Association of Cerebrovascular and Alzheimer Disease Biomarkers With Cholinergic White Matter Degeneration in Cognitively Unimpaired Individuals

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ABSTRACT:

Objectives: Several pathological processes might contribute to the degeneration of the cholinergic system in aging. We aimed to determine the contribution of amyloid, tau, and cerebrovascular biomarkers towards the degeneration of cholinergic white matter (WM) projections in cognitively unimpaired individuals.

Methods: The contribution of amyloid and tau pathology was assessed through cerebrospinal fluid (CSF) levels of the Aβ42/40 ratio and phosphorylated tau (p-tau). CSF Aβ38 levels were also measured. Cerebrovascular pathology was assessed using automatic segmentations of WM lesions on magnetic resonance imaging (MRI). Cholinergic WM projections (i.e., cingulum and external capsule pathways) were modeled using tractography based on diffusion tensor imaging data. Sex and APOE ε4 carriership were also included in the analysis as variables of interest.

Results: We included 203 cognitively unimpaired individuals from the H70 Gothenburg Birth Cohort Studies (all individuals 70 years old, 51% female). WM lesion burden was the most important contributor to the degeneration of both cholinergic pathways (Increase in mean square error (IncMSE)=98.8% in external capsule pathway and IncMSE=93.3% in the cingulum pathway). Levels of Aβ38 and p-tau also contributed to cholinergic white matter degeneration, especially in the external capsule pathway (IncMSE=28.4% and IncMSE=23.4%, respectively). The
Aβ_{42/40} ratio did not contribute notably to the models (IncMSE<3.0%). APOE ε4 carriers showed poorer integrity in the cingulum pathway (IncMSE=21.33%). Women showed poorer integrity of the external capsule pathway (IncMSE=21.55%), which was independent of amyloid status as reflected by the non-significant differences in integrity when comparing amyloid positive versus amyloid negative women participants (T_{201}=-1.55; p=0.123).

**Conclusions:** In cognitively unimpaired older individuals, WM lesions play a central role in the degeneration of cholinergic pathways. Our findings highlight the importance of WM lesion burden in the elderly population, which should be considered in the development of prevention programs for neurodegeneration and cognitive impairment.
1. INTRODUCTION

The cholinergic neurons located in the nucleus basalis of Meynert (NBM) provide the major cholinergic input to the cerebral cortex and are essential to cognitive functioning. Postmortem studies have traced two principal cholinergic projection pathways from the NBM to the neocortex: the medial and the lateral pathways. The medial pathway advances through the white matter (WM) axons of the rectus gyrus, bends at the rostrum of the corpus callosum and enters the cingulum bundle, projecting to the paraolfactory, cingulate and restrosplenial cortices. The lateral pathway advances both through the claustrum and the extreme capsule (i.e., perisylvian division), projecting to the frontoparietal operculum, insula, and superior temporal gyrus; as well as through the external capsule and uncinate fasciculus (i.e., capsular division), projecting to the remaining parts of the frontal, parietal, and temporal neocortex. Recent diffusion tensor imaging (DTI)-based tractography studies have examined these pathways, providing the opportunity to study the integrity of the cholinergic system and its potential association with cognitive performance and pathophysiological processes in vivo.

The strategic location of the NBM and its connective circuitry to the cortex results in increased vulnerability to brain pathology. For example, cholinergic neurons are affected in early stages of Alzheimer’s disease (AD)-related tauopathy due to their proximity to heavily affected basotemporal regions, which likely also alters their connective circuitry to the cortex. Further, other age-related pathologies can also impact the integrity of the cholinergic system. WM lesions (WML), which are thought to be a marker of cerebrovascular disease, are commonly found on magnetic
resonance images (MRI) in the elderly. A recent study showed that WML are associated with worse integrity of the cholinergic projections in cognitively unimpaired older individuals, and cholinergic projections influenced cognitive performance. Interestingly, despite the association of WML with the integrity of the cholinergic projection system, neither WML burden itself nor NBM volume contributed to cognitive performance. These findings raised the question of whether other age-associated pathologies apart from WML might be affecting the integrity of the cholinergic projections in cognitively unimpaired individuals.

In this study, we investigated the contribution of amyloid and tau pathology in combination with cerebrovascular disease towards the degeneration of cholinergic WM projections in cognitively unimpaired individuals. It is important to address these research questions to assess whether and how other pathologies apart from cerebrovascular disease may affect the integrity of cholinergic projections in cognitively unimpaired individuals.

2. METHODS

2.1. Participants

The study sample belongs to the Gothenburg H70 Birth Cohort Studies. Every 70-year-old listed in the Swedish Population Registry as a resident in Gothenburg (Sweden) was invited to a comprehensive examination on aging and age-related factors. A total of 1203 individuals born in 1944 (response rate 72.2%; mean age 70.5 years) agreed to participate, of whom 430 consented to a lumbar puncture
Lumbar puncture was considered as contraindicated in participants under anticoagulant therapy, immune-modulated therapy, and cancer therapy. After excluding participants not suitable for a lumbar puncture, the cerebrospinal fluid (CSF) extraction was conducted in 322 (26.8%) individuals. Every participant was also invited to take part in a brain MRI examination of which 792 individuals (response rate 65.8%) underwent MRI conducted at Aleris in Gothenburg. The MRI examination was conducted within 3 months from the initial study visit. The lumbar puncture was conducted within 2 months from the MRI examination. The general examinations and other procedures have previously been described in detail. General cognitive status was measured using the Mini-Mental State Examination (MMSE) and the Clinical Dementia Rating (CDR) scale.

For the current study, inclusion criteria were: (1) a CDR score of 0; (2) MMSE >24; (3) availability of CSF biomarkers; and (4) availability of MRI data, yielding a final sample of 203 individuals (51% female).

2.2. MRI data acquisition, image processing, and assessment of WML

MRI data were acquired in a 3.0T Philips Achieva system (Philips Medical Systems), using a 3D T1-weighted Turbo Field Echo (TFE) sequence (Repetition time (RT)=7.2 ms, Echo time (TE)=3.2 ms, flip angle=9°, matrix size=250x250 mm, field of view=256x256, slice thickness=1.0 mm); a 3D FLAIR sequence (RT=48000 ms., TE=280 ms., TI=1650 ms., flip angle=90°, number of slices=140, matrix size=250x237 mm, slice thickness=2.0 mm); a Susceptibility-Weighted Imaging (SWI) sequence (RT=14.59-17.60 ms., TE=20.59-24.99 ms., flip angle=10°, matrix size=229x222
mm, slice thickness=1.0 mm); and a DTI sequence encoded with 1 b-value shell: 800ks/mm², along with 32 directions and 1 b=0 image (RT=7340 ms, ET=83 ms, flip angle=90°, matrix size=112x112 mm, field of view= 224x224, slice thickness=3.0 mm).

WML were measured as WM hypointensities and WM hyperintensities in T1-weighted and FLAIR sequences, respectively. WML and total intracranial volume (TIV) were automatically segmented using FreeSurfer 6.0.0. FreeSurfer detects hypointense WM signal abnormalities and automatically labels WML volumes for each participant using a probabilistic procedure. Hyperintense WML were automatically segmented using the open source segmentation toolbox LST 2.0.15. It has previously been shown that hypointense and hyperintense WML are strongly correlated. Previous findings revealed that hypointense WML might represent necrotic damage closer to accumulated cerebrovascular pathology, while hyperintense WML might also represent acute damage including peri-inflammatory processes. Due to the aim of the current study, we focused on hypointense WML, but all the analyses were replicated using hyperintense WML and are reported in supplementary material. MRI data management and processing was done using theHiveDB database system. WML volumes in milliliters (ml) were adjusted by TIV to account for variability in head size.

Previously established ROI masks for the cholinergic WM pathways (i.e., cingulum and external capsule pathways) were used. Briefly, the masks were created using probabilistic diffusion-based fiber tracking of the NBM WM projections. These ROI masks of the cholinergic WM pathways were transferred from MNI standard space to
each individual DTI image (b0) in native space using the non-linear SyN registration algorithm\textsuperscript{14} from advanced normalization tools (ANTs, http://stnava.github.io/ANTs/). Native space mean diffusivity (MD) maps were calculated for each subject using the FMRIB’s Diffusion Toolbox from FSL (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki). Microstructural properties of each participant’s cholinergic WM tracts were then calculated by averaging the MD values within the back-transformed ROI masks in native space. The MD index was preferred over the fractional anisotropy (FA) index since MD is more robust in the influence of crossing fibers\textsuperscript{15}.

2.3. Complementary MRI markers of cerebrovascular disease and vascular risk factors

In addition to the automated measure of WML, we assessed cerebral microbleeds, lacunes, and superficial siderosis, for completeness of information. The presence/absence of cerebral microbleeds was visually assessed on SWI, lacunes (3-15 mm) were assessed on FLAIR images, and superficial siderosis were assessed on SWI. All visual assessments were performed by an experience neuroradiologist blinded to clinical data,\textsuperscript{17} according to the Standards for Reporting Vascular Changes on Neuroimaging and standard scales and standardized scales\textsuperscript{18}. We also recorded and described the frequency of vascular risk factors, including hypertension, diabetes, smoking and ischemia as assessed through a semi-structured interview and clinical examination by research nurses or medical doctors.\textsuperscript{7}
2.4. CSF sampling and biomarker analysis

Lumbar puncture for CSF sampling and determination of APOE e4 carriership were conducted following standard procedures. CSF biomarker levels were determined by a commercially available assay. CSF tau phosphorylated at threonine 181 (p-tau) was determined by immunoassay ELISA (INNOTEST® PHOSPHO_TAU (181P)). The \( \text{A}\beta_{42/40} \) ratio, and CSF A\( \beta_{38} \) were determined by the V-PLEX A\( \beta \) Peptide Panel 1 (6E10) Kit (Meso Scale Discovery, Rockville, MD). We used p-tau to assess tau neurofibrillary tangle (NFT) pathology. The CSF A\( \beta_{42/40} \) ratio was used as a marker of amyloidosis. For descriptive purposes, each individual was classified as positive (+; i.e., abnormal) or negative (-; i.e., normal) according to CSF biomarkers for A\( \beta \) (CSF A\( \beta_{42} \)) and p-tau (CSF p-tau) following cohort-specific cut-off values: \( \leq 530 \) pg/ml for A\( \beta_{42} \) and p-tau>80. A\( \beta_{38} \), a shorter isoform of A\( \beta \) that can also be found in the CSF, is still poorly understood. A previous study suggested that A\( \beta_{38} \) could be a marker of AD. Another study reported a predominant localization of A\( \beta_{38} \) within the vascular vessels in Alzheimer’s disease (AD) patients. In addition, there is also evidence showing the presence of A\( \beta_{38} \) in other non-AD dementias and patients with chronic neuroinflammation. These diverse findings reflect the view that the role of A\( \beta_{38} \) still needs to be elucidated. Hence, we included CSF A\( \beta_{38} \) in this study in order to determine its association with AD biomarkers as well as cerebrovascular disease in the general population.
2.5. Statistical analysis

Statistical analyses were conducted using the R statistical software (http://www-R-project.org). A p-value <0.05 (two-tailed) was deemed significant in all the analyses. We used random forest (RF) regression models to assess the differential contributions of the different pathology-specific biomarkers towards the integrity of NBM projections. Two separate RF regression models, treated as the outcome variables, were fitted for the prediction of MD in the cingulum and the external capsule pathway, respectively. MD values were multiplied by a constant ($c=10000$) to facilitate the visualization of the data. WML, CSF Aβ42/40 ratio, Aβ38, and p-tau were included as predictors in all RF models, along with sex (i.e., male/female) and APOE status (i.e., at least one ε4 allele to be treated as carrier, otherwise non-carrier). RF is a machine learning method that estimates multiple decision trees via bootstrap aggregation (bagging). Each tree predicts a classification independently and votes for the corresponding class. The majority of the votes decides the overall prediction. A conditional importance score is computed for each tree in RF analysis. This is done by measuring the change in the prediction error when the values of a certain variable are permuted within a grid defined by the included covariates. Then this conditional score is averaged across the entire ensemble. These conditional importance scores are designed to reduce the undesirable effects of collinearity among predictor variables. The final importance of each predictor denotes its contribution to the model. Importance values below or equal to zero denote no contribution. A conditional regression tree is produced as a graphical representation of the model. The RF was comprised of 5000 conditional inference trees. R2 was computed to assess the quality of the RF models. Although aging is associated with WM neurodegeneration and
greater WML volumes\textsuperscript{4,28}, age was not included as a covariate in the models since it was controlled from the design (i.e. all participants were 70 years old). For completeness of information, we also report Pearson correlation coefficients among the predictor variables included in the random forest models and independent sample t-tests for categorical variables that resulted important in the RF analysis. The randomForest\textsuperscript{29} and Party packages\textsuperscript{30} were used for these analyses.


The H70 study was approved by the Regional Ethical Review Board in Gothenburg (Approval Numbers: 869-13, T076- 14, T166-14, 976-13, 127-14, T936-15, 006-14, T703-14, 006-14, T201-17, T915-14, 959-15, T139-15), and by the Radiation Protection Committee (Approval Number: 13-64) in concordance with the 1964 Helsinki declaration and its later amendment.

2.7. Data availability statement

The authors state that anonymized data on which the article is based will be shared by request from any qualified investigator.

3. RESULTS

Demographic, clinical data, vascular risk factors, and MRI markers of cerebrovascular disease are shown in Table 1. In our sample of 203 cognitively unimpaired individuals (all 70 years old, 51% female), 2% had an AD biomarker profile (i.e. A+T+), 43% had abnormal CSF levels of amyloid-beta only (i.e. A+T-) and 4.4% had
abnormal CSF levels of p-tau only (i.e. A-T+). Results are shown for hypointense WML volume from T1-weighted 3D images. Virtually the same results were obtained when including hyperintense WML instead of hypointense WML in the models (see Supplementary Material).

The RF models showed that WML volume was the most important predictor for the average MD of the cingulum pathway (see Figure 1). P-tau, Aβ38 and APOE ε4 carriernship were also important predictors in the model. Aβ42/40 ratio received low importance score. Sex did not contribute to the MD in the cingulum pathway. The RF tree revealed that WML volume was the best predictor splitting individuals according to their MD in the cingulum pathway. Four groups were distinguished (see Figure 2). P-tau, Aβ38, Aβ42/40 ratio, sex and APOE ε4 carriernship did not separate any of the groups based on their association with MD in the cingulum pathway.

Regarding the prediction of the MD in the external capsule pathway, WML volume was again the most important predictor (see Figure 1). Aβ38, p-tau and sex were also important in the model. Women showed poorer integrity in the external capsule pathway. This finding was independent of amyloid status, as reflected by the non-significant differences in integrity when comparing amyloid positive versus amyloid negative women participants (T_{201}=-1.55; p=0.123). APOE ε4 carriernship received a low importance score and Aβ42/40 ratio did not contribute to the MD in the external capsule pathway. The RF tree revealed that WML volume, Aβ38 and p-tau were important predictors to split individuals according to their MD in the external capsule. Five groups were distinguished at the end of the tree (see Figure 3).
Figure 4 shows the correlation matrix for all pairs of continuous predictors in the RF models. WML volume was negatively correlated with $\text{A}_\beta^{38}$. P-tau was also correlated with $\text{A}_\beta^{38}$ and $\text{A}_\beta^{42/40}$ ratio.

4. DISCUSSION

In our study, we investigated the contribution of cerebrovascular disease compared with amyloid pathology and tau pathology towards the degeneration of cholinergic WM pathways in cognitively unimpaired individuals. We demonstrated the role of WML burden as a central contributor to the degeneration of the cholinergic projections.

The NBM is well-known for its key role in cognitive functioning and its deterioration is linked to cognitive impairment in AD\(^1\). It is important to determine the pathological processes contributing towards degeneration of the cholinergic system as it has previously been demonstrated to be associated with cognitive impairment in advanced aging \(^4\). In this sample of cognitively unimpaired aged individuals, we demonstrated that WML were the most important contributor towards the degeneration of the studied cholinergic pathways, followed by CSF $\text{A}_\beta^{38}$ and p-tau levels. Conversely, the $\text{A}_\beta^{42/40}$ ratio did not show a substantial contribution.

The integrity of the cholinergic system is crucial for proper cognitive functioning \(^1\). The cholinergic hypothesis of cognitive aging postulates that age-related memory decline and other cognitive problems may arise due to declining cholinergic activity \(^31,32\). In a previous study, we demonstrated that the WM integrity of cholinergic
projections was closely associated with attention and memory performance in an independent aging cohort of cognitively unimpaired individuals \(^4\). The influence of WML burden on cortical disconnection of the cholinergic system might be associated with subclinical cognitive impairments in the elderly. Longitudinal studies have shown that a high WML burden increases the risk of future cognitive impairment \(^33\). Future studies should determine the disruptive role of WML in the association between cholinergic projections and cognitive performance in normal aging and the continuum of AD.

Although WML burden was the most important predictor in our RF models, we found that Aβ\(_{38}\) also contributed to the integrity of the cholinergic system. In contrast, the Aβ\(_{42/40}\) ratio was not an important predictor of neurodegeneration of cholinergic WM projections. The role of CSF Aβ\(_{38}\) and its association with neurodegeneration is still under debate \(^34,35\). CSF Aβ\(_{38}\) levels are lower in frontotemporal dementia (FTD) \(^23\) and dementia with Lewy bodies (DLB) \(^24,25\) than in AD patients. Furthermore, Aβ\(_{38}\) has previously been linked to increased counts of lacunes and cerebral microbleeds, two markers of cerebrovascular disease \(^36\). Deposits of Aβ\(_{38}\) in vascular vessels have also been found in postmortem AD studies \(^22\). Therefore, several studies suggest a potential association of Aβ\(_{38}\) with cerebrovascular pathology. In line with this, we showed that lower CSF Aβ\(_{38}\) levels were associated with a higher WML burden. In our study, both WML burden and CSF Aβ\(_{38}\) were the most important predictors of WM neurodegeneration of the cholinergic system as compared with AD biomarkers (CSF Aβ\(_{42/40}\) and p-tau). These findings suggest an association between Aβ\(_{38}\) and cerebrovascular disease in normal aging, and their predilection for the cholinergic WM. Recent reports have demonstrated that higher levels of Aβ\(_{38}\) in the CSF may
have a protective effect against future cognitive decline and AD dementia in individuals with a positive AD biomarker profile at baseline\textsuperscript{37}. In support of this, decreased CSF Aβ\textsubscript{38} levels have previously been linked to reduced cingulate and insula cortex volumes in our cohort\textsuperscript{34}. The cingulate cortex receives important cholinergic input from the medial cholinergic pathway and the insula from the lateral cholinergic pathway\textsuperscript{1}. These areas are well-known for their role in emotion regulation, behavior and executive functioning\textsuperscript{38}. Future studies should test whether Aβ\textsubscript{38}, neurodegeneration of the cholinergic system, and reduced cingulate and insula gray matter volumes are associated with subclinical changes in emotion regulation and executive functioning in the elderly.

The cholinergic circuitry is highly vulnerable to brain pathology. In our study, we found pathway-dependent associations of WML, Aβ\textsubscript{38}, and tau (p-tau) pathological markers with cholinergic WM projections. Our results show that individuals with decreased Aβ\textsubscript{38} and high WML burden had the poorest integrity of the external capsule pathway. Interestingly, women also showed poorer integrity in the external capsule pathway, independently of amyloid status. In contrast, WML burden was the only predictor of the integrity in the cingulum pathway. These pathway-dependent findings point to a greater vulnerability of the cingulum pathway to vascular pathology, in comparison to amyloid/tau pathologies. Regionally, the cingulum pathway is located in periventricular regions, where the presence of WML increases with aging\textsuperscript{39}. Periventricular WML have previously been associated with lower cortical cholinergic activity in normal aging\textsuperscript{40}. Conversely, the external capsule pathway might be more vulnerable to cerebrovascular disease and pathologies associated with Aβ\textsubscript{38}.
Regarding tau pathology, our results showed a negative association between p-tau and degeneration of cholinergic WM projections (i.e. a poorer integrity of WM projections was associated with lower levels of CSF p-tau). This counterintuitive finding might be the result of a selection bias in our sample. All our participants were cognitively unimpaired 70-year-olds, and only 6.4% had abnormal CSF p-tau levels. It is important to take into consideration that the combination of abnormal levels of p-tau with other brain pathologies such as WML will most probably result in cognitive impairment and therefore those individuals may have been excluded from our study. Whether increased CSF p-tau levels are associated with degeneration of cholinergic WM projections needs to be further tested in more diverse populations of older individuals, including patients with cognitive impairment.

The data provided by this study describes the contribution of CSF Aβ42/40 ratio, Aβ38 and p-tau levels in combination with WML burden towards the degeneration of the cholinergic system in cognitively unimpaired elderly from a population-based cohort. However, all individuals included were 70 years old, therefore results can only be partially generalized to other age groups. A limitation of the current study, intrinsic to the tractography approach used to generate the cholinergic WM projection masks, is the existence of transverse crossing WM fibers that can lead to distorted information about the WM integrity. We aimed to partly overcome this limitation by using the MD index instead of fractional anisotropy (FA), since MD is less affected by crossing fibers. The associations between amyloid/tau biomarkers and WML might lead to collinearity problems. Using RF regression with conditional inference trees, we were able to handle multicollinearity to some degree. Alternative information about the
spatial location of WML as well as cholinergic functional activity profiles based on fMRI could complement the findings of our current study \(^2\). We demonstrated an association between A\(\beta\)38 and the degeneration of the cholinergic system. Nevertheless, the literature about the role of A\(\beta\)38 in neurodegenerative processes is still limited and further research is needed. There is currently a discussion ongoing as to whether the validated biomarker cut-offs for dementia diagnosis are clinically relevant for preclinical stages of the disease \(^41\). Subthreshold pathology in individuals exhibiting normal biomarker profiles might already be affecting the brain integrity leading to WM degeneration. Thus, in our study we used continuous values as the input for the analysis. The integrity of the cholinergic projections across abnormal amyloid/tau profiles in clinical stages of AD needs to be further elucidated. Finally, a previous study demonstrated that WML can also be related to AD pathology \(^42\). However, in our study WML were not associated with CSF levels of A\(\beta\)42/40 and p-tau, which suggests that our WML measure likely does not reflect AD pathology \(^18\).

This study highlights the importance of cerebrovascular pathology relative to amyloid and tau pathology in their contribution to cholinergic neurodegeneration, in cognitively unimpaired individuals. WML within cholinergic pathways correlate with cognitive impairment \(^43\) and executive dysfunction \(^44\) in dementia patients. Given the central role of the cholinergic system in cognition, our study suggests that management of cholinergic WML and vascular risk factors should be considered in the development of prevention programs for neurodegeneration and cognitive impairment. As these data are replicated in independent cohorts, it may help in clinical considerations with regards to cerebrovascular and AD biomarkers,
cholinergic dysfunction, and cognitive impairment. This knowledge could eventually support therapeutic decisions in the context of acetylcholinesterase inhibitors.

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6. CONFLICTS OF INTEREST

HZ has served on scientific advisory boards and/or as a consultant for Alector, Eisai, Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, Nervgen, AZTherapies, CogRx and Red Abbey Labs, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). SK has served on a
scientific advisory board/ or as a consultant for Geras Solutions and Biogen, unrelated to the present study. ME has served as a consultant for Biogen unrelated to present study. The other authors report no disclosures.


7. REFERENCES


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Figure 1. The contribution of amyloid, tau and cerebrovascular biomarkers towards the integrity of cholinergic WM pathways.

The plot represents the percentage of increase in the prediction error (%IncMSE) when removing each variable from the random forest model. NBM ROI is represented in red. The cingulum pathway is represented in blue (Orange for the cingulum mask). The external capsule pathway is represented in green (Brown for the external capsule mask). WML volume: white matter lesions based on hypointense signal abnormalities of T1-weighted-3D images; $A\beta_{38}$: amyloid $\beta_{38}$; $A\beta_{42/40}$: amyloid $\beta_{42/40}$ ratio; P-tau: phosphorylated tau; $APOE \epsilon 4$: apolipoprotein E $\epsilon 4$. 

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**Figure 2. Random forest regression tree for the MD in the cingulum pathway.**

The figure represents the recursive partitioning for the MD index in the cingulum pathway and the contribution of WML volume to the portioning. Correspondent p-values are given for each inner node. Boxplot for MD distribution are shown for each final group. WML volume: white matter lesions based on hypointense signal abnormalities of T1-weighted-3D images; MD: mean diffusivity.

**Figure 3. Random forest regression tree for the MD in the external capsule pathway.**

The figure represents the recursive partitioning for the MD index in the external capsule pathway and the contribution of WML volume, p-tau and Aβ38 to the portioning. Correspondent p-values are given for each inner node. Boxplot for MD distribution are shown for each final group. WML volume: white matter lesions based on hypointense signal abnormalities of T1-weighted-3D images; Aβ38: Amyloid β38; P-tau: phosphorylated tau; MD: mean diffusivity.
Figure 4. Correlation matrix for the predictors included in the random forest models.

Background of significant correlations ($p < 0.05$) was colored according to the value of the correlation coefficient and shaped accordingly to the association distribution. Otherwise left empty.
Table 1. Study sample demographic and clinical data.

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>203</td>
</tr>
<tr>
<td>Sex (% Female)</td>
<td>51</td>
</tr>
<tr>
<td>APOE status (% ε4 carriers)</td>
<td>35</td>
</tr>
<tr>
<td>MMSE</td>
<td>29.23(0.98)</td>
</tr>
<tr>
<td>Education (years)</td>
<td>13.22 (3.95)</td>
</tr>
<tr>
<td>WML volume (ml)</td>
<td>3.01 (2.30)</td>
</tr>
<tr>
<td>Aβ₃₈ (pg/ml)</td>
<td>2498(679.15)</td>
</tr>
<tr>
<td>Aβ₄₂/₄₀ ratio</td>
<td></td>
</tr>
<tr>
<td>p-tau (pg/ml)</td>
<td>49.45(17.56)</td>
</tr>
<tr>
<td>NBM volume (TIV corrected)</td>
<td>0.20 (0.03)</td>
</tr>
<tr>
<td>MD in cingulum pathway</td>
<td>0.00097 (0.00006)</td>
</tr>
<tr>
<td>MD in external capsule pathway</td>
<td>0.00107 (0.00008)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>73.8</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>11.3</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>61.7</td>
</tr>
<tr>
<td>Ischemia (%)</td>
<td>6.04</td>
</tr>
<tr>
<td>Cerebral microbleeds (%)</td>
<td>16.7</td>
</tr>
<tr>
<td>Lacunes (%)</td>
<td>8.4</td>
</tr>
<tr>
<td>Superficial siderosis (%)</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Note: values represent Mean (SD) unless another parameter is specified. % represents the percentage of individuals with presence of vascular risk factors of presence of cerebral microbleeds, lacunes, or superficial siderosis. MMSE: Mini-mental state examination; WML: white matter lesions; Aβ: Amyloid β; p-tau: phosphorylated tau 181; NBM: nucleus basalis of Meynert; MD: mean diffusivity.
Association of Cerebrovascular and Alzheimer Disease Biomarkers With Cholinergic White Matter Degeneration in Cognitively Unimpaired Individuals

Nira Cedres, Daniel Ferreira, Milan Nemy, et al.

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