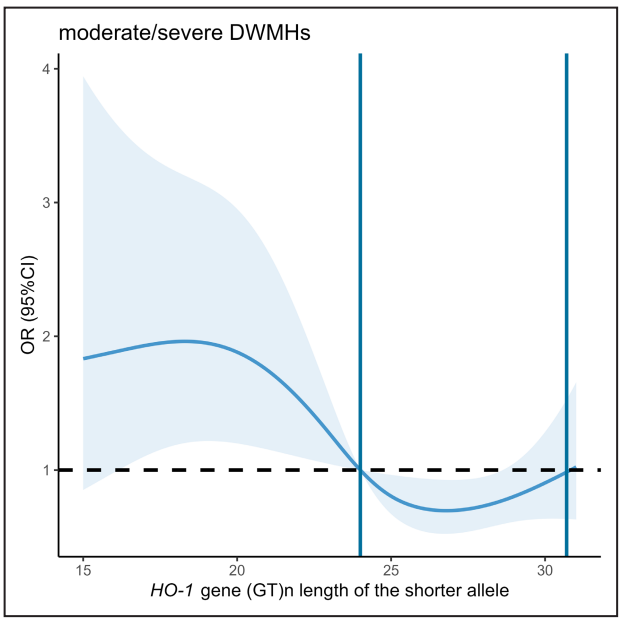

Figure 3. Restricted cubic spline of the association of the *HO-1* variable number tandem repeat length with the moderate/severe DWMHs.
Blue lines show the cutoffs we applied. DWMHs indicates deep white matter hyperintensities; HO-1, heme oxygenase-1; and OR, odds ratio.

Synergistic Effects of *HO-1* (GT)n With Age on the Progression of WMHs

To elucidate the interaction between traditional risk factors for moderate/severe DWMHs and the *HO-1* (GT)n genotype, stratified analyses were conducted. In the younger population aged <60 years, subjects with the *S/S* genotype exhibited an OR of 1.711 (95% CI, 0.632–4.628; *P*=0.290) for moderate/severe DWMHs in comparison to subjects with non-*S/S* genotypes. In contrast, among the older adult population (aged ≥60 years), subjects with the *S/S* genotype had a significantly higher OR of 2.033 (95% CI, 1.304–3.168; *P*=0.002) for moderate/severe DWMHs. Considering the disparate risks observed in relation to *S/S* genotype exposure, we proceeded with further interaction analyses. The summed interaction regression model revealed significant interaction between age and *S/S* genotype, namely, the relative excess risk attributable to interaction (6.032 [95% CI, 0.149–11.915]), the attributable proportion due to interaction (0.512 [95% CI, 0.239–0.784]), and the synergy index (2.268 [95% CI, 1.188–4.330]). In contrast, no significant interaction effects were observed for sex, hypertension, diabetes, and stroke history with *S/S* genotype (Figure 4).

Subgroups	Number	Adjusted OR (95%CI)	P value	RERI (95%CI)	AP (95%CI)	S (95%CI)
All patients	849	2.001 (1.323, 3.027)	0.001			
Age , y				6.032 (0.149, 11.915)	0.512 (0.239, 0.784)	2.268 (1.188, 4.330)
< 60	272	1.711 (0.632, 4.628)	0.290			
≥60	577	2.033 (1.304, 3.168)	0.002			
Sex				0.303 (–1.853, 2.458)	0.099 (–0.574, 0.773)	1.173 (0.372, 3.702)
Female	364	2.224 (1.165, 4.245)	0.015			
Male	485	1.880 (1.095, 3.227)	0.022			
Hypertension				3.084 (–0.207, 6.376)	0.503 (0.173, 0.833)	2.506 (0.982, 6.394)
no	263	1.713 (0.720, 4.077)	0.224			
yes	586	2.330 (1.426, 3.807)	0.001			
Diabetes				1.216 (–0.974, 3.406)	0.404 (–0.107, 0.915)	2.529 (0.496, 12.890)
no	592	1.707 (1.016, 2.867)	0.043			
yes	257	2.554 (1.270, 5.138)	0.009			
Stroke				–0.558 (–3.935, 2.819)	–0.137 (–1.054, 0.780)	0.846 (0.293, 2.448)
no	602	2.732 (1.653, 4.515)	< 0.001			
yes	247	1.327 (0.610, 2.885)	0.476			

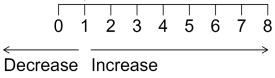

Figure 4. Forest plot of the OR of predictors for moderate/severe deep white matter hyperintensities, stratified by age, hypertension, diabetes, and stroke history.
AP indicates attributable proportion due to interaction; OR, odds ratio; RERI, relative excess risk attributable to interaction; and S, synergy index.

In further survival analysis, the Kaplan-Meier curve demonstrated the cumulative probability of moderate/severe DWMHs with aging in *S/S* genotype subjects was significantly increased compared with non-*S/S* genotype subjects (log-rank test, *P*=0.050) (Figure 5).

DISCUSSION

The main finding of this study was that the *HO-1* gene promotor (GT)n repeat polymorphism was independently associated with the progression of moderate/severe DWMHs, whereas there was no significant correlation with PVHs. Specifically, *SS*-type homozygotes were positively associated with moderate/severe DMWHs, with shorter (GT)n being associated with a higher risk of DWMHs; and there was a synergetic effect of *S/S* homozygotes with age on the progression of DWMHs.

WMHs represent a notable imaging characteristic of cerebral small-vessel disease. The higher prevalence of WMHs in Asian populations may be predominantly influenced by genetic predisposition.[39,40] Although genome-wide association studies have identified multiple polymorphic loci linked to WMHs,[41,42] the available

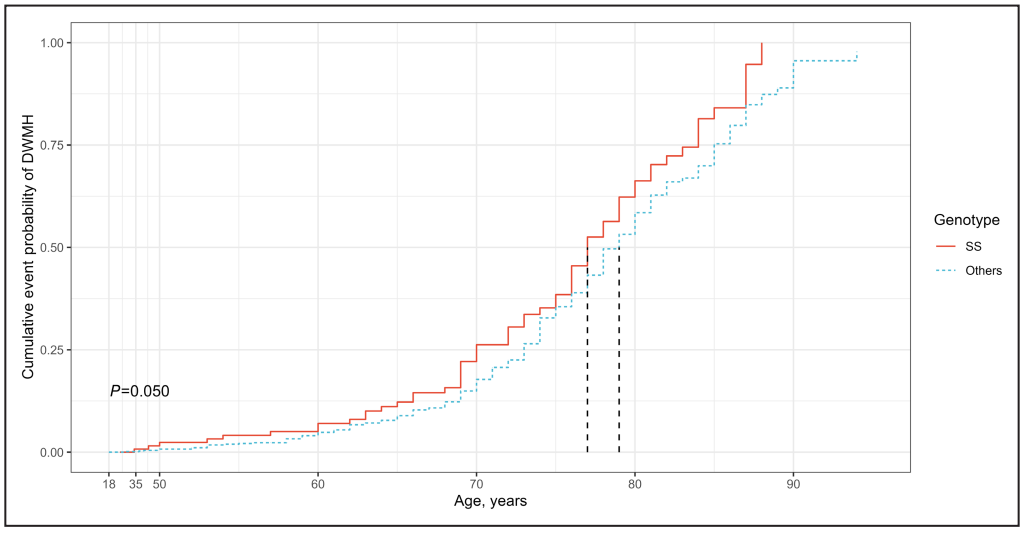

Figure 5. Kaplan-Meier curve demonstrates the cumulative probability of moderate/severe DWMHs with aging in *SS* genotype subjects is significantly increased compared with non-*SS* genotype subjects (log-rank test, *P*=0.050). DWMHs indicates deep white matter hyperintensities.

evidence fails to offer intricate insights into the underlying gene function. By using a candidate gene screening approach, we discovered that (GT)n polymorphic loci in the *HO-1* promoter region were associated with DWMHs, representing a novel finding in the pathogenesis of WMHs. Notably, individuals carrying the *SS* genotype showed a significantly elevated risk of moderate/severe DWMHs. This is consistent with the genetic heterogeneity featuring the WMHs. However, this polymorphic locus was not significantly associated with PVHs, consistently suggesting that WMHs in different brain regions may not be entirely homogeneous in terms of pathogenesis.[43,44]

HO-1 plays an important role in cellular protection against oxidative damage through its scavenging of heme and production of bioactive molecules. Previous studies have reported that the *HO-1* gene promoter *S* allele is protective against a variety of diseases. Gill et al analyzed 544 autopsy brain tissue samples and found that the shorter *HO-1* (GT)n allele increased *HO-1* promoter activity, which could provide neuroprotection by reducing neuroimmune activation and reduce the risk of HIV-related encephalitis.[26] In a large sample case-control study, Rueda et al reported the protective effect of the *HO-1* promoter (rs3074372) *S* allele against rheumatoid arthritis.[29] Clinical studies from cardiovascular diseases have also reported that the *HO-1* (GT)n *S* allele possesses a certain degree of protection against disease susceptibility, ejection fraction, and mortality in subjects with coronary artery disease.[23,24,27] In contrast to earlier investigations, the present study revealed disparate findings, possibly attributable to the fact that the cytoprotective effects of HO-1 may vary depending on the specific cells, tissues, and pathologic conditions.

Previous studies have reported conflicting outcomes on the impact of HO-1, revealing both neuroprotective and detrimental effects.[20,45] One possible mechanism by which the induction of HO-1 provides cellular protection is through the catabolism of pro-oxidative hemoglobin and production of Fe^{2+}, CO, and bilirubin to scavenge reactive oxygen species. On the other hand, heme-derived iron and CO may exacerbate intracellular oxidative stress and cellular damage by promoting the generation of free radicals within the mitochondrial compartment. Experimental studies have found that sustained activation of the (Nuclearfactor erythroidderived 2-like 2) Nrf2/HO-1 pathway in mouse astrocytes induced oligodendrocyte apoptosis and hindered myelin regeneration.[46] In contrast, *HO-1* knockout mice had reduced reactive oxygen species and cell apoptosis in brain tissue.[47] Not only has the *HO-1* gene been identified as a major relevant differentially expressed gene promoting iron death, but the promotion of HO-1 overexpression in translational research has emerged as a promising potential target for tumor therapy.[48,49]

Aging is an important cause of WMHs. Our results also suggested that aging and the *HO-1* gene might act synergistically on the progression of WMHs. Myelin degeneration and loss occur in all primates with age.[50] The effect of *HO-1* on the WMH progression becomes more prominent with the aging process, which is consistent with the finding that high expression of *HO-1* affects myelin repair, as found on the mouse model of AD.[46] In addition, compared with youth, in the aged brain, microglia are overactivated and astrocytes readily undergo conversion to a proinflammatory phenotype, and an increase in inflammatory factors may be 1 of the intrinsic reasons why the *HO-1* promoter region is activated and thus involved in the progression of WMHs in the aging population.[51]

This study has some limitations. First, it was a single-center study from a memory clinic. Because of the lack of AD phenotype assessment, it cannot be excluded that the association of *HO-1* with WMHs may be confounded by potential influence of other factors, such as AD phenotypes, and some caution is needed in generalizing the results in the general population, which needs to be further validated in other cohorts. Second, our study only examined the (GT)n repeat of *HO-1* gene. There is a possibility that the observed association was actually attributable to other polymorphisms that were correlated with the (GT)n repeat. Further study is warranted to perform a validation of HO-1 enzyme activity in the brain, and elucidation of the mechanism needs to be analyzed in the laboratory using cellular or animal models. Third, we performed a semiquantitative assessment of WMHs using the Fazakas scale and did not further analyze more detailed anatomic burdens of WMHs, whereas spatial distribution specificity of WMHs may vary during pathophysiological processes. This needs to be validated in a larger sample size of clinical imaging cohorts.

CONCLUSIONS

The polymorphic locus of the *HO-1* gene (GT)n in the Chinese population was significantly correlated with DWMHs. Future studies need to be conducted in other populations with larger sample sizes. Further investigation is necessary to elucidate the physiological function of HO-1 and unravel the molecular mechanisms that contribute to the development of WMHs, as this could unveil a promising therapeutic target for WMHs.

ARTICLE INFORMATION

Received December 13, 2023; accepted May 1, 2024.

Affiliations

Department of Neurology and Memory Center, The 10th Affiliate Hospital, Southern Medical University, Dongguan, China (J.C., J.L., X.F., S.L.,

Z.S.); Postgraduate School, Guangdong Medical University, Zhanjiang, Guangdong, China (J.C., X.W., J.W., K.C., Z.S.); and The 1st Clinical Medical School, Southern Medical University, Dongguan, China (J.L., J.K., J.L., Z.S.).

Acknowledgments

Author contributions: Junting Chen and Zhu Shi conceived the study. Junting Chen, Jinrui Li, and S.L. initiated the study design. Junting Chen, Jinrui Li, and Jianxia Ke helped to conduct the data collection and statistical analysis. Xiaomian Wang, Xiaoli Fu, Jintao Li, Jia Wen, and Kailin Cheng conducted the neuroimaging analysis and genetic testing. All authors contributed to the refinement of the study protocol and approved the final manuscript.

Sources of Funding

This work was supported by the Basic and Applied Basic Research Foundation of Guangdong Province Fund (2021B1515120089) and the Special Fund for Postgraduate Training, Dongguan People's Hospital (K202035).

Disclosures

None.

Supplemental Material

Table S1

REFERENCES

1. Longstreth WT Jr, Arnold AM, Beauchamp NJ Jr, Manolio TA, Lefkowitz D, Jungreis C, Hirsch CH, O'Leary DH, Furberg CD. Incidence, manifestations, and predictors of worsening white matter on serial cranial magnetic resonance imaging in the elderly: the cardiovascular health study. *Stroke*. 2005;36:56–61. doi: 10.1161/01.STR.0000149625.99732.69
2. Schmidt R, Schmidt H, Haybaeck J, Loitfelder M, Weis S, Cavalieri M, Seiler S, Enzinger C, Ropele S, Erkinjuntti T, et al. Heterogeneity in age-related white matter changes. *Acta Neuropathol*. 2011;122:171–185. doi: 10.1007/s00401-011-0851-x
3. Prins ND, Scheltens P. White matter hyperintensities, cognitive impairment and dementia: an update. *Nat Rev Neurol*. 2015;11:157–165. doi: 10.1038/nrneurol.2015.10
4. Kaskikallio A, Karrasch M, Koikkalainen J, Lötjönen J, Rinne JO, Tuokkola T, Parkkola R, Grönholm-Nyman P. White matter hyperintensities and cognitive impairment in healthy and pathological aging: a quantified brain MRI study. *Dement Geriatr Cogn Disord*. 2019;48:297–307. doi: 10.1159/000506124
5. Maillard P, Carmichael O, Fletcher E, Reed B, Mungas D, DeCarli C. Coevolution of white matter hyperintensities and cognition in the elderly. *Neurology*. 2012;79:442–448. doi: 10.1212/WNL.0b013e3182617136
6. Alber J, Alladi S, Bae HJ, Barton DA, Beckett LA, Bell JM, Berman SE, Biessels GJ, Black SE, Bos I, et al. White matter hyperintensities in vascular contributions to cognitive impairment and dementia (VCID): knowledge gaps and opportunities. *Alzheimer's Dement (New York, N Y)*. 2019;5:107–117. doi: 10.1016/j.trci.2019.02.001
7. Hu HY, Ou YN, Shen XN, Qu Y, Ma YH, Wang ZT, Dong Q, Tan L, Yu JT. White matter hyperintensities and risks of cognitive impairment and dementia: a systematic review and meta-analysis of 36 prospective studies. *Neurosci Biobehav Rev*. 2021;120:16–27. doi: 10.1016/j.neubiorev.2020.11.007
8. Hilal S, Mok V, Youn YC, Wong A, Ikram MK, Chen CL. Prevalence, risk factors and consequences of cerebral small vessel diseases: data from three Asian countries. *J Neurol Neurosurg Psychiatry*. 2017;88:669–674. doi: 10.1136/jnnp-2016-315324
9. de Leeuw FE, de Groot JC, Achten E, Oudkerk M, Ramos LM, Heijboer R, Hofman A, Jolles J, van Gijn J, Breteler MM. Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam scan study. *J Neurol Neurosurg Psychiatry*. 2001;70:9–14. doi: 10.1136/jnnp.70.1.9
10. Jin H, Ding Z, Lian S, Zhao Y, He S, Zhou L, Zhuoga C, Wang H, Xu J, Du A, et al. Prevalence and risk factors of white matter lesions in Tibetan patients without acute stroke. *Stroke*. 2020;51:149–153.
11. Maniega SM, Valdés Hernández MC, Clayden JD, Royle NA, Murray C, Morris Z, Aribisala BS, Gow AJ, Starr JM, Bastin ME, et al. White matter hyperintensities and normal-appearing white matter integrity in the aging brain. *Neurobiol Aging*. 2015;36:909–918. doi: 10.1016/j.neurobiolaging.2014.07.048
12. Zhuang FJ, Chen Y, He WB, Cai ZY. Prevalence of white matter hyperintensities increases with age. *Neural Regen Res*. 2018;13:2141–2146. doi: 10.4103/1673-5374.241465
13. Al-Mashhadi S, Simpson JE, Heath PR, Dickman M, Forster G, Matthews FE, Brayne C, Ince PG, Wharton SB; Medical Research Council Cognitive Function and Ageing Study. Oxidative glial cell damage associated with white matter lesions in the aging human brain. *Brain Pathology (Zurich, Switzerland)*. 2015;25:565–574. doi: 10.1111/bpa.12216
14. Shimamura N, Katagai T, Fujiwara N, Ueno K, Watanabe R, Fumoto T, Naraoka M, Ohkuma H. Intra-arterial anti-oxidant power negatively correlates with white matter injury, and oxidative stress positively correlates with disability in daily activities. *Exp Neurol*. 2021;336:113539. doi: 10.1016/j.expneurol.2020.113539
15. Kang PYC, Chen Y, Ford AL, An H, Lee JM. Oxygen metabolic stress and white matter injury in patients with cerebral small vessel disease. *Stroke*. 2022;53:1570–1579. doi: 10.1161/STROKEAHA.121.035674
16. Chang TC, Chen YC, Huang YC, Lin WC, Lu CH. Systemic oxidative stress and cognitive function in Parkinson's disease with different PWMH or DWMH lesions. *BMC Neurol*. 2021;21:16. doi: 10.1186/s12883-020-02037-z
17. Jung YH, Park S, Lee NK, Han HJ, Jang H, Kim HJ, Seo SW, Na DL. White matter lesions predominantly located in deep white matter represent embolic etiology rather than small vessel disease. *Dement Neurocogn Disord*. 2023;22:28–42. doi: 10.12779/dnd.2023.22.1.28
18. Bach FH. Heme oxygenase-1 as a protective gene. *Wien Klin Wochenschr*. 2002;114(suppl 4):1–3.
19. Abraham NG, Kappas A. Pharmacological and clinical aspects of heme oxygenase. *Pharmacol Rev*. 2008;60:79–127. doi: 10.1124/pr.107.07104
20. Wu J, Li S, Li C, Cui L, Ma J, Hui Y. The non-canonical effects of heme oxygenase-1, a classical fighter against oxidative stress. *Redox Biol*. 2021;47:102170. doi: 10.1016/j.redox.2021.102170
21. Exner M, Minar E, Wagner O, Schillinger M. The role of heme oxygenase-1 promoter polymorphisms in human disease. *Free Radic Biol Med*. 2004;37:1097–1104. doi: 10.1016/j.freeradbiomed.2004.07.008
22. Drummond GS, Baum J, Greenberg M, Lewis D, Abraham NG. HO-1 overexpression and underexpression: clinical implications. *Arch Biochem Biophys*. 2019;673:108073. doi: 10.1016/j.abb.2019.108073
23. Liang KW, Lee WJ, Lee WL, Wu JP, Lee IT, Wang JS, Sheu WHH. Subjects with coronary artery disease and reduced ejection fraction have longer (GT)(n) repeats in the heme-oxygenase 1 gene promoter. *Heart Vessel*. 2021;36:615–620. doi: 10.1007/s00380-020-01733-7
24. Chen YH, Lin SJ, Lin MW, Tsai HL, Kuo SS, Chen JW, Charng MJ, Wu TC, Chen LC, Ding P, et al. Microsatellite polymorphism in promoter of heme oxygenase-1 gene is associated with susceptibility to coronary artery disease in type 2 diabetic patients. *Hum Genet*. 2002;111:1–8. doi: 10.1007/s00439-002-0769-4
25. Taha H, Skrzypek K, Guevara I, Nigisch A, Mustafa S, Grochot-Przeczek A, Ferdek P, Was H, Kotlinowski J, Kozakowska M, et al. Role of heme oxygenase-1 in human endothelial cells: lesson from the promoter allelic variants. *Arterioscler Thromb Vasc Biol*. 2010;30:1634–1641. doi: 10.1161/ATVBAHA.110.207316
26. Gill AJ, Garza R, Ambegaokar SS, Gelman BB, Kolson DL. Heme oxygenase-1 promoter region (GT)n polymorphism associates with increased neuroimmune activation and risk for encephalitis in HIV infection. *J Neuroinflammation*. 2018;15:70. doi: 10.1186/s12974-018-1102-z
27. Liang KW, Lee WJ, Lee WL, Wu JP, Sheu WH. Shorter (GT)(n) repeats in the haem-oxygenase 1 gene promoter are associated with better mid-term survival in subjects with coronary artery disease and abnormal ejection fraction. *Biomarkers*. 2021;26:732–736. doi: 10.1080/1354750X.2021.1992650
28. Pechlaner R, Willeit P, Summerer M, Santer P, Egger G, Kronenberg F, Demetz E, Weiss G, Tsimikas S, Witztum JL, et al. Heme oxygenase-1 gene promoter microsatellite polymorphism is associated with progressive atherosclerosis and incident cardiovascular disease. *Arterioscler Thromb Vasc Biol*. 2015;35:229–236. doi: 10.1161/ATVBAHA.114.304729
29. Rueda B, Oliver J, Robledo G, López-Nevot MA, Balsa A, Pascual-Salcedo D, González-Gay MA, González-Escribano MF, Martín J. HO-1 promoter polymorphism associated with rheumatoid arthritis. *Arthritis Rheum*. 2007;56:3953–3958. doi: 10.1002/art.23048

30. Yang CM, Lin CC, Hsieh HL. High-glucose-derived oxidative stress-dependent Heme Oxygenase-1 expression from astrocytes contributes to the neuronal apoptosis. *Mol Neurobiol*. 2017;54:470–483. doi: 10.1007/s12035-015-9666-4
31. Yu W, Tu Y, Long Z, Liu J, Kong D, Peng J, Wu H, Zheng G, Zhao J, Chen Y, et al. Reactive oxygen species bridge the gap between chronic inflammation and tumor development. *Oxidative Med Cell Longev*. 2022;2606928:1–22. doi: 10.1155/2022/2606928
32. Gupta A, Lacoste B, Pistel PJ, Ingram DK, Hamel E, Alaoui-Jamali MA, Szarek WA, Vlahakis JZ, Jie S, Song W, et al. Neurotherapeutic effects of novel HO-1 inhibitors in vitro and in a transgenic mouse model of Alzheimer's disease. *J Neurochem*. 2014;131:778–790. doi: 10.1111/jnc.12927
33. Fazekas F, Chawluk JB, Alavi A, Hurtig HI, Zimmerman RA. MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. *AJR Am J Roentgenol*. 1987;149:351–356. doi: 10.2214/ajr.149.2.351
34. DeCarli C, Fletcher E, Ramey V, Harvey D, Jagust WJ. Anatomical mapping of white matter hyperintensities (WMH): exploring the relationships between periventricular WMH, deep WMH, and total WMH burden. *Stroke*. 2005;36:50–55. doi: 10.1161/01.STR.0000150668.58689.f2
35. Griffanti L, Jenkinson M, Suri S, Zsoldos E, Mahmood A, Filippini N, Sexton CE, Topiwala A, Allan C, Kivimäki M, et al. Classification and characterization of periventricular and deep white matter hyperintensities on MRI: a study in older adults. *NeuroImage*. 2018;170:174–181. doi: 10.1016/j.neuroimage.2017.03.024
36. Iordanishvili E, Schall M, Loução R, Zimmermann M, Kotetishvili K, Shah NJ, Oros-Peusquens AM. Quantitative MRI of cerebral white matter hyperintensities: a new approach towards understanding the underlying pathology. *NeuroImage*. 2019;202:116077. doi: 10.1016/j.neuroimage.2019.116077
37. Haller S, Kövari E, Herrmann FR, Cuvinciuc V, Tomm AM, Zulian GB, Lovblad KO, Giannakopoulos P, Bouras C. Do brain T2/FLAIR white matter hyperintensities correspond to myelin loss in normal aging? A radiologic-neuropathologic correlation study. *Acta Neuropathol Commun*. 2013;1:14. doi: 10.1186/2051-5960-1-14
38. Yao D, Li S, Jing J, Cai X, Jin A, Yang Y, Wang S, Meng X, Lin J, Mei L, et al. Association of serum cystatin C with cerebral small vessel disease in community-based population. *Stroke*. 2022;53(10):3123–3132. doi: 10.1161/STROKEAHA.122.039277
39. Atwood LD, Wolf PA, Heard-Costa NL, Massaro JM, Beiser A, D'Agostino RB, DeCarli C. Genetic variation in white matter hyperintensity volume in the Framingham study. *Stroke*. 2004;35:1609–1613. doi: 10.1161/01.STR.0000129643.77045.10
40. Bhagat R, Marini S, Romero JR. Genetic considerations in cerebral small vessel diseases. *Front Neurol*. 2023;14:1080168. doi: 10.3389/fneur.2023.1080168
41. Fornage M, Debette S, Bis JC, Schmidt H, Ikram MA, Dufouil C, Sigurdsson S, Lumley T, DeStefano AL, Fazekas F, et al. Genome-wide association studies of cerebral white matter lesion burden: the CHARGE consortium. *Ann Neurol*. 2011;69:928–939. doi: 10.1002/ana.22403
42. Traylor M, Zhang CR, Adib-Samii P, Devan WJ, Parsons OE, Lanfranconi S, Gregory S, Cloonan L, Falcone GJ, Radmanesh F, et al. Genome-wide meta-analysis of cerebral white matter hyperintensities in patients with stroke. *Neurology*. 2016;86:146–153. doi: 10.1212/WNL.0000000000002263
43. Habes M, Sotiras A, Erus G, Toledo JB, Janowitz D, Wolk DA, Shou H, Bryan NR, Doshi J, Völzke H, et al. White matter lesions: spatial heterogeneity, links to risk factors, cognition, genetics, and atrophy. *Neurology*. 2018;91:e964–e975. doi: 10.1212/WNL.0000000000006116
44. Armstrong NJ, Mather KA, Sargurupremraj M, Knol MJ, Malik R, Satizabal CL, Yanek LR, Wen W, Gudnason VG, Dueker ND, et al. Common genetic variation indicates separate causes for periventricular and deep white matter hyperintensities. *Stroke*. 2020;51:2111–2121. doi: 10.1161/STROKEAHA.119.027544
45. Waza AA, Hamid Z, Ali S, Bhat SA, Bhat MA. A review on heme oxygenase-1 induction: is it a necessary evil. *Inflamm Res*. 2018;67:579–588. doi: 10.1007/s00011-018-1151-x
46. Molina-Gonzalez IHR, Jiwaji Z, Dando O, Kent SA, Emelianova K, Lloyd AF, Forbes LH, Mahmood A, Skripuletz T, Gudi V, et al. Astrocyte-oligodendrocyte interaction regulates central nervous system regeneration. *Nat Commun*. 2023;14:3372. doi: 10.1038/s41467-023-39046-8
47. Fernández-Mendívil C, Luengo E, Trigo-Alonso P, García-Magro N, Negredo P, López MG. Protective role of microglial HO-1 blockade in aging: implication of iron metabolism. *Redox Biol*. 2021;38:101789. doi: 10.1016/j.redox.2020.101789
48. Wei R, Zhao Y, Wang J, Yang X, Li S, Wang Y, Yang X, Fei J, Hao X, Zhao Y, et al. Tagitinin C induces ferroptosis through PERK-Nrf2-HO-1 signaling pathway in colorectal cancer cells. *Int J Biol Sci*. 2021;17:2703–2717. doi: 10.7150/ijbs.59404
49. Zhao P, Song H, Gao F, Chen L, Qiu J, Jin J, Pan C, Tang Y, Chen M, Pan Y, et al. A novel derivative of curcumol, HCL-23, inhibits the malignant phenotype of triple-negative breast cancer and induces apoptosis and HO-1-dependent ferroptosis. *Molecules*. 2023;28:3389. doi: 10.3390/molecules28083389
50. Peters A, Kemper T. A review of the structural alterations in the cerebral hemispheres of the aging rhesus monkey. *Neurobiol Aging*. 2012;33:2357–2372. doi: 10.1016/j.neurobiolaging.2011.11.015
51. Norden DM, Godbout JP. Review: microglia of the aged brain: primed to be activated and resistant to regulation. *Neuropathol Appl Neurobiol*. 2013;39:19–34. doi: 10.1111/j.1365-2990.2012.01306.x