Brain MRI fiber-tracking reveals white matter alterations in hypertensive patients without damage at conventional neuroimaging

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Aims	Hypertension is one of the main risk factor for dementia. The subtle damage provoked by chronic high blood pressure in the brain is usually evidenced by conventional magnetic resonance imaging (MRI), in terms of white matter (WM) hyperintensities or cerebral atrophy. However, it is clear that by the time brain damage is visible, it may be too late hampering neurodegeneration. Aim of this study was to characterize a signature of early brain damage induced by hypertension, before the neurodegenerative injury manifests.
Methods and results	This work was conducted on hypertensive and normotensive subjects with no sign of structural damage at conventional neuroimaging and no diagnosis of dementia revealed by neuropsychological assessment. All individuals underwent cardiological clinical examination in order to define the hypertensive status and the related target organ damage. Additionally, patients were subjected to DTI-MRI scan to identify microstructural damage of WM by probabilistic fiber-tracking. To gain insights in the neurocognitive profile of patients a specific battery of tests was administered. As primary outcome of the study we aimed at finding any specific signature of fiber-tracts alterations in hypertensive patients, associated with an impairment of the related cognitive functions. Hypertensive patients showed significant alterations in three specific WM fiber-tracts: the anterior thalamic radiation, the superior longitudinal fasciculus and the forceps minor. Hypertensive patients also scored significantly worse in the cognitive domains ascribable to brain regions connected through those WM fiber-tracts, showing decreased performances in executive functions, processing speed, memory, and paired associative learning tasks.
Conclusions	Overall, WM fiber-tracking on MRI evidenced an early signature of damage in hypertensive patients when otherwise undetectable by conventional neuroimaging. In perspective, this approach could allow identifying those patients that are in initial stages of brain damage and could benefit of therapies aimed at limiting the transition to dementia and neurodegeneration.
Keywords	White matter tractography • Hypertension • Cognitive impairment • Presymptomatic diagnosis

1. Introduction

High blood pressure (BP) is a chronic condition that causes progressive target organ damage.[1,2] Decades of studies in hypertension allowed the identification of typical features of preclinical lesions in target organs of chronic increased BP, making available to clinicians clear markers of disease progression and complications in peripheral organs. However, high BP also profoundly challenges the brain. Besides contributing to acute forms of brain damage like ischemic and hemorrhagic stroke, hypertension is also the major risk factor for chronic brain injury, manifesting with alterations of cognitive functions, ranging from subtle deficits to dementia.[3,4] It is well known that the vast majority of cases of Alzheimer's Disease and related dementia in elderly are not ascribable to genetic predisposition but rather to the chronic exposure to vascular risk factors in midlife.

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That is said, the clinical approach to this category of patients usually starts when symptoms are clearly evident and progression of dementia is perceived even by themselves. Indeed, the clinically relevant brain damage can be evidenced by conventional imaging, like structural MRI, which assesses the brain injury associated to the symptomatology and usually characterized as cerebral atrophy and white matter (WM) hyperintensities.[5–9] However, it has becoming increasingly clear that when these macroscopic signs of brain damage are manifest, it may be too late to reverse the neurodegenerative process and we still lack procedures for assessing progression markers that could reveal pre-symptomatic alterations and identify patients at risk of developing dementia lately.

A conceptual advancement that could help in overcoming these limitations, comes from novel neuroimaging approaches in MRI, capable to recognize alterations in the brain when conventional methods of analysis fail in detecting signs of damage.[10] To this aim, WM microstructural alterations began to be studied using DTI.[11–13] The basic concept standing behind this approach exploits the water diffusion properties in WM and can reveal localized abnormalities in specific brain areas. The quantitative analysis of these diffusion properties let us calculate different indexes, which can describe the characteristics of integrity and organization of WM fiber bundles. The advantage over conventional methods stands on the possibility to identify damage at microscopic level instead of macroscopic lesions, making it possible staging damage progression.

A main limitation in the use of this approach came from the evidence that the initial region of interest (ROI)-based or atlas-based methods of DTI analysis evidenced many technical flaws. More recently, the technological advancement allowed to overcome these limitations by developing methods to spatially characterize WM diffusion abnormalities along the pathway of a specific tract of WM instead of larger brain areas, and modelling appropriate diffusion schemes based on probabilistic assumptions[14] that produce a set of trajectories linked to a specific voxel.[14,15] Thus far, however, no study exploited this strategy to examine microstructural WM damage in hypertensive patients, at a stage where they have normal brain imaging at conventional MRI and no diagnosis of dementia at the neurocognitive assessment. The goal of our work was to identify a specific signature of subtle and early alterations in the brain of hypertensive patients, through the combined use of probabilistic fiber-tracking of WM and neuropsychological assessment.

2. Methods

2.1 Experimental design

This study was conducted at IRCCS Neuromed, Department of Angiocardioneurology and Translational Medicine. The protocol was performed in compliance with the ethical standards established in the Declaration of Helsinki and approved by the Ethical Committee of our Institution, as registered in clinicaltrials.gov (NCT02310217). Informed consent was obtained from all individual participants included in the study.

Between November 2014 and September 2017, we screened subjects admitted at our outpatients' facility—Regional Excellence Hypertension Center of the Italian Society of Hypertension—affiliated to the Department of Neurological Complications of Cardiovascular Diseases of our Institute. We recruited individuals conforming to the following criteria: aged 40 to 65 years, compliant to give written informed consent, possibility to perform a dedicated 3 Tesla-MRI scan. The resulting 143 eligible patients were subjected to the assessment of strict inclusion/exclusion criteria by two independent raters. The exclusion criteria were the following: stroke, dementia, schizophrenia, seizures, Parkinson's disease, bipolar

disorder, and any other diagnosed neurologic or psychiatric disease, claustrophobia, secondary hypertension, end-stage heart disease, renal failure, dialysis treatment, diabetes, atrial fibrillation, and drugs that could affect cognitive functions. In the end, 70 patients met the criteria and entered the study. Then, in order to have a homogeneous sample of individuals, we further excluded subjects with already manifest signs of damage or alterations at conventional MRI and/or diagnosis of dementia, assessed as detailed in the specific sections below. After this latter screening, 28 patients reporting WM hyperintensities or lacunar damage ($n = 19$), neurinoma ($n = 1$), meningioma ($n = 1$), angioma ($n = 1$), brain calcifications ($n = 2$), and artefacts due to movement ($n = 4$) were further excluded, resulting in a final sample of 42 individuals.

Each subject included in the study protocol underwent clinical examinations to assess covariates as office BP, smoking, body mass index (BMI), and education, and were categorized in hypertensive ($n = 23$) and normotensive ($n = 19$) individuals accordingly to their history of hypertension, current anti-hypertensive therapy and target organ damage, determined as detailed below in specific sessions. Since the protocol was designed as an observational study, there was no unified anti-hypertensive schedule for hypertensives and actual anti-hypertensive therapy was recorded for all patients and included the following main classes of drugs: angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers (ACE-I/ARBs), calcium channel blockers, diuretics, and β-blockers.

2.2 Clinical procedures

2.2.1 Neuroimaging, DTI processing, and fiber-tracking

Brain MRI was performed on a 3 T GE Signa Horizon scanner (General Electric Medical Systems, Milwaukee, WI). After localization and calibration routines, the sequences used were as follows: T1-weighted images in axial orientation, acquired using a 3D SPGR sequence (TR/TE/TI = 5.6/1.7/400 ms, flip angle = 11°, FOV = 256 mm, slice thickness = 1 mm, pixel spacing = 1.0 mm × 1.0 mm) with a 256 × 256 in-plane resolution; AX DP/T2 scan (TR/TE/TE2 = 3320/10/103.0 ms, FOV = 220 mm, slice thickness = 4 mm); DTI was performed with a diffusion weighted spin echo-planar imaging sequence (TR/TE = 89.7/12200 ms, slice thickness 3 mm, 50 contiguous slices, maximum b-value = 1000 s/mm2 in 30 optimized non-collinear directions; one volume was acquired without diffusion weighting). All images were anonymized; radiological assessment was performed by a blinded radiologist; subsequent DTI and structural image analysis was performed by a blinded operator through an automated analysis protocol.

Conventional MRI on T1 and AX DP/T2 scans were used to assess the presence of macroscopic brain damage, presence of WM hyperintensity load, lacunae, or other pathological alterations. A negative radiological result was considered an indispensable criterion to be eligible and the inclusion cut-off used for vascular-related damage was set at a Fazekas score ≤ 1.

Diffusion images were processed using the standardized pipeline with the DTIfit tool. After pre-processing, analysis was performed using the PROBTRACK tool of probabilistic Bayesian Framework for tractography. PROBTRACK exploits a diffusion model estimated by BEDPOSTX, a tool for probabilistic diffusion parameters estimation capable of modelling crossing fibers. A standard tractography pipeline has been implemented using the plugin AutoPTX, following parameters and quality check procedures as described.[16] Tracts were categorized in associative, projection, limbic, and callosal. Diffusion parameters were quantified with Fractional Anisotropy (FA), Mean Diffusivity (MD), Axial Diffusivity (AD), and Radial Diffusivity (RD). The tract segmentation was achieved

by thresholding the normalized tract density images. The threshold was selected according to the FA reproducibility criterion,[17] segmented tract volumes were computed to exclude WM tract atrophies. FA, MD, AD, and RD were averaged for voxels inside tract segmentations. All tools were available from FSL Suite (version 5.0.8).[18–20]

The analysis of brain cortex assessed cortical thinning or atrophy (exclusion criteria for the study) and was performed with FSL-VBM (Voxel based morphometry),[21] an optimized VBM protocol[22] carried out with FSL tools.[18] First, structural images were brain-extracted and grey matter-segmented before being registered to the MNI152 standard space using non-linear registration.[23] Second, all native grey matter images were non-linearly registered to this study-specific template and 'modulated' to correct for local expansion (or contraction) due to the non-linear component of the spatial transformation. The modulated grey matter images were then smoothed with an isotropic Gaussian kernel with a sigma of 3 mm. Finally, voxelwise General Linear Model (GLM) was applied using permutation-based non-parametric testing, correcting for multiple comparisons across space with significance threshold of corrected P-value ($P < 0.05$).

2.2.2 Hypertension diagnosis and blood pressure measurement

Hypertension diagnosis was performed based on a systolic blood pressure >140 mmHg, a diastolic blood pressure >90 mmHg or they were previously diagnosed with hypertension and treated with anti-hypertensive drugs. BP was measured in sitting position after 5 minutes of rest, using an automated validated device (OMRON-705IT) with an appropriately sized cuff on the dominant arm. Three consecutive measurements were taken at 2 minutes apart and average values were recorded.

2.2.3 Ultrasonographic analyses for cardiac and vascular imaging

The overall one-dimensional left ventricle (LV) measurements and the two-dimensional views were obtained following the American Society of Echocardiography guidelines.[24] Left Ventricular Mass (LVM) was calculated using the Devereux's formula and normalized for the height elevated to 2.7 (indexed LVMI). Hypertrophic remodeling was diagnosed when LVMI > 50 g/m$^{2.7}$.[24,25] Then we calculated Relative Wall Thickness (RWT) at end-diastole as 2 PWT/LVIDD. Diastolic function was assessed as the ratio of early to late ventricular filling and patients were categorized as diastolic dysfunction with a E/A ratio < 1.

The measures of intima media thickness (IMT) were obtained at the distal 1.0 cm below the carotid bifurcation in common carotid and at the proximal 1.0 cm of the internal carotid artery.[26–28]

Two blinded cardiologists reviewed the ultrasonographic analyses, to assess cardiac and vascular remodeling.

2.2.4 Renal function

Renal function was assessed by evaluating serum creatinine levels, albuminuria, and estimated glomerular filtration (eGFR) through standardized procedures.

2.2.5 Cognitive assessment

Patients underwent cognitive assessment administered by a certified psychologist, blinded to the clinical condition. The Instrumental Activities of Daily Living (IADL) test was used to assess potential impairment of daily life activities, by using a scale that measures emotional, cognitive, and physical functions.[29] Score ranges were from 0 (low function,

dependent) to 8 (high function, independent), with a cut off value set at 8 for females and 5 for males. Normal performance on this test was considered an indispensable criterion to exclude severe cognitive dysfunction.

MoCA battery of tests was used as the gold standard neuropsychological evaluation in cerebrovascular diseases.[30–32] According to validation studies for patients with vascular dementia,[30,33] patients who reported a MoCA inferior to 17 were considered demented and consequently excluded from the study protocol. Subdomains of MoCA were categorized according to normative data and were defined as following: visuospatial, executive functions, language, attention, and memory. Verbal paired-associate learning test was administered to evaluate subjects' anterograde learning.[34] Semantic Verbal Fluency Test was used to assess the capability to access to semantic store through working memory[35] and lastly Stroop Test was administered to patients to evaluate the ability of interfering stimuli interdiction.[36] The execution of all the neuropsychological tests was completed always in the same order and allowed to obtain a complete profiling of subjects' cognitive functions.

2.3 Statistics

Based on preliminary data and previous studies assessing the impact of cardiovascular risk factors on cognitive functions[37,38] and hypothesizing a predicted difference in MoCA score of 4 corrected points between mean values obtained for normotensive (NT) and hypertensive patients (HT), we calculated the power analysis for the study with SPSS SamplePower 3 software (version 3; IBM Solutions). Our computation analysis, assuming a mean difference between the two populations of 4.0 points and a common within-group standard deviation (SD) of 3.0, estimated to enroll at least 13 cases for each group to achieve an alpha of 0.05 and a beta power of 0.90, by setting a two-tailed test for comparison of independent samples. With our actual sample size of 19 NT and 23 HT subjects for the two groups, the study showed a power exceeding 99% to yield a statistically significant result, with a confidence interval of 2.72 to 5.28.

Depending on the type of variables, characteristics of patients were presented as numbers (%) or means ± SD.

Shapiro–Wilk Test was used to assess the normality of continuous variables. Comparisons between NT and HT were performed using t-test for independent samples for continuous variables or Mann–Whitney test for non-normally distributed variables. Categorical data were compared using Fisher exact χ^2 test. Multivariate analysis was performed to assess the influence of relevant covariates in the significant differences emerged in the univariate analysis. Bivariate correlation analyses were conducted with Pearson correlation. When specified and accordingly to the datasets, partial correlation analyses or linear regressions were performed to assess the possibility that relevant covariates could influence the significant results observed. All the analyses were performed with SPSS (version 23; IBM Solutions). $P < 0.05$ was considered statistically significant.

3. Results

3.1 General characteristics of subjects and hypertension-induced target organ damage

In order to stage the level of hypertensive disease's progression, recruited patients with general characteristics showed in *Table 1* were subjected to the evaluation of typical signs of target organ damage due to chronic high BP. All the HT patients arriving at our observation were already on anti-hypertensive therapy, as listed in Supplementary material online, *Table S1*. Estimated duration of hypertensive condition was also

recorded, accordingly to anamnestic history of patients (see Supplementary material online, *Table S1*).

At echocardiography, HT displayed signatures of hypertrophic remodeling, evidenced by increased thickness of LV walls, LVMI[2,7], and RWT (*Table 1*). Hypertensive patients showed a significant diastolic dysfunction, even though the preserved cardiac ejection fraction (EF) observed in HT was indicative of an adaptive remodeling without loss of contractility, thus excluding organ failure (*Table 1*). In measuring carotid arteries' IMT, HT displayed a moderate wall thickening (*Table 1*), usually reflecting a status of chronic increased BP,[39] reaching the significance only for the left internal carotid artery. On the other hand, the absence of atherosclerotic plaques excluded obstructive diseases that could compromise cerebrovascular functioning. Lastly, we evaluated renal function and observed that patients with hypertension had no alterations in microalbuminuria, serum creatinine, and estimated glomerular filtration rate (eGFR) (*Table 1*).

3.2 Fiber-tracking highlights a signature of WM microstructural alterations in hypertensive patients

Accordingly to our inclusion/exclusion criteria, all individuals recruited had no damage at conventional T2-MRI scans, assessed by a blinded neuroradiologist. We further ascertained absence of topical cortical thinning of the grey matter, which could negatively impact on cognitive function, independently of WM damage. To unravel this issue we performed VBM analysis on T1-scan that showed normal grey matter, with no significant cluster of differences between NT and HT individuals, with lowest *P*-value equal to 0.274 across the volume (*Figure 1*). Additionally, we checked whether the WM tracts segmented by fiber-tracking DTI-MRI had any difference in the size, by evaluating single tracts' volumes. No difference between HT and NT emerged, meaning that no tract evidenced malformation or atrophy (see Supplementary material online, *Table S2*).

Table 1 General characteristics of population under investigation and clinical assessment evidencing a mild peripheral organ damage in HT

Sample Characteristics	Normotensive $n=19$	Hypertensive $n=23$	*P*-value
Demographic			
Age—mean (SD)	52 (8)	55 (7)	0.272
Sex—number of females (percentage)	11 (57.86%)	11 (47.82%)	0.527
Smokers—number (percentage)	3 (15.78%)	4 (17.39%)	0.893
BMI—mean (SD)	26 (4.9)	30.2 (4.5)	**<0.01
Blood Pressure			
Systolic blood pressure—mmHg mean (SD)	122 (10.02)	138 (10.11)	***<0.001
Diastolic blood pressure—mmHg mean (SD)	77 (6.22)	87 (9.01)	***<0.001
Cardiac Remodeling			
LV end-diastolic diameter—mm. mean (SD)	4.86 (0.30)	5.04 (0.45)	0. 777
IV septal thickness—mm. mean (SD)	0.91 (0.12)	1.14 (0.14)	***<0.001
LV posterior wall thickness—mm. mean (SD)	0.90 (0.13)	1.10 (0.13)	***<0.001
LV mass index (LVMI[2,7])—g/m^2. mean (SD)	37.85 (8.88)	55.29 (9.65)	***<0.001
Relative wall thickness—RWT. mean (SD)	0.37 (0.04)	0.44 (0.05)	***<0.001
Dyastolic disfunction—*E/A'*<1 (percentage)	4 (21%)	17 (73%)	***<0.001
LV Ejection fraction—%. mean (SD)	65.47 (6.03)	67.43 (7.26)	0.276
Carotid Arterial Thickening			
Internal Carotid Artery (right)—IMT. mean (SD)	0.74 (0.17)	0.86 (0.21)	0.052
Common Carotid Artery (right)—IMT. mean (SD)	0.80 (0.13)	0.88 (0.12)	0.059
Internal Carotid Artery (left)—IMT. mean (SD)	0.76 (0.16)	0.87 (0.19)	*<0.05
Common Carotid Artery (left)—IMT. mean (SD)	0.79 (0.13)	0.87 (0.23)	0.088
Renal damage			
Creatinine—mg/dL. mean (SD)	0.74 (0.17)	0.76 (0.15)	0.343
Albuminuria—mg/24 h. mean (SD)	11.56 (15.67)	16.02 (16.63)	0.250
Estimated GFR—mL/min. mean (SD)	115.24 (34.00)	124.65 (39.39)	0.383
Cognitive Assessment			
IADL—score. mean (SD)	7.7 (0.73)	7.65 (0.71)	0.618
MoCA—score, mean (SD)	26.00 (2.35)	22.08 (2.60)	***<0.001
Semantic Verbal Fluency—score. mean (SD)	49.47 (11.6)	44.08 (11.5)	0.129
Paired-Associate Learning—score. mean (SD)	13.78 (3.9)	9.6 (4.9)	**<0.01
Stroop Color Word Test—score. mean (SD)	0.21 (0.6)	0.85 (1.4)	*<0.05
Stroop Interference Test—time. mean (SD)	16.45 (7.73)	25.5 (11.04)	***<0.001

General characteristics of patients' sample under investigation are listed in the table, together with BP data. Evaluation of cardiac and vascular remodeling revealed a moderate hypertrophy induced by hypertension. Assessment of renal function evidenced no sign of organ failure. Evaluation of general cognitive performance obtained by administration of the following tests: IADL (Instrumental Activities of Daily Living), MoCA battery, Semantic Verbal Fluency, Paired-Associate Learning, Stroop Color Test.

When we assessed the diffusion parameters of FA, MD, AD, and RD of the WM tracts segmented by fiber-tracking DTI-MRI, we revealed significant differences between HT and NT patients in specific tracts (*Figure 2* and *Table 2*). Concomitant FA and MD variations are usually considered for extrapolation of specific tracts' alterations. In brief, lower FA indicates disorganized fascicles, affected by microstructural processes such as demyelination, axonal degradation, or gliosis.[13] MD, is a more sensitive measure even though less specific, and it results increased by pathological processes affecting neuronal membranes.[13] We observed a specific pattern of alterations in the WM fiber tracts of HT as compared to NT (*Figure 2* and *Table 2*). In particular, HT showed a significant deterioration of WM connections in projection fibers of the Right Anterior Thalamic Radiation (r-ATR) (*Figure 2A*), association fibers of the Right

Superior Longitudinal Fasciculus (r-SLF) (*Figure 2B*) and callosal fibers of the Forceps Minor (FMI) (*Figure 2C*).

Thus, over the whole WM tracts analysed (*Figure 3A*), HT patients displayed a significant decrease in FA and concomitant increase in MD in the above-described specific tracts (i.e. ATR, SLF, and FMI), resulting in the signature represented in *Figure 3B*. Interestingly, while concurrent alterations of FA and MD were displayed only by right WM bundles of the specific tracts, MD alone revealed a damage progressing in the contralateral side (*Table 2*). In addition, AD and RD DTI parameters were considered, to take into account the potential impact of different neural mechanisms on WM abnormalities. Conventionally, incremental variations in RD are associated with myelin breakdown,[13] whereas in AD describe secondary processes of axon degeneration.[13] Our data also
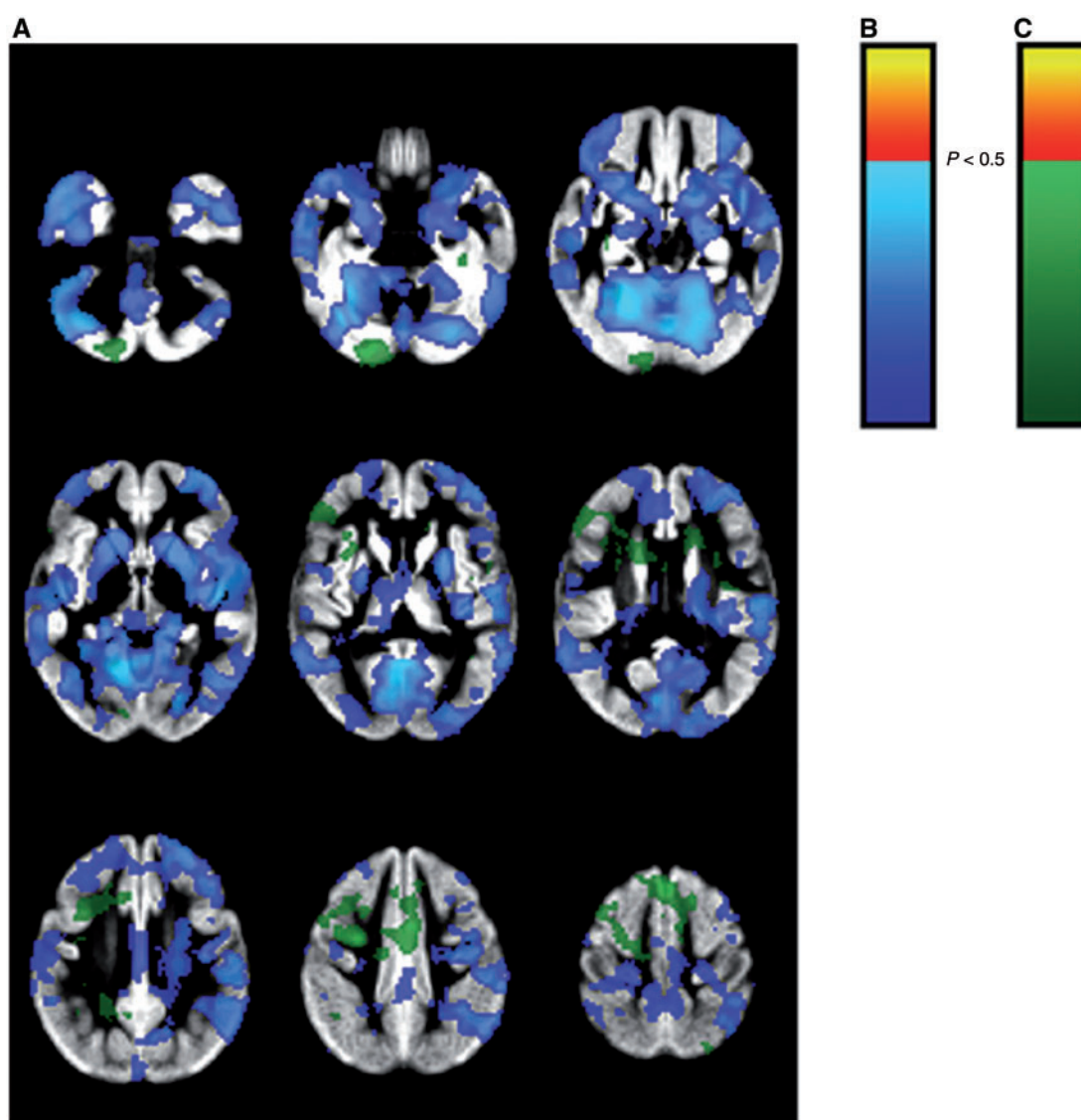

Figure I Voxel-based morphometric analysis of grey matter. (*A*) Voxel-Based Morphometric (VBM) Analysis of the grey matter in normotensive (NT) and hypertensive (HT) subjects showing in a colour-code the regions of brain cortex where (*B*) NT differed from HT (blue scale for non-significant differences—red scale for significant differences) and where (*C*) HT differed from NT (green scale for non-significant differences—red scale for significant differences). No cluster of voxels showed significant difference, being the lowest *P*-value =0.274.
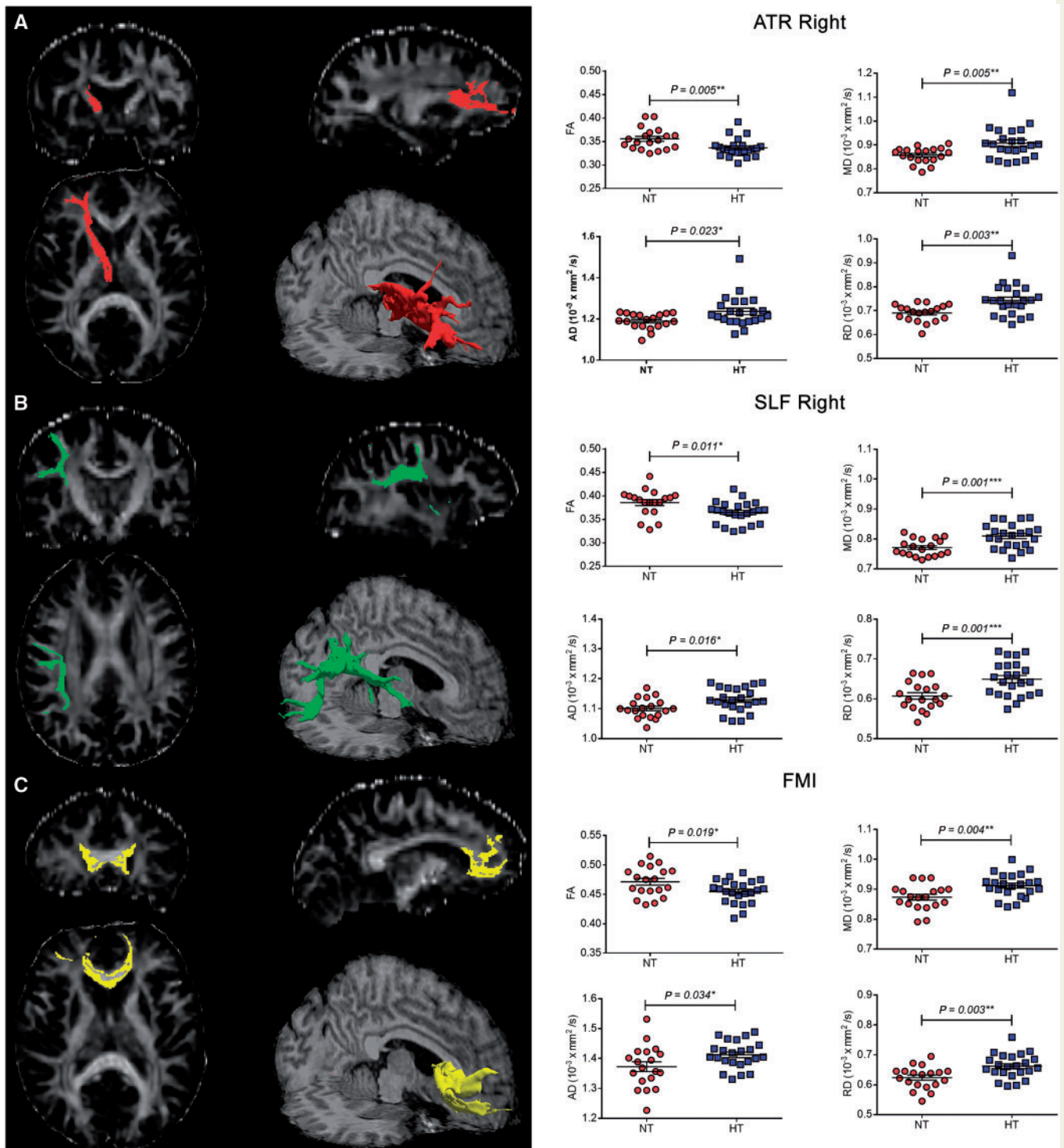
Figure 2 Tractographic reconstruction of relevant WM tracts altered in hypertensive patients. Coronal, Sagittal, and Axial projections were obtained with a 3D rendering of reconstructed regions. (A–C) A representative tractographic reconstruction of the Right Anterior Thalamic Radiation (A), Right Superior Longitudinal Fasciculus (B), and Forceps Minor (C) are shown on the left. Graphs on the right report the individual mean values of FA, MD, AD, RD assessed for each specific WM tract.

report a significant alteration of RD and AD in the tracts of interest (Table 2).

Though showing no significant difference between the two categories of subjects, age is a known factor affecting diffusion parameters of WM.[40] In order to test whether the significant differences between HT

and NT patients emerged in the r-ATR, r-SLF, and FMI WM tracts were affected by aging, we performed a multivariate analysis with age as covariate. The difference in DTI parameters emerged in the univariate between HT and NT subjects was still significant in the ANCOVA, independently of age (see Supplementary material online, Table S3).

Table 2 Average tract-specific measurements of DTI parameters obtained by probabilistic tractography segmentation

	FA		MD		AD		RD	
	NT	HT	NT	HT	NT	HT	NT	HT
Associative								
SLF R	0.39 (0.03)	0.36 (0.02)*	0.77 (0.03)	0.81 (0.04)***	1.10 (0.03)	1.13 (0.04)*	0.61 (0.04)	0.65 (0.04)***
SLF L	0.39 (0.03)	0.39 (0.02)	0.76 (0.03)	0.80 (0.04)**	1.12 (0.03)	1.14 (0.04)*	0.61 (0.03)	0.63 (0.04)*
ILF R	0.39 (0.02)	0.38 (0.02)	0.86 (0.02)	0.87 (0.04)	1.24 (0.03)	1.25 (0.03)	0.66 (0.03)	0.68 (0.04)
ILF L	0.39 (0.02)	0.39 (0.02)	0.86 (0.02)	0.87 (0.03)	1.25 (0.03)	1.26 (0.03)	0.66 (0.02)	0.68 (0.04)
IFO R	0.40 (0.02)	0.39 (0.02)	0.85 (0.02)	0.88 (0.04)*	1.24 (0.05)	1.27 (0.04)	0.66 (0.02)	0.69 (0.04)*
IFO L	0.40 (0.02)	0.39 (0.03)	0.86 (0.02)	0.88 (0.04)	1.25 (0.04)	1.28 (0.05)	0.67 (0.02)	0.69 (0.05)
UNC R	0.33 (0.02)	0.32 (0.02)	0.89 (0.04)	0.93 (0.05)**	1.22 (0.05)	1.25 (0.05)*	0.73 (0.04)	0.77 (0.05)**
UNC L	0.33 (0.03)	0.33 (0.02)	0.88 (0.04)	0.91 (0.06)*	1.20 (0.05)	1.24 (0.06)*	0.73 (0.05)	0.75 (0.06)
Limbic								
CGC R	0.35 (0.03)	0.34 (0.02)	0.81 (0.03)	0.84 (0.04)*	1.12 (0.05)	1.15 (0.05)	0.65 (0.03)	0.68 (0.04)**
CGC L	0.39 (0.04)	0.37 (0.03)	0.81 (0.03)	0.83 (0.05)	1.17 (0.05)	1.18 (0.05)	0.63 (0.04)	0.66 (0.05)*
CGH R	0.23 (0.02)	0.22 (0.02)	0.99 (0.04)	1.03 (0.06)*	1.23 (0.05)	1.26 (0.07)	0.88 (0.04)	0.92 (0.06)*
CGH L	0.23 (0.02)	0.21 (0.03)	0.99 (0.06)	1.02 (0.10)	1.22 (0.07)	1.24 (0.10)	0.88 (0.06)	0.92 (0.10)
Projection								
CST R	0.45 (0.02)	0.44 (0.03)	0.89 (0.05)	0.90 (0.06)	1.33 (0.06)	1.33 (0.06)	0.67 (0.06)	0.68 (0.06)
CST L	0.44 (0.03)	0.44 (0.03)	0.90 (0.06)	0.89 (0.06)	1.33 (0.06)	1.32 (0.05)	0.68 (0.07)	0.68 (0.06)
AR R	0.30 (0.02)	0.30 (0.02)	0.93 (0.07)	0.98 (0.07)	1.22 (0.08)	1.27 (0.07)*	0.79 (0.07)	0.83 (0.07)
AR L	0.29 (0.02)	0.30 (0.02)	0.95 (0.06)	0.97 (0.06)	1.22 (0.06)	1.27 (0.07)	0.81 (0.06)	0.83 (0.06)
ATR R	0.36 (0.02)	0.34 (0.02)**	0.86 (0.03)	0.91 (0.07)**	1.19 (0.04)	1.24 (0.08)*	0.69 (0.04)	0.74 (0.06)**
ATR L	0.36 (0.02)	0.35 (0.02)	0.86 (0.04)	0.89 (0.05)	1.19 (0.05)	1.23 (0.05)*	0.69 (0.04)	0.72 (0.05)*
STR R	0.38 (0.02)	0.37 (0.02)	0.82 (0.05)	0.83 (0.06)	1.16 (0.04)	1.17 (0.07)	0.64 (0.05)	0.66 (0.06)
STR L	0.39 (0.02)	0.38 (0.02)	0.81 (0.04)	0.82 (0.05)	1.15 (0.03)	1.16 (0.05)	0.64 (0.04)	0.65 (0.05)
PTR R	0.37 (0.02)	0.36 (0.03)	0.88 (0.04)	0.88 (0.04)	1.24 (0.05)	1.24 (0.05)	0.70 (0.04)	0.70 (0.04)
PTR L	0.37 (0.02)	0.37 (0.02)	0.89 (0.04)	0.90 (0.04)	1.26 (0.06)	1.26 (0.04)	0.71 (0.04)	0.71 (0.05)
Callosal								
FMI	0.47 (0.02)	0.45 (0.02)*	0.87 (0.04)	0.91 (0.04)**	1.37 (0.07)	1.41 (0.05)*	0.62 (0.04)	0.66 (0.04)**
FMA	0.45 (0.03)	0.45 (0.03)	1.04 (0.08)	0.99 (0.07)*	1.57 (0.09)	1.52 (0.08)	0.78 (0.08)	0.73 (0.07)

Significance values are as follows: *$P<0.05$; **$P<0.01$; ***$P<0.001$.

FA (fractional anisotropy), MD (mean diffusivity), RD (radial diffusivity), and AD (axial diffusivity) are reported for the following fiber-tracts: Association Fibers (left and right SLF, ILF, IFO, UNC), Limbic System (left and right CGC, CGH), Projection Fibers (left and right CST, AR, ATR, STR, PTR), and Callosal Fibers (FMI and FMA).

To further exclude that other potentially influencing covariates could interfere with the effects of hypertension on DTI parameters, we also tested gender, smoke, and BMI by ANCOVA. None of these factors influenced the significant difference between HT and NT for the WM tracts reported in the univariate analysis (see Supplementary material online, Table S3). More important, when we tested the potential influence of some anti-hypertensive drugs on the observed effects, we did not find any significant interference for none of the medications used (see Supplementary material online, Table S4).

3.3 Hypertensive patients showed a specific pattern of cognitive alterations

Cognitive assessment began with the administration of IADL test for all individuals. Each patient showed normal performance on this test (Table 1) and, hence, was subjected to MoCA test.[31] HT displayed significantly impaired performance on MoCA, as compared to NT (Table 1). Further analysis of specific cognitive subdomains tested by the MoCA revealed significantly impaired memory, executive functions, attention, and language domains (see Supplementary material online, Table S5).

The significantly worse score reported by HT patients in the verbal paired-associate learning revealed difficulties in retaining new information through working memory (Table 1). On the Stroop Test, HT did not perform as well as NT, thus indicating that hypertension negatively affects executive functions (Table 1). The Semantic Verbal Fluency Test showed comparable performance in HT and NT, thereby indicating no alteration in language abilities that could affect performance on other tasks (Table 1).

3.4 WM microstructural alterations scale with cognitive impairment and target organ damage

In the end, we generated correlation models among microstructural WM alterations, cardiac remodeling, hypertensive condition and cognitive profile. We found a significant positive correlation between MoCA scores and FA of the projection and association fibers (ATR and SLF) (Figure 3C and D). FA values of the same projection and association fibers negatively correlated with the estimated duration of hypertension (Figure 3E) as MoCA scores did (Figure 3F). It is interesting to notice that the sample of hypertensive patients (n = 18), excluded because of already manifest neurological damage, had a significantly longer estimated
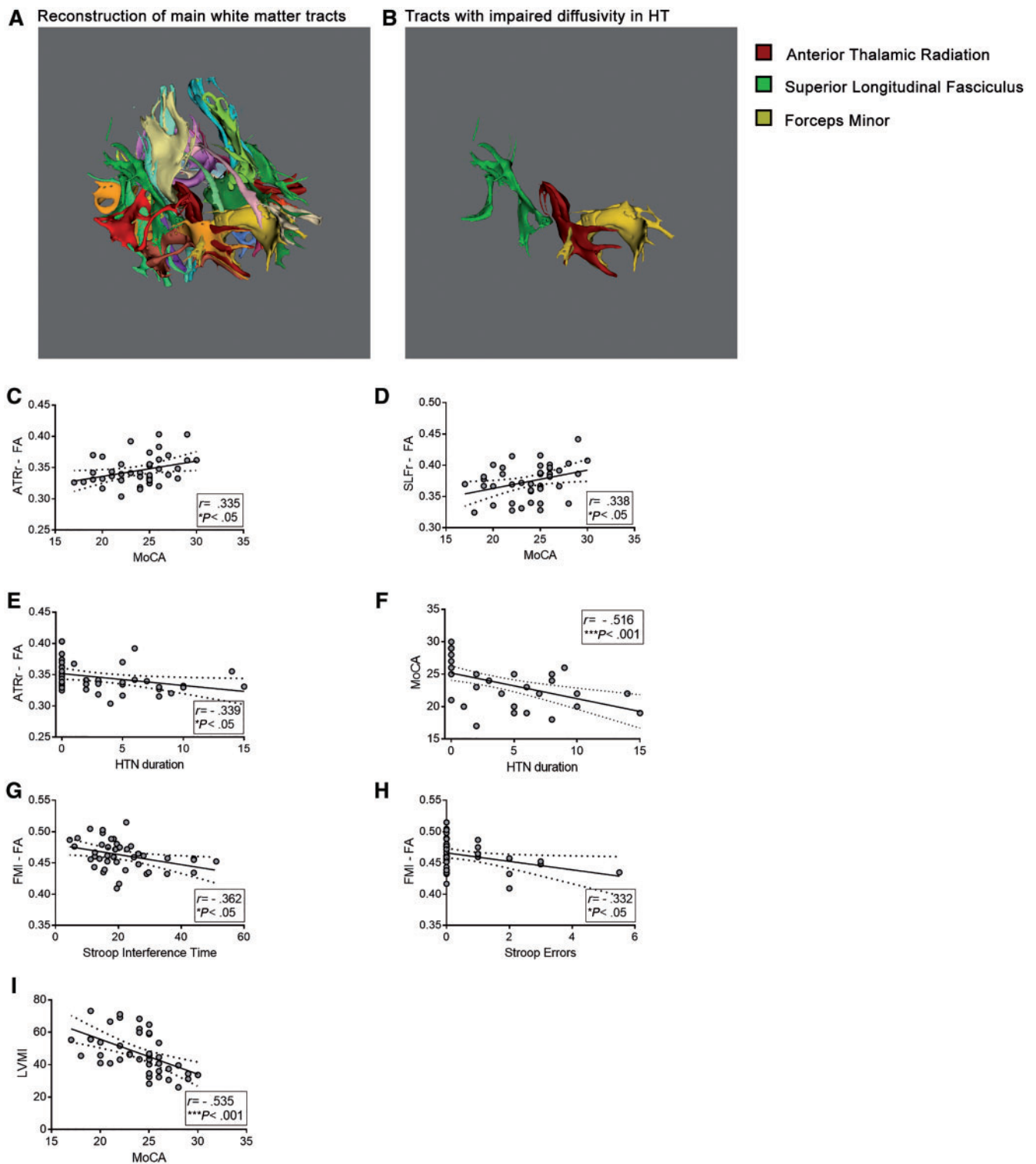
Figure 3 WM microstructural alterations scale with cognitive functions and target organ damage of hypertension. (*A*) Global 3D rendering of the overall tracts reconstructed by fiber-tracking in all the subjects enrolled. (*B*) 3 D rendering of the sole tracts showing altered parameters of WM in hypertensive patients (in red ATR, in green SLF, in yellow FMI). (*C* and *D*) FA of the specific fiber tracts involved in memory and executive functions, namely the ATR (in *C*) and the SLF (in *D*) showed a positive correlation with scores reported at the MoCA test. (*E* and *F*) FA of the ATR and score at the MoCA test negatively correlated with the estimated duration of hypertension. (*G* and *H*) FA of the FMI, a fiber tract typically involved in processing speed, negatively correlated with Stroop interference time (*G*) and Stroop errors (*H*). (*I*) MoCA performance negatively correlated also with LVMI, index of hypertension peripheral organ damage. Dashed lines indicate 95% CI.

duration of hypertension when compared to the group of included patients (mean ± SD = 9.61 ± 5.57 vs. 6.13 ± 3.73 years; *P < 0.05), thus suggesting that with disease advancement, brain damage progressively evolve toward an increasingly manifest injury. Further supporting this hypothesis, both the included and excluded patients had comparable blood pressure control (data not shown).

Hence, in order to test whether the observed correlations between DTI parameters and cognitive scores was modulated by hypertensive condition, we also performed a partial correlation analyses controlling for SBP and years of hypertensive conditions. There were no significant influences of SBP levels and overall duration of hypertension in the correlation observed neither between MoCA and FA r-ATR [SBP: $df(39) = 0.239$, $n = 42$, $P = 0.132$; years of hypertension: $df(39) = 0.199$, $n = 42$, $P = 0.213$] nor between MoCA and FA r-SLF [SBP: $df(39) = 0.287$, $n = 39$, $P = 0.069$; years of hypertension: $df(39) = 0.276$, $n = 42$, $P = 0.081$].

Since altered FMI may be involved in impaired processing speed tasks,[41] we tested the correlation between Stroop interference time, Stroop test errors and FMI-FA, finding a significant relationship suggestive of an impact of hypertension in inhibiting interfering stimuli, represented by the time performance (*Figure 3G* and *H*). Even the correlation observed between FA-FMI and Stroop Interference Time was controlled for the interaction with both SBP, duration of hypertension and age, given that this latter parameter emerged as influencing the deterioration of FA-FMI observed in HT. Interestingly, while there was a statistically significant negative partial correlation between Stroop Interference Time and FA-FMI while controlling for SBP [$df(39) = -0.321$, $n = 39$, $P = 0.040$] no effect was observed for overall duration of hypertension [$df(39) = -0.282$, $n = 42$, $P = 0.074$] or when controlling for age [$df(39) = -0.126$, $n = 42$, $P = 0.431$]. When controlling the correlation between Stroop test errors and FMI-FA for the same parameters it emerged a significant negative correlation when controlling for overall duration of hypertension [$df(39) = -0.312$, $n = 42$, $P = 0.047$] while no effect were observed controlling for SBP [$df(39) = -0.300$, $n = 39$, $P = 0.057$] or when controlling for age [$df(39) = -0.196$, $n = 42$, $P = 0.219$].

In the end, the significant negative correlation observed between indexed LVMI and MoCA (*Figure 3I*) revealed parallel progression of early cognitive alterations and initial peripheral organ damage.

4. Discussion

This is the first study that highlights how hypertensive patients present microstructural alterations of specific WM tracts, at a stage where no sign of macroscopic structural brain damage was evidenced by conventional methods. The WM abnormalities, displayed in tracts segmented by probabilistic fiber-tracking and associated to projection/association fibers and callosum system, were not visible on structural MRI and were associated with impairment of the related cognitive functions, typically prodromal of later dementia.

Our results have been obtained in a hypertensive population characterized by the absence of comorbidities and an initial stage of peripheral organ damage, as shown by modest cardiac and vascular hypertrophic remodeling, but no overt sign of end-organ failure. The lack of structural brain injury induced by hypertension makes assessing prodromal signs quite challenging. DTI-MRI revealed itself as an important step toward using advanced neuroimaging as a diagnostic and prognostic biomarker.[11,12]

WM links different functional brain areas through bundles of fibers established between different cortical structures or between subcortical areas and the cortex. The fiber bundles could be thus defined, following anatomical projections. Based on this *a priori* knowledge of the whole connections in the brain, we can track the WM bundles relating different functional areas and creating a unique tract identification for each subject.[16,42] By applying this method, we performed DTI-MRI tractography, probabilistically estimating fiber orientation and number per each voxel, and extracting quantitative diffusion parameters.[14,15]

Despite HT subjects in our study were on anti-hypertensive therapy, the analysis evidenced a specific signature of their brains, characterized by a deterioration of projection fibers (ATR), association fibers (SLF), and callosum (FMI) systems. Interestingly, the injured tracts are related to specific cognitive functions that were concomitantly impaired, as envisaged by performance on cognitive assessment. The ATR is a projection fiber that connects the thalamus's anterior nucleus to the anterior cingulate gyrus and frontal cortex, and ATR damage has been linked to impaired memory,[43] as revealed also in our HT patients by poor performance on MoCA test, and specifically in the memory subscale. The SLF, a tract composed of two long bi-directional bundles of neuronal axons, connects the cerebrum parietal, occipital, and temporal lobes with the ipsilateral frontal cortices.[43] Characterized primarily as an association fiber, the SLF facilitates the formation of a bidirectional neural network necessary for cognitive functions such as attention, memory, emotions, and language,[43] functions as well impaired in HT under investigation in our study. Lastly, the FMI, also known as the anterior forceps, is a fiber bundle that connects the frontal lobes' lateral and medial surfaces, crosses the midline via the genu of the corpus callosum, and has been linked to processing speed tasks.[41] This latter is some way measured by the results reported in the Stroop interference test, significantly worse in HT. Taken together, the altered WM tracts recapitulate a spatial signature of microstructural alterations that could be prodromal of the brain areas typically reported as affected by lacunar damage or hyperintensities in studies performed in patients at later stages of the disease and subjected to conventional MRI. Interestingly, this pattern of alteration has been extracted from a sample of patients with adequate range of blood pressure control, suggesting that an efficient anti-hypertensive therapy is not sufficient to exclude the onset of vascular dementia or protect the brain from WM microstructural damage. Further supporting this concept, the sample of patients that we excluded because of macroscopic WM damage evidenced by conventional MRI had a significantly longer estimated duration of hypertensive condition, despite showing ranges of blood pressure control similar to those of the samples of patients included in the study protocol.

In addition, it should be further considered that several other risk factors, typically associated with hypertension as well, as aging, increased BMI and smoking, may influence *per se* WM integrity. The results obtained in the covariate analyses conducted in subjects under investigation in this work, would suggest a negligible role of potential interactions established between hypertension and confounding factors, thus strengthening the relevance of the signature identified. Likely, the strict inclusion/exclusion criteria chosen in this study allowed to study a population of HT as deprived as possible of confounding factors for the analysis of WM alterations.

To fully understand which kind of treatment can protect WM and cognitive functions, studies aimed at addressing the mechanisms generating the damage need to be carried on. On this notice, two recent works added important knowledge in this field of research, showing how salt intake can affect cerebral blood flow[44] and how pericyte loss can alter the

vascular function in WM regions,[45] suggesting new targets or medical recommendations for adequate protection from hypertension-induced brain damage.

4.1 Conclusions, study limitations, and perspectives

Main strengths of this study consist in: 1) the comprehensive scan protocol executed on a 3T-MRI for the acquisition of high-resolution diffusion parameters of WM tracts obtained by fiber-tract probabilistic analysis, instead of less efficient atlas selections-based protocols; 2) restriction of the analysis to individuals with no evidence of brain damage at conventional MRI nor diagnosis of dementia. Overall these criteria allowed us to reveal early alterations of WM in patients where the conventional diagnostic methods failed to evidence an initial stage of brain injury.

At same time, this study also presents some limitations that deserve discussion, in order to place our findings in the appropriate context and pave the way for future studies. First, we acknowledge that the strict inclusion criteria resulted in a quite limited sample size of patients under investigation. Secondly, we have only considered a baseline analysis of these patients, having no chance at this stage of predicting the evolution of the damage identified in HT patients.

In conclusion, although larger studies are needed to better define the impact of such WM alterations on the evolution toward dementia, our findings support the possibility that non-invasive tool of advanced brain imaging coupled with cognitive assessment has the potential to provide invaluable information, which could possibly aid in the early prediction of long-term deficits in patients with hypertension. The current trend of personalized treatments calls for the acquisition of more data, to give a wider spectrum of information based not only on clinical assessments but depicting the lifestyle and habits of patients potentially linked to the etiology of hypertension. In future studies, clinical, tractographical, and cognitive data will be complemented by lifestyle and dietary habits information, in order to tailor an anti-hypertensive treatment on a per patient basis.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

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