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ARTICLE

The association of matrix metalloproteinase 9 (MMP9) with hippocampal volume in schizophrenia: a preliminary MRI study

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Matrix metalloproteinases 9 (MMP9) are enzymes involved in regulating neuroplasticity in the hippocampus. This, combined with evidence for disrupted hippocampal structure and function in schizophrenia, has prompted our current investigation into the relationship between MMP9 and hippocampal volumes in schizophrenia. 34 healthy individuals (mean age = 32.50, male = 21, female = 13) and 30 subjects with schizophrenia (mean age = 33.07, male = 19, female = 11) underwent a blood draw and T1-weighted magnetic resonance imaging. The hippocampus was automatically segmented utilizing FreeSurfer. MMP9 plasma levels were measured with ELISA. ANCOVAs were conducted to compare MMP9 plasma levels (corrected for age and sex) and hippocampal volumes between groups (corrected for age, sex, total intracranial volume). Spearman correlations were utilized to investigate the relationship between symptoms, medication, duration of illness, number of episodes, and MMP9 plasma levels in patients. Last, we explored the correlation between MMP9 levels and hippocampal volumes in patients and healthy individuals separately. Patients displayed higher MMP9 plasma levels than healthy individuals ($F(1, 60) = 21.19$, $p < 0.0001$). MMP9 levels correlated with negative symptoms in patients ($R = 0.39$, $p = 0.035$), but not with medication, duration of illness, or the number of episodes. Further, patients had smaller left ($F(1,59) = 9.12$, $p = 0.0040$) and right ($F(1,59) = 6.49$, $p = 0.013$) hippocampal volumes. Finally, left ($R = -0.39$, $p = 0.034$) and right ($R = -0.37$, $p = 0.046$) hippocampal volumes correlated negatively with MMP9 plasma levels in patients. We observe higher MMP9 plasma levels in SCZ, associated with lower hippocampal volumes, suggesting involvement of MMP9 in the pathology of SCZ. Future studies are needed to investigate how MMP9 influences the pathology of SCZ over the lifespan, whether the observed associations are specific for schizophrenia, and if a therapeutic modulation of MMP9 promotes neuroprotective effects in SCZ.

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INTRODUCTION

Neuroplasticity refers to the brain's ability to reorganize neuronal pathways in response to internal and external stimuli and comprises several levels, including micro-and macro connectivity, activity-dependent brain function, and synaptic transmission [1–3]. While previous studies have characterized altered neuroplasticity as one of the core pathologies of schizophrenia [4, 5], recent research tries to identify which factors might underly these abnormalities. Interestingly, several lines of evidence highlighted the extracellular matrix's role in modulating neuronal pathways in response to stimuli. Specifically, matrix metalloproteinases (MMPs), a large family of extracellularly acting zinc-dependent proteases [6], have been implicated in the dysregulation of forming and eliminating synapses in schizophrenia.

Matrix metalloproteinase 9 (MMP9) is the most prevalent MMP in the central nervous system [7]. It is expressed in neural and glial cells in multiple brain regions, including the hippocampus [8, 9]. It is released under the influence of glutamate and can bind to various substrates, including beta-amyloid, precursors of growth factors, chemokines, tissue inhibitors, and adhesion molecules [10]. At the physiological level, it controls hippocampal [11–16] and dendritic development and maintains the extracellular environment of the postsynaptic part of excitatory synapses (by modifying both NMDA and AMPA receptors) [9, 17], which are vital for synaptic plasticity and long-term potentiation [18, 19]. Long-term potentiation is a physiological readout of neuroplasticity that a) reflects the functional ability for memory acquisition and storage and b) is often disturbed in schizophrenia [4, 20].

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Interestingly, increased MMP9 levels are associated with structural (e.g., abnormally long and thin dendritic spines [7]) and functional (e.g., impaired long-term potentiation [14, 21, 22]) synaptic plasticity abnormalities.

MMP9 has repeatedly been implicated in the pathophysiology of schizophrenia [23]. Several studies have demonstrated MMP9 upregulation in the blood of patients with schizophrenia when compared to healthy individuals [24, 25]. Upregulation of mRNA expression of MMP9 was reported in peripheral blood mononuclear cells in treatment-naive schizophrenia patients, with a marked downregulation following treatment [26]. Preliminary evidence also suggests that minocycline, an antibiotic and MMP9 inhibitor, ameliorates symptom severity in patients with schizophrenia - further pointing towards the potential role of MMP9 as a treatment target [27–29].

While this accumulating evidence suggests a possible relationship between abnormal MMP9 regulation and brain structural and functional pathology in schizophrenia, no studies have investigated this link in humans. In this preliminary study, we propose to combine and analyze the relationship between imaging markers of the hippocampus structure, the blood marker MMP9, and clinical variables (including symptom severity, medication, duration of illness, number of episodes). Given the crucial role of the hippocampus for schizophrenia [30–33], the role of MMP9 in regulating hippocampus structure and function [8, 9, 12–15], and its role in the regulation of dendritic and synaptic plasticity, we postulate that a) patients with schizophrenia will present with higher plasma levels of MMP9 than healthy individuals and b) MMP9 abnormalities will be associated with smaller hippocampal volumes in patients.

MATERIALS AND METHODS

Participants

The present study includes data from 34 healthy individuals and 30 patients with schizophrenia. Patients were recruited in the Department of Psychiatry at University Hospital Brno, Brno, Czech Republic, following hospital admission for an acute psychotic episode. Patients were diagnosed utilizing the Structured clinical interview for DSM5 - Research version (SCID-RV) criteria [34]. The Positive and Negative Symptoms Scales (PANSS) [35] were administered to determine symptom severity. We recruited healthy individuals via advertising within the local community. Exclusion criteria for healthy individuals and patients were a history of neurological injury, brain disorder, substance abuse, and an inability to undergo MRI. Additional exclusion criteria for healthy individuals were any history of psychiatric illness themselves, first or second-degree relatives (assessed with Mini-International Neuropsychiatric Interview [36]).

All participants provided written informed consent for participation, and the Institutional Ethical Committee of the University Hospital Brno approved the study.

Data collection

MMP9. Venous blood from people with schizophrenia was collected approximately three weeks after hospitalization for an acute psychotic episode, on the day of MRI scanning. At this time, patients have been intensively treated with antipsychotics, and positive symptoms started to subside. The blood was collected at 7 am in a fasting state to account for possible circadian fluctuations. Blood was collected into tubes with EDTA and immediately stored on ice (0 °C). Plasma was separated by centrifugation within 30 min in a refrigerated centrifuge, and aliquots were immediately stored at −75 °C until the time of analysis.

Plasma MMP-9 values were obtained using the Quantikine sandwich ELISA kit (#DMP900, R&D Systems, Minneapolis, MN). The Quantikine immunoassay control group 130 (#QC130) was used as a quantitative control for the determination of MMP9. The MMP9 immunoassay recognizes both the pro-MMP9 (92 kDa) and the active MMP9 (82 kDa), but not the 65 kDa form. Before the analysis, plasma samples were diluted 33 times according to the manufacturer's specifications for the assay and measured in duplicate. The detection limit was 0.156 ng/mL, and the intra-assay coefficient of variation was <2%. The optical densities were measured using the Spectramax 340PC microplate reader (Molecular Devices, San Jose, CA).

Image acquisition. 3T Siemens Magnetom Prisma (Erlangen, Germany) with 64 channel Head-Neck coil was utilized to collect the structural MRI data. A whole brain, high-resolution three-dimensional T1-weighted magnetization prepared rapid gradient echo (MPRAGE) sequence was used to collect 240 sagittal slices (field of view = 224×224 mm^2, 1 mm^3 isotropic voxel, TR = 2.3 s, TE = 2.33 ms, flip angle = 8).

Image processing

Structural T1-weighted images underwent visual quality control (for artifacts). Next, images were axis-aligned and centered. Consecutively, brain masks were automatically generated using 3D Slicer (software version 4.5; www.slicer.org) and manually edited to guarantee anatomical precision. FreeSurfer 5 was run on the structural imaging data, and the output was checked for quality.

Statistical analyses

All statistical analyses were performed with SPSS version 26 and GraphPad Prism 8.

MMP9

Group comparison: To test for group differences in MMP9, we conducted an ANCOVA with MMP9 plasma level as the dependent variable, group as the independent variable (healthy individuals versus patients with schizophrenia), and age and sex as covariates. Please note that (a) MMP9 plasma values were not normally distributed in healthy individuals (healthy: $D(34) = 0.20$, $p < 0.001$; patients: $D(30) = 0.11$, $p < 0.20$) and (b) the variance of MMP9 plasma levels was different between groups ($F = 8.51$, $p < 0.005$). Thus, we repeated the group comparisons for MMP9 levels with a non-parametric Mann–Whitney *U* test.

Association between MMP9 and clinical measures: Post hoc Spearman's correlation analyses between positive/ negative symptom severity, chlorpromazine equivalent dosage (CPZ), duration of illness, number of episodes, and MMP9 plasma levels were carried out for patients. We chose Spearman's rank order because not all variables were normally distributed. We utilized Fisher's Exact Score to test if the correlation coefficients significantly differed from zero.

Hippocampus

Group comparison: To test for group differences between the patients and the HC groups in the hippocampus, we used two ANCOVAs with left or right hippocampal volume as the independent variable, group (healthy individuals versus patients with schizophrenia) as the dependent variable, and age, sex, and total intracranial volume as covariates.

Association between MMP9 and hippocampus. For patients with schizophrenia and healthy individuals separately, we applied Spearman's correlation analyses to correlate the left and right hippocampus volume with MMP9 plasma levels. Fisher's Exact Score was utilized to test whether the correlation coefficient significantly differed from zero.

Table 1. Demographics.

	Healthy individuals ($N = 34$)	Individuals with schizophrenia ($N = 30$)	Test-statistic
Age in years (mean ± std)	32.50 ± 9.10	33.07 ± 9.48	$T = -0.24$, df = 62, $p < 0.81$
Sex	Male = 21, Female = 13	Male = 19, Female = 11	X2 = 0.017, df = 1, $p < 0.90$
Education in years (mean ± std)	14.91 ± 2.71	13.93 ± 2.95	$T = 1.38$, df = 62, $p < 0.17$
Duration of illness in years (mean ± std)		7.96 ± 6.45	
Number of episodes		3.37 ± 2.39	
Chlorpromazine equivalent dosage in mg		740.77 ± 385.08	
PANSS Negative Symptoms		15.60 ± 6.31	
PANSS Positive Symptoms		11.80 ± 4.77	

Table 1 shows that healthy individuals and patients were matched for age, sex, and education years and displays clinical characteristics for patients.
std standard deviation, *PANSS* Positive and Negative Symptom Scale [74].

RESULTS

Demographics

Patients with schizophrenia and healthy individuals did not show group differences in critical demographic variables (see Table 1).

MMP9

Group comparison: ANCOVA with MMP9 plasma levels as the dependent variable showed a significant effect of group ($F(1,60) = 21.19$, $p < 0.0001$, Cohen's $d = 1.09$). The average MMP 9 plasma levels of patients with schizophrenia were almost twice as high as those of healthy individuals (89.76% increase, patients = 119.72 ± 62.65 ng/ml; healthy individuals = 63.09 ± 37.89 ng/ml) (Fig. 1). In addition to a significant group effect, we also observed a significant sex effect F(1,60) = 8.46, $p < 0.005$). Age did not have a significant effect (F(1,60) = 0.006, $p < 0.94$). To follow up on the significant sex effect, we conducted a sex x group interaction analysis, which did not yield a significant result (F(1,60) = 2.85, $p < .097$). Please note that we still observed significant group differences when repeating the analyses with a non-parametric Mann–Whitney U test ($U = 188.00$, $p < 0.0001$).

Association between MMP9 and clinical measures: To assess whether MMP9 levels were associated with clinical measures, we performed correlations in patients with schizophrenia. Positive symptom severity did not significantly correlate with MMP9 plasma levels ($R = -0.23$, $p = 0.23$). However, there was a statistically significant correlation between MMP9 plasma levels and negative symptom severity ($R = 0.39$, $p = 0.035$). MMP9 plasma levels were not correlated with CPZ ($R = -0.015$, $p = 0.94$), duration of illness ($R = -0.014$, $p < 0.94$), or number of episodes ($R = 0.036$, $p < 0.85$).

Hippocampus. ANCOVA with the left hippocampal volume as dependent variable ($F(1,59) = 9.12$, $p = 0.004$, Cohen's $d = 0.44$) and the ANCOVA with the right hippocampal volume as dependent variable ($F(1,59) = 6.49$, $p = 0.013$, Cohen's $d = 0.33$) both demonstrated a significant group effect. Patients with schizophrenia had smaller left (4.35% decrease, healthy individuals = 3.91 cm^3 ± 0.37 cm^3, patients = 3.74 cm^3 ± 0.40 mm^3) and right (3.50% decrease, healthy individuals = 4.00 cm^3 ± 0.38 cm^3, patients = 3.86 mm^3 ± 0.46 cm^3) hippocampal volumes than healthy individuals. For associations between hippocampal volumes and clinical variables please see Supplementary Table 1.

Association between MMP9 and hippocampus. For healthy individuals we did not observe an association between MMP9 plasma levels and either left ($R = -0.030$, $p = 0.97$) or right ($R = 0.003$, $p = 0.99$) hippocampal volumes. However, in patients MMP9 plasma levels were negatively correlated with left ($R = -0.39$, $p = 0.034$) and right ($R = -0.37$, $p = 0.046$) hippocampal volumes (Fig. 2).

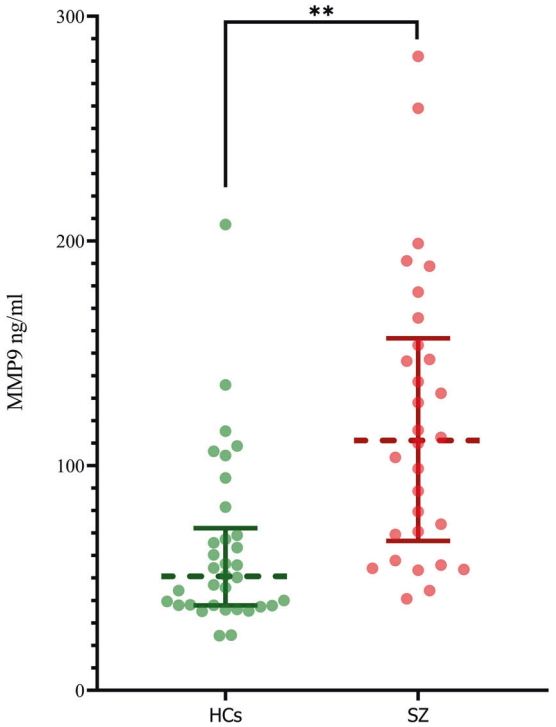

Fig. 1 Group comparison of MMP9 values. Group comparisons for matrix metalloproteinase 9 (MMP9) plasma values (in ng/ml) for healthy individuals (HC) and patients with schizophrenia (SCZ). The dotted lines display the median, the whiskers the interquartile range. Single dots represent individual values. An ANCOVA (corrected for age and sex) demonstrates that patients with SCZ display significantly higher MMP9 plasma values than HC ($F(1,60) = 21.19$, $p < .0001$, Cohen's $d = 1.09$). This result was confirmed by a non-parametric Mann–Whitney U test ($U = 188.00$, $p < 0.0001$).

DISCUSSION

The present exploratory study characterizes the association between MMP9 and the hippocampus in schizophrenia. We observed lower hippocampal volumes and higher MMP9 plasma levels in patients. Most interestingly, in patients, hippocampal volumes and MMP9 plasma levels were negatively correlated.

Group comparisons

MMP9. Our finding of elevated MMP9 plasma levels is in line with previous studies demonstrating higher peripheral activity of MMP9 [37] and its increased blood concentration [24, 25] in patients with schizophrenia. It has been proposed that abnormal MMP9 levels might be a useful peripheral biomarker of

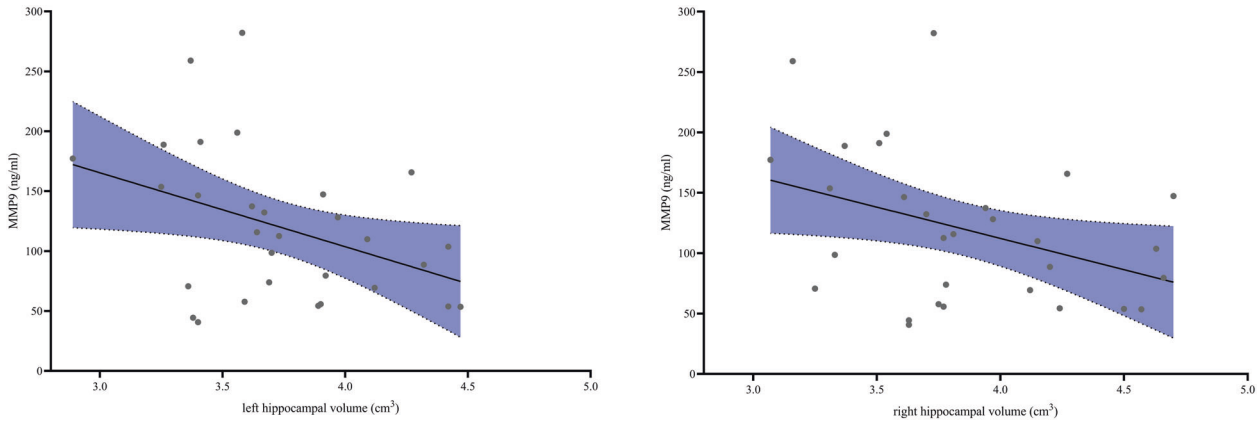

Fig. 2 Correlation of MMP9 levels and hippocampal volume. Correlation between left and right hippocampal volume (in cm^3) and matrix metalloproteinase 9 MMP9 plasma levels (in ng/ml) in patients with schizophrenia. In patients MMP9 plasma levels were negatively correlated with both left ($R = -0.39$, $p = 0.034$) and right ($R = -0.37$, $p = 0.046$) hippocampal volumes.

schizophrenia [24], potentially discriminating patients from healthy individuals [38].

Indeed, given MMP9 distribution and function, upregulation of this protein might explain several processes that are disturbed throughout the lifespan in schizophrenia. MMP9 is an essential regulator of neuroplasticity. Both up- and downregulation of MMP9 can lead to impaired neuroplasticity [19], possibly in part by regulating the development and integrity of perineuronal nets. Moreover, a recent animal study suggests that MMP9 upregulation might be a core mechanism that translates oxidative stress into neuroinflammatory dysregulation and cytotoxic responses [39]. In that paper, the authors propose a "vicious cycle" between oxidative stress and neuroinflammation, especially during development, controlled and modulated by MMP9 expression, leading to long-term detrimental effects on PNNs and PVI neurons.

While our study, correlational in nature, is not designed to test for consequences of MMP9 upregulation, we see an association between MMP9 plasma levels and negative symptoms in patients with schizophrenia. Previous research suggested that negative symptoms may indicate neuronal damage [40] and that predominantly negative symptoms are associated with neurodevelopmental abnormalities [41]. Additionally, our finding is in line with recently published studies reporting that MMP9 mRNA expression is more pronounced in deficit schizophrenia [38, 42]. Last, it has previously been demonstrated that MMP9 influences hippocampal and prefrontal N-methyl-D-aspartate receptor (NMDA) activity [38, 42]. We speculate that this modulation of NMDA activity might mediate the association between MMP9 and negative symptoms in schizophrenia.

Hippocampal volume. Given the crucial role of the hippocampus for memory, emotion processing, and higher-level cognition [43], which are all affected in schizophrenia, it is one of the most studied brain structures in psychosis. Evidence from postmortem [32, 44, 45] and imaging research [43, 46, 47] implies structural and functional abnormalities of the hippocampus and the hippocampal circuits in schizophrenia. Our finding of overall hippocampal atrophy is, thereby, in line with a recent meta-analysis that showed that the hippocampus is the most affected subcortical brain region in SCZ [33].

Association between MMP9 and brain structure

We observed a negative association between MMP9 plasma levels and hippocampal volume. We have focused our analyses on the hippocampus, given the (1) vital role of the hippocampus for the schizophrenia pathophysiology and (2) known importance of MMP9 for hippocampal development and function [11]. MMP9 regulates dendritic and synaptic morphology during development in the hippocampus [9, 14, 48–50] and is expressed in the adult human hippocampus [9]. Additionally, animal studies have demonstrated the role of MMP9 expression in the hippocampus for memory performance [18, 19] and its upregulation in reaction to stress, drugs, or fear [51–53]. Our study supports these previous findings by linking MMP9 plasma levels with macrostructural volumetric measurements in humans. However, more extensive studies are needed to examine whether the observed associations are specific for schizophrenia, how they develop over the trajectory of the disorder, and if therapeutic modifications of MMP9 plasma levels and activity can influence the pathology of SCZ [54]. Last, given the small sample size and exploratory nature of the present study, we did not explore if the association between MMP9 and hippocampal volume might be specific to subregions of the hippocampus. However, some animal studies imply that MMP9 is upregulated explicitly in CA1 and not in the whole hippocampus [19, 52] and experiments in ischemic insult mice models reveal that MMP9 inhibitors [55, 56] have spatially specific neuroprotective effects in the striatum and CA1/CA2 subfields of the hippocampus.

Limitations and future directions

We acknowledge several limitations of the present study. First, this is a preliminary study testing a novel approach of linking MMP9 plasma levels and brain pathologies. Given its novelty and relatively small sample size, we only assessed one brain region. However, there is evidence suggesting a broader role of MMP9 for brain structure and SCZ pathology. Specifically, MMP9 might also play a pivot role in modulating the central inflammatory response [7, 53, 57]. Future studies are needed to examine how MMP9 and brain pathologies' association develops with age [58] and over the SCZ trajectory. Additionally, we acknowledge that while the reported correlations are medium strong, the statistical tests were not corrected for multiple comparisons, and the significance would not survive a Bonferroni multiple-comparison correction (likely due to the small sample size). While we demonstrated that MMP9 is not associated with antipsychotic medication, more extensive studies should examine additional factors, such as sex [59, 60], nicotine [61, 62], alcohol [63], and metabolic dysregulations [64]. Furthermore, while we do not see an association between MMP9 levels and hippocampal volumes in healthy individuals, this does not mean that our finding is specific to SCZ. While some previous studies have suggested that MMP9 might be an excellent marker to discriminate patients with SCZ from healthy individuals [25], trans-diagnostic studies are missing. Importantly there is preliminary evidence that MMP9 might also

be involved in the pathology of mood disorders [65, 66], autism [67], multiple sclerosis [68, 69], and stroke [70, 71]. Last, while most studies propose that peripheral MMP9 levels can directly be associated with MMP9 levels in the brain [72], this assumption needs further validation.

CONCLUSION

We provide evidence for the association between MMP9 and hippocampal volume in patients with schizophrenia. Additionally, we show abnormal MMP9 plasma levels and hippocampal volumes in patients with SCZ, hinting towards the role of MMP9 in the pathology of SCZ. For example, MMP9 inhibitors such as minocycline can regulate MMP9 and promote neuroplasticity, reverse neurodegeneration and improve negative symptoms in SCZ [73]. More extensive studies are needed to replicate our findings and investigate the association between MMP9 and brain pathology along the SCZ spectrum.

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AUTHOR CONTRIBUTIONS

JS-H Ph.D. MD: Substantial contributions to the design of the work, analysis, and interpretation of data; drafting the work; final approval of the version to be published; agreement to be accountable for all aspects of the work. MS MD: Substantial contributions to the analysis of data; revising the draft; final approval of the version to be published; agreement to be accountable for all aspects of the work. NM Ph.D. MD: Substantial contributions to the analysis of data; revising the draft; final approval of the version to be published; agreement to be accountable for all aspects of the work. JR Ph.D.: Substantial contributions to the analysis of data; revising the draft; final approval of the version to be published; agreement to be accountable for all aspects of the work. K-IKC Ph.D.: Substantial contributions to the analysis of data; revising the draft; final approval of the version to be published; agreement to be accountable for all aspects of the work. ER: Substantial contributions to the analysis of data; revising the draft; final approval of the version to be published; agreement to be accountable for all aspects of the work. MV Ph.D.: Substantial contributions to the analysis of data; revising the draft; final approval of the version to be published; agreement to be accountable for all aspects of the work. OSS MD: Substantial contributions to the analysis of data; revising the draft; final approval of the version to be published; agreement to be accountable for all aspects of the work. CH: Substantial contributions to the analysis of data; revising the draft; final approval of the version to be published; agreement to be accountable for all aspects of the work. OP Ph.D.: Substantial contributions to the analysis of data; revising the draft; final approval of the version to be published; agreement to be accountable for all aspects of the work. FAS Ph.D.: Substantial contributions to the analysis of data; revising the draft; final approval of the version to be published; agreement to be accountable for all aspects of the work. C-FW Ph.D.: Substantial contributions to the analysis of data; revising the draft; final approval of the version to be published; agreement to be accountable for all aspects of the work. JL Ph.D. MD: Substantial contributions to the acquisition of data; revising the draft; final approval of the version to be published; agreement to be accountable for all aspects of the work. LU Ph.D. MD: Substantial contributions to the acquisition of data; revising the draft; final approval of the version to be published; agreement to be accountable for all aspects of the work. JT Ph.D. MD: Substantial contributions to the acquisition of data; revising the draft; final approval of the version to be published; agreement to be accountable for all aspects of the work. LV Ph.D.: Substantial contributions to the acquisition of data; revising the draft; final approval of the version to be published; agreement to be accountable for all aspects of the work. PKI: Substantial contributions to the acquisition of data; revising the draft; final approval of the version to be published; agreement to be accountable for all aspects of the work. MJ: Substantial contributions to the acquisition of data; revising the draft; final approval of the version to be published; agreement to be accountable for all aspects of the work. TWW Ph.D. MD: Substantial contributions to the design of the work, analysis, and interpretation of data; revising the draft; final approval of the version to be published; agreement to be accountable for all aspects of the work. TK Ph.D. MD: Substantial contributions to the acquisition of data; revising the draft; final approval of the version to be published; agreement to be accountable for all aspects of the work. ZK Ph.D.: Substantial contributions to the design of the work, analysis, and interpretation of data; revising the draft; final approval of the version to be published; agreement to be accountable for all aspects of the work. MK Ph.D. MD: Substantial contributions to the design of the work, analysis, and interpretation of data; drafting the work; final approval of the version to be published; agreement to be accountable for all aspects of the work

ADDITIONAL INFORMATION

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