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Subtle Gray Matter Changes in Temporo-Parietal Cortex Associated with Cardiovascular Risk Factors

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Abstract. Vascular risk factors may play an important role in the pathophysiology of Alzheimer's disease (AD). While there is consistent evidence of gray matter (GM) abnormalities in earlier stages of AD, the presence of more subtle GM changes associated with vascular risk factors in the absence of clinically significant vascular events has been scarcely investigated. This study aimed to examine GM changes in elderly subjects with cardiovascular risk factors. We predicted that the presence of cardiovascular risk would be associated with GM abnormalities involving the temporal-parietal cortices and limbic structures. We recruited 248 dementia-free subjects, age range 66–75 years, from the population-based "São Paulo Ageing and Health Study", classified in accordance to their Framingham Coronary Heart Disease Risk (FCHDR) score to undergo an MRI scan. We performed an overall analysis of covariance, controlled to total GM and APOE4 status, to investigate the presence of regional GM abnormalities in association with FCHDR subgroups (high-risk, medium-risk, and low-risk), and followed by *post hoc* *t*-test. We also applied a co-relational design in order to investigate the presence of linear progression of the GM vulnerability in association with cardiovascular risk factor. Voxel-based morphometry showed that the presence of cardiovascular risk factors were associated with regional GM loss involving the temporal cortices bilaterally. Those results retained statistical significance after including APOE4 as a covariate of interest. We also observed that there was a negative correlation between FCHDR scores and rGM distribution in the parietal cortex. Subclinical cerebrovascular abnormalities involving GM loss may provide an important link between cardiovascular risk factors and AD.

Keywords: Alzheimer's disease, elderly, MRI, population-based, vascular risk factors

INTRODUCTION

Dementia is an increasingly common diagnosis in the aging population, and the number of affected individuals is expected to rise exponentially in coming years [1]. Alzheimer's disease (AD) is the leading cause of dementia in the world, imposing a great social burden [2].

Over the past decades, *in vivo* brain imaging studies have consistently detected the presence of structural and functional abnormalities in association with the diagnosis of AD [3], involving preferentially the lateral

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temporo-parietal neocortices, precuneus, posterior cingulate gyrus, and medial temporal structures including the hippocampus, parahippocampal gyrus, and amygdala [3–5]. Such abnormalities can be detected at early stages of AD [6], as well as in subjects who are carriers of the major genetic risk factor for AD, the $\varepsilon 4$ variant of the gene encoding apolipoprotein (APOE $\varepsilon 4$ allele) [7, 8].

Vascular risk factors [9], such as hypertension [10], diabetes [11], dyslipidemia [12], smoking [13], and obesity [14], are now recognized as important risk factors for both AD and vascular dementia (VaD) [15–22]. There is also evidence from *postmortem* and *in vivo* neuroimaging studies using magnetic resonance imaging (MRI) indicating that brain lesions of vascular origin, such as white matter hyperintensities and infarcts, may be significantly associated with the diagnosis of AD [23–25]. The presence of the APOE $\varepsilon 4$ allele is associated with an increased risk not only of dementia, but also cardiovascular disease [17, 26]. Taken together, these observations have provided support for the view that vascular-related mechanisms could be of critical relevance to the pathophysiology of AD [27–29].

One strategy that may provide further support to the "vascular hypothesis" of AD involves the use of neuroimaging techniques to investigate, in non-demented individuals with prominent vascular-related risk factors, the presence of atrophy and/or hypofunction affecting the same brain regions known to be critical to the pathophysiology of AD. For instance, there are MRI reports associating arterial hypertension with late-life atrophy of the hippocampus [10] and decreased neocortical gray matter (GM) volumes [30]. Smoking habits [31] and type II diabetes [32, 33] have also been associated with GM volume reductions in the medial temporal cortex as well as in other brain regions previously implicated in AD, such as the precuneus and posterior cingulate gyrus. Recently, our group conducted a single photon emission computerized tomography (SPECT) study comparing regional cerebral blood flow (rCBF) patterns between healthy elderly subjects and individuals suffering from heart failure (HF), one other important cerebrovascular risk factor; we found significant rCBF reductions in HF patients circumscribed to the precuneus and posterior cingulate gyrus [34], the brain regions most often detected as hypofunctional in incipient stages of AD [3, 35].

The above imaging studies that investigated the relationship between vascular risk factors and subclinical brain structural and functional changes in non-demented individuals have a number of limitations. These studies have often recruited hospital-based samples [31, 34, 36–38], rather than large, community-based populations identified using epidemiological designs. Also, the lack of concurrent assessments of the presence of the APOE $\varepsilon 4$ allele in the majority of MRI studies has prevented investigations of the degree to which such genetic factor contributes to the brain morphological deficits detected in association with vascular risk factors in non-demented elderly subjects. Finally, the strategy of isolating a single vascular risk factor may be less than ideal [30, 39, 40], as vascular risk factors seldom appear isolated in the elderly population [41].

The Framingham Coronary Heart Disease Risk (FCHDR) [42] is a measure that was devised to synthesize the combination of different cardiovascular risk factors in the prediction of a major vascular-related event. Those cardiovascular risk factors include age, blood pressure, diabetes mellitus, smoking status, and cholesterol levels [43]. This risk-score is widely used in epidemiological studies [44–46], and it is therefore important to conduct imaging studies employing such composite index. In one of the few MRI investigations that used this strategy, Seshadri and colleagues observed a significant inverse correlation between Framinghan Stroke Risk Profile (FSRP) scores and a measure of total brain volume in dementia-free subjects [47].

A recent epidemiological investigation carried out in Brazil [48], the São Paulo Ageing & Health study (SPAH), identified a population of 2,072 individuals aged 65 and above living in a circumscribed area from the city of São Paulo, with the primary purpose of determining the prevalence and risk factors for dementia using transcultural protocols developed by the 10/66 Dementia Research Group [48–50]. This population has now been followed-up two years after their initial evaluation. During the second wave of the SPAH study, we have conducted a cross-sectional MRI investigation, with the primary purpose of investigating the contribution of vascular risk factors, as assessed using a composite measure (the FCHDR profile), to the presence and distribution of regional GM abnormalities in a large subsample ($n = 248$) of dementia-free elderly subjects from the above population. We hypothesized that exposure to elevated levels of vascular risk factors would be associated with lower regional GM (rGM) volumes in brain regions previously implicated in pathophysiology of AD, namely the lateral temporo-parietal neocortices, precuneus, posterior cingulate gyrus, and medial temporal structures. We also wished

to investigate whether such GM abnormalities would remain detectable in subjects with high levels of vascular risk factors after controlling for the presence of the APOE ε4 allele. The inclusion of APOE ε4 allele status was based on the fact that genetic predisposition to AD can enhance the effect of cardiovascular risk factors over the GM distribution in the general population [51].

METHODS

Study sample

The current MRI study received approval from the local Committee for Ethics and Research (CAPpesq) of the Faculty of Medicine, University of São Paulo (protocol 450/05) and written consent was obtained from all subjects. The characteristics of the SPAH study have been described in detail elsewhere [48]. In brief, all residents aged 65 or above ($n = 2{,}072$) of pre-defined census sectors of an economically disadvantaged area of São Paulo were contacted with the aim of determining the prevalence and risk factors for dementia [48–50].

The pool of potential candidates for the present MRI study was created after inspection of the epidemiological databank from the first wave of the SPAH study with two purposes: assessment of inclusion and exclusion; and exclusion of cases for which one or more of the variables needed to generate the FCHDR profile (gender, age, total cholesterol (CLT) level, total high density lipoprotein (HDL) level, hypertension (blood pressure), diabetes, and smoking habits) were not available. Risk estimates were derived from the experience of the Framingham Heart Study and predict coronary heart disease in individuals from 30 to 74 years [52]. Information on participants' age was obtained by asking dates of birth and confirmed from identity cards. The presence of diabetes mellitus was defined by a fasting blood glucose level ≥ 126 mg/dl and/or current use of insulin or hypoglycemic oral drug treatment. Levels of CLT and HDL were obtained using the cholesterol-oxidase method. Three measurements of blood pressure were performed with an OMRON digital sphygmomanometer, model HEM-712-C. Measurements were taken at least one hour without ingestion of caffeine and/or smoking, with participants seated. The first measurement was taken after 5 minutes of rest, and the two remaining measurements were taken at intervals of 5 minutes. For the calculation of the arterial pressure value, the first measurement was discarded, and the arithmetic mean of the second and third measurements was calculated. Finally, participants were asked about their smoking habits. The FCHDR calculated for each subject was then used in order to subdivide the sample in the following three groups according to their cardiovascular risk: low-risk (FCHDR < 9%), medium-risk (FCHDR = 10–19%), and high-risk (FCHDR > 20%).

We initially excluded, from the pool of potentially eligible subjects, all individuals aged above 75 years at time of recruitment for MRI scanning ($n = 996$), as well as those who had either not completed the 2-year clinical follow-up, present any missing data that prevented FCHDR scoring ($n = 107$), or who fulfilled diagnostic criteria for neuropsychiatric disorders ($n = 52$). This led to the identification of 917 potentially eligible individuals who were classified in terms of their cardiovascular risk according to the FCHDR in low-risk (24%), medium-risk (36.4%), and high-risk (39.1%) subjects. Telephone contacts were then made with each potentially eligible subject in order to invite him or her to take part in the brain imaging study, and to check for the presence of contra-indications for MRI scanning (carrying cardiac pacemaker, valvular prosthesis, or internal electrical magnetic device, history of neurosurgery or presence of metal fragment in brain, eye, or spinal cord). We failed to successfully contact 103 subjects, and for those who were reachable, we excluded 206 subjects (132/74 female/male) who fulfilled exclusion criteria for our study (presence of cognitive decline, mild cognitive impairment (MCI), dementia history of stroke, epilepsy, brain trauma, and transitory ischemic event), thus resulting in a total of 608 potential subjects to be invited to undergo the brain imaging session. The Fig. 1 presents the data regarding eligibility criteria and selection from our sample.

In order to reach sufficient statistical power we used the software G*Power3 [53, 54] to perform *a priori* power analysis for the identification of significant between group rGM differences. Therefore, collection of MRI data was devised for a minimum sample size of 156 in order to reach a power of 0.80 with α error probability of 0.05 and effect size of 0.40 for the identification of significant rGM differences between the three groups, with calculations conducted using G*Power 3[53, 54].

We consecutively contacted the potential subjects from our pool ($n = 608$) in order to fulfill our sample. As we estimated that there would be several subjects with silent brain lesions, therefore we opted to perform a MRI scan in 248 dementia-free elderly subjects aged between 66–75 years (female/male [134/114]) divided according to cardiovascular risk scanning (FCHDR low-risk [$n = 58$]; medium-risk

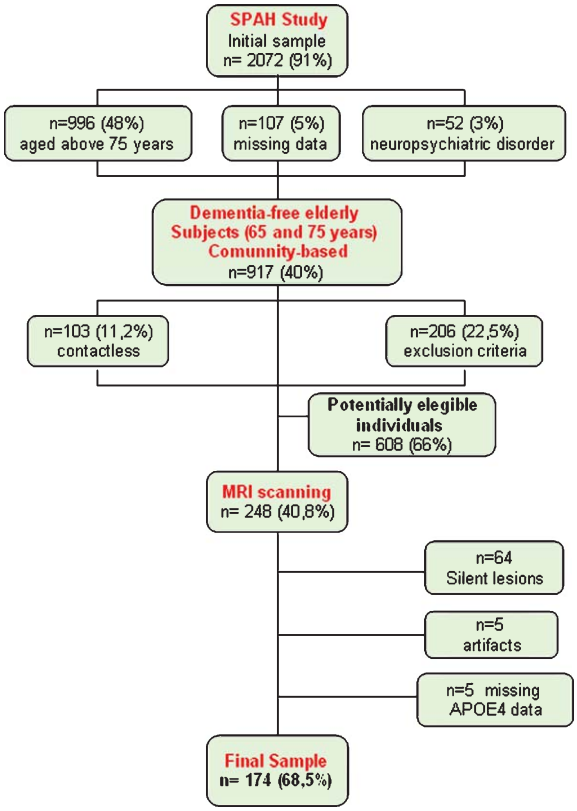

Fig. 1. Flowchart of sample selection in this study.

[$n = 88$]; high-risk [$n = 102$]) in order to guarantee the statistical power. The contacts were done consecutively until a total of 248 scans were performed. A total of 52 from those individuals refused to participate in the MRI investigation. There were significant difference between those included in the present MRI study ($n = 248$) and those in the excluded ($n = 1118$) from the SPAH database in terms of age distribution ($p < 0.001$), years of education ($p < 0.001$), and familiar income ($p < 0.001$).

Clinical and demographic measurements

The identification of cases of dementia and other major psychiatric disorders by the epidemiological team followed the protocol developed by the 10/66 Dementia Research Group [48–50]. This protocol included: the Community Screening Instrument for Dementia (CSI-D); an adapted version of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) ten-word list; the animal naming verbal fluency task from the CERAD; the Geriatric Mental State (GMS); a structured neurological assessment; and a structured cardiological evaluation. The protocol had specific questions regarding Parkinson's disease, epilepsy, symptomatic or transient ischemic attacks; and severe head trauma. The criterion for the exclusion of subjects with MCI, after excluding cases of dementia, was defined as a performance of 1.5 standard deviation below the mean performance in the cognitive battery described above obtained from all subjects between 66 and 75 years of age from the original SPAH sample. Schooling data of each subject was extracted from the SPAH study database. In brief, we considered subjects as having 4 years of education if they had completed the 4th grade, 8 years if having completed the 8th grade, 11 years if having completed high school, and 15 years if having completed college. When one of these educational periods was not completed, the number of years until drop out was used as the estimate of mean number of years of education.

Genotyping: APOE4 measures

Genomic DNA was extracted from EDTA-anticoagulated venous blood using standard salting-out method. The concentration of extracted DNA was determined by spectrophotometric measurements. Five microliters of the extracted DNA was diluted 1 : 50 in 0.2 × TE buffer. Absorption was measured for both blank (only 0.2 × TE) and diluted DNA solutions at 260 nm using the GeneQuant (Amersham Pharmacia). An absorbance (A260) of 1.0 corresponds to 50 ug of double stranded DNA per milliliter. After quantification, the DNA was diluted to a working level of 10 ng/uL. The single nucleotide polymorphisms (SNPs) rs429358 and rs7412 that determine the APOE isoforms were used to genotype all subjects under contract by Prevention Genetics (http://www.preventiongenetics.com/).

Brain imaging data acquisition

MRI images were acquired using a 1.5 T General Electric Signa LX CVi scanner (Milwaukee, WI, USA), using the following standardized acquisition protocol: a) a dual-spin echo sequence of 120 transaxial slices across the entire brain (axial PD/T2); b) a T2-weighted fast spin-echo transaxial sequence with 88 slices; and c) a three-dimension gradient echo (Spoiled Gradient Recalled Acquisition–SPGR) sequence of 124 slices with TR/TE of 21.7/5.2 msec, flip angle of 20 degrees, 220 mm field of view (FOV), 1.5 mm slice thickness, number of measures (NEX) of 01, 256 × 192 matrix.

Qualitative analysis

Data sets (SPGR, T2, and PD) were readily reconstructed and visually checked by a radiologist for identification of major artifacts and presence of any gross brain lesions, such as tumors and silent infarcts (stroke or lacunar infarcts).

Image processing: Voxel-based morphometry

Quantitative analyses were performed using the above SPGR sequence after exclusion of MRI scans in which silent brain lesions or artifacts were detected. The presence of rGM reductions in association with cardiovascular risk factors was investigated using the voxel-based morphometry (VBM) approach with Statistical Parametric Mapping (SPM2) software (Wellcome Department of Imaging Neuroscience, London, UK), running in Matlab version 6.1 (Mathworks, Sherborn, Massachusetts). An optimized VBM protocol was applied, in order to take account of the structural characteristics of elderly brains. First, a customized template was created specifically for the study, consisting on an average T1-weighted image and a priori GM, WM, and CSF templates, based on the images of all subjects included in the study, in order to more closely match the population under investigation [55]. In order to build this template, images were spatially normalized to the standard SPM T1-MRI template, based on 152 healthy subjects from the Montreal Neurological Institute (MNI). This spatial normalization step was restricted to linear 12-parameter affine transformations, to minimize deformations of our original images. Spatially normalized images were then segmented into GM, WM, and CSF compartments, with a modified mixture model cluster analysis technique. This used the MNI *prior* probability maps provided in the SPM2 package, overlaid onto the images to classify voxels in terms of their probability of belonging to a particular tissue class. The segmentation method also included an automated brain extraction procedure to remove non-brain tissue and an algorithm to correct for image intensity non-uniformity. Finally, images were smoothed with an isotropic Gaussian kernel (8 mm full width at half maximum) and averaged to provide the GM, WM, and CSF templates in stereotactic space.

Subsequently, the processing of the original images from all subjects was carried out, beginning by image segmentation with the study-specific, a *priori* GM, WM, and CSF templates. Extracted GM and WM images were then spatially normalized to the customized GM and WM templates with 12-parameter linear and non-linear (7 ˗ 9 ˗ 7 basis functions) transformations. The parameters resulting from this spatial normalization step were then reapplied to the original structural images. These fully normalized images were then re-sliced with trilinear interpolation to a final voxel size of 2 ˗ 2 ˗ 2 mm^3 and segmented into GM, WM, and CSF partitions. Voxel values were modulated by the Jacobian determinants derived from the spatial normalization, thus allowing brain structures that had their volumes reduced after spatial normalization to have their total counts decreased by an amount proportional to the degree of volume shrinkage. When performed using modulated images, statistical analyses test for differences in the total volume of GM [55]. All images were smoothed with a Gaussian kernel before statistical analyses, using two choices of width. First, analyses were conducted with images smoothed with the Gaussian kernel most usually employed in VBM studies (12-mm at full-width half maximum - FWHM). Subsequently, analyses were repeated with images smoothed with a 4-mm FWHM Gaussian kernel, as this is more appropriate for the investigation of GM deficits in specific, small-sized temporo-limbic structures such as the hippocampus and amygdala [54].

Statistical analyses

Using the Statistical Package for Social Sciences (SPSS) for Windows (10.0 version), group comparisons of clinical and demographic data (between FCHDR low-risk, medium-risk, and high-risk subjects) were conducted using analyses of variance (ANOVA) for continuous variables (age and mean years of education), and chi-square tests for other, categorical variables. For the investigation of the association between vascular risk factor and the presence of gross brain lesions, we directly compared clinical and demographic characteristics of subjects with and without silent brain infarct (SI) as detected in the qualitative, visual inspection of images.

Initially all analyses on the images processed using VBM routines were conducted using the degree of vascular risk as a continuous variable rather than subdividing the sample in three groups. Voxelwise linear correlation indices across the whole brain were calculated between rGM values and FCHDR scores using the entire sample of elderly individuals (excluding those with gross brain lesions). Seven masks were used in each hemisphere, involving, respectively, the: lateral temporal cortex; lateral parietal cortex, hippocampus; parahippocampal gyrus; amygdala; precuneus;

and posterior cingulate gyrus. In this exploratory correlational analysis, we only considered findings as significant findings if they survived correction for multiple comparisons at a FWE-corrected $p \leq 0.05$ threshold over the whole brain [56].

Subsequently, between-group differences in rGM distribution across the three FCHDR groups (high-risk, medium-risk, and low-risk) were assessed with an overall analysis of covariance (ANCOVA) model, including a measure of the total amount of GM and gender as covariate of no interest. The total amount of GM was given by the total number of voxels within the GM compartment of each subject. Only voxels with values above an absolute GM threshold of 0.05 were entered in such analyses, resulting in a searching volume of approximately 250,000 voxels. Resulting statistics were threshold at the $p < 0.001$ level of significance, and displayed as a statistical parametric map (SPM) into standard anatomical space at a threshold of $Z = 3.09$. Firstly, the ANCOVA SPM was inspected on a hypothesis-driven fashion, searching for clusters of voxels in regions where GM between-group differences had been predicted a *priori* (lateral temporal-parietal cortices, hippocampus, parahippocampal gyrus, amygdala, precuneus, and posterior cingulate gyrus). This hypothesis-driven analysis was conducted using the small volume correction (SVC) approach, with the purpose of constraining the total number of voxels included in the analysis. Each region was circumscribed by merging the spatially normalized region-of-interest masks that are available within the Anatomical Automatic Labeling SPM toolbox. Anatomical masks were used in each hemisphere, resulting in search volumes of: 3141 voxels for the lateral temporal cortex (encompassing the superior, middle, and inferior temporal gyri); 3455 voxels for the lateral parietal cortex (encompassing the supramarginal, posterior, and angular gyri), 946 voxels for the hippocampus; 978 voxels for the parahippocampal gyrus; 248 voxels for the amygdala; 3140 voxels for the precuneus; and 335 voxels for the posterior cingulate gyrus. Post hoc evaluation of significant ANCOVA findings in these regions was then performed with secondary two-tailed, independent-sample *t*-tests. Findings in those areas were reported as significant only if surviving FWE correction for multiple comparisons over the whole brain ($p < 0.05$). Subsequently, the ANCOVA map was inspected again, in order to identify any additional significant between-group rGM differences in unpredicted regions across the entire brain. Findings in these additional areas would only be reported as significant if surviving FWE correction for multiple comparisons over the whole brain ($p < 0.05$) and cluster size >100 voxels. In order to investigate whether our VBM findings were related to a genetic predisposition, we repeated the above analysis including the presence of APOE $\varepsilon 4$ alleles as a covariate of interest in the voxelwise ANCOVA model outlined above. For the reporting of significant findings of all voxel-based analyses above, we converted MNI coordinates of voxels of maximal statistical significance to the Talairach and Tournoux system [57].

RESULTS

Clinical and demographic characteristics of the entire sample

Table 1 presents the demographic and clinical characteristics for the whole sample ($n = 248$). We observed a gender imbalance across the three groups ($p < 0.001$), with a predominance of women in the low-risk group and a greater prevalence of men in the high-risk group. Regarding to individual cardiovascular risk factors, we found that the presence of history of diabetes was significantly different between the three groups ($p = 0.002$) (Table 1).

Qualitative brain imaging analysis: Presence of silent brain lesions

The blind qualitative visual inspection of MRI datasets by a radiologist identified a total of 53 patients (22.37% from the total sample) who presented at least one silent brain lesion. The presence of SIs were detected in 48 cases (19.35% from the total sample), while the remaining cases ($n = 5$) were defined as silent brain tumors ($n = 3$) and silent congenital vascular malformations ($n = 2$). Most SI detected were lacunar infarcts. They were preferentially located at the basal ganglia and/or thalamus (61%), followed by white matter (16%), cerebellum and/or brainstem (17%), temporo-occipital (4%), parietal (1%), and frontal (1%) cortices. There were no difference in those subjects with and without SI regarding the presence of APOE4 allele ($p = 0.354$), diabetes ($p = 0.858$), hypertension ($p = 0.317$), level of education ($p = 0.061$), and gender distribution ($p = 0.744$).

Clinical and demographic characteristics of the subsample that underwent VBM processing

After excluding subjects with presence of any gross lesion ($n = 53$), movement artifacts during image

Table 1
Demographic and clinical characteristics for overall MRI sample classified according to their cardiovascular risk using FCHDR scores

	Low-risk *(n = 58)*	Medium-risk *(n = 88)*	High-risk *(n = 102)*	Statistical *test*	p value
Mean age (±SD) in years	70.93 ± 2.67	70.25 ± 2.34	71.01 ± 2.70	ANOVA	0.101
Male/female	13\45	28\60	73/29	χ^2	<0.001
Mean years of education (±SD)	4.24 ± 3.12	4.94 ± 3.80	3.83 ± 3.34	ANOVA	0.089
Hypertension *n* (%)	34 (58.6)	60 (68.2)	68 (66.7)	χ^2	0.461
Diabetes *n* (%)	8 (13.8)	24 (27.3)	40 (39.6)	χ^2	0.002
Dyslipidemia *n* (%)	9 (15.5)	3 (3.4)	5 (4.9)	χ^2	0.581
APOE ε4 allele *n* (%)	12 (20.7)	26(29.5)	17 (17.7)	χ^2	0.156

FCHDR: Framingham Coronary Heart Disease Risk; SD: Standard Deviation.

acquisition ($n = 4$), presence of cognitive or major psychiatric disorder ($n = 16$) identified during the clinical examination at the day of MRI scanning, a total of 174 subjects entered the quantitative analysis of rGM volumes, using the VBM protocol. Table 2 presents the demographic and clinical characteristics for the subjects who underwent VBM processing. The same gender imbalance across the three groups described above remained present after excluding subjects with silent brain lesions or movement artifacts, with a predominance of females in the low-risk and males in the high-risk groups ($p = 0.017$) (Table 2). Regarding individual cardiovascular risk factors, between-group differences in the prevalence of diabetes remained significant between the three groups ($p = 0.003$).

At the time of MRI scanning, subjects that entered the VBM analysis were being treated with the following medications: antihypertensive drugs ($n = 108$); hypoglycemic agents ($n = 34$); statins ($n = 20$); hormones ($n = 11$); antidepressants ($n = 3$); benzodiazepines ($n = 2$); digitalis ($n = 2$); corticosteroids ($n = 1$); acetylsalicylic acid ($n = 25$); calcium carbonate ($n = 13$); and angiotensin-converting enzyme inhibitor ($n = 1$).

Gray matter volumes: Correlation analysis between rGM distribution and FCHDR scores

The voxelwise linear correlation analysis between GM volumes and FCHDR scores for the whole sample revealed the presence of significant negative correlation involving the bilaterally the parietal cortex; namely the right (cluster 650 voxels, Z = 3.98, pFWE = 0.006) and left (cluster 26 voxels, Z = 3.92, pFWE = 0.007) superior parietal cortices, and the right (cluster 1688 voxels, Z = 3.96, pFWE = 0.004) and left (cluster 298 voxels, Z = 4.05, pFWE = 0.005) inferior parietal cortices.

Gray matter volumes: Voxelwise between-group rGM comparisons

The ANCOVA comparing the three groups revealed several clusters of significant between-group rGM differences involving brain regions where abnormalities had been predicted *a priori*. These encompassed the right and left temporal cortex ($p = 0.009$ corrected for multiple comparisons [pFWE]) and pFWE = 0.007); the hippocampus

Table 2
Demographic and clinical characteristics for the three subgroups of subjects classified according to their cardiovascular risk using FCHDR scores that entered VBM analysis

	Low-risk *(n = 47)*	Medium-risk *(n = 67)*	High-risk *(n = 64)*	Statistical *test*	p value
Mean age (±SD) in years	70.94 ± 2.47	70.91 ± 2.69	70.44 ± 2.60	ANOVA	0.492
Male/female	17/40	41/37	36/27	χ^2	0.017
Mean years of education (±SD) in years	4.79 ± 3.21	3.64 ± 2.60	4.21 ± 2.81	ANOVA	0.112
Hypertension *n* (%)	23 (40.35)	50 (64.10)	43 (68.25)	χ^2	0.450
Diabetes *n* (%)	7 (12.29)	21 (26.92)	29 (46.03)	χ^2	0.003
Dyslipidemia *n* (%)	9 (15.79)	8 (10.26)	5 (7.94)	χ^2	0.294
APOE ε4 allele *n* (%)	9 (15.79)	16 (20.52)	12 (18.75)	χ^2	0.708

FCHDR: Framingham Coronary Heart Disease Risk; SD: Standard Deviation.

Table 3
Voxel based morphometry investigation of brain volume abnormalities in a dementia-free community of elderly subjects classified according to their cardiovascular risk including total gray matter as covariate

Region name	BA	P_{FWE} corrected[a]	Size of Cluster[b]	Peak Z Score[c]	Coordinates x,y,z[d]
Significant rGM reductions in high-risk group in comparison to low-risk group (*t*-test/FWHM 12 mm)					
Right temporal cortex	21/22/42	0.015	596	4.08	−48−38 6
Middle temporal gyrus					
Superior temporal gyrus					
Left temporal cortex		0.017	835	4.00	63−17 5
Middle temporal gyrus					
Superior temporal gyrus					
Trend of rGM reductions in medium-risk group in comparison to low-risk group (*t*-test/FWHM 12 mm)					
Right superior temporal cortex	21/22	0.061	190	3.37	−47−33 5
Significant rGM reductions in low-risk group in comparison to high-risk group (*t*-test/FWHM 4 mm)					
Left Amygdala		0.005	236	4.06	25 1−24
Right Parahippocampal Gyrus		0.016	412	4.22	−23−4 −28
Left Parahippocampal Gyrus		0.001	430	4.83	24−2−35

[a] Statistical significance after correction for multiple comparisons; inferences made at the level of individual voxels (family-wise error correction). [b] Number of contiguous voxels that surpassed the initial threshold of $p < 0.01$ (uncorrected) in the statistical parametric maps. [c] Z scores for the voxel of maximal statistical significance in the correlation between rGM and age. [d] Talairach & Tournoux (1998) coordinates of the voxel of maximal statistical significance within each cluster. BA = approximate Broadmann area.

(pFWE = 0.019); the right and left parahipocampal gyrus (pFWE = 0.001 and pFWE = 0.005); and the left amygdala (pFWE = 0.004). Table 3 shows the results of the *post-hoc* analyses comparing two groups using unpaired *t*-tests. In comparison to the low-risk group, the high-risk group presented rGM reductions in the right and left temporal cortices (pFWE = 0.015 and pFWE = 0.017, respectively). Trends toward between-group rGM differences in the right and left superior temporal cortices also emerged in the comparison of the medium-risk group in comparison to the low-risk one (pFWE = 0.061 and pFWE = 0.083, respectively). The *post hoc* analyses in temporolimbic structures, applying a FWHM of 4 mm, showed significantly greater rGM values in the high-risk group relative to the low-risk group in the right hippocampus (pFWE = 0.037), the right and left parahippocampal gyri (pFWE = 0.016 and pFWE = 0.001, respectively), and in the left amygdala (pFWE = 0.005).

APOE4 effect on regional gray matter distribution

The APOE4 allele was found in 37 subjects. We did not observed significant differences in APOE4 prevalence across the three groups. In comparison to the low-risk group, the high-risk group presented rGM reductions in the right and left temporal cortices (pFWE = 0.015 and pFWE = 0.017, respectively). The *post hoc* analyses in temporolimbic structures, applying a FWHM of 4 mm, showed significantly greater rGM values in the high-risk group relative to the low-risk group in the right hippocampus (pFWE = 0.038), the right and left parahippocampal gyri (pFWE = 0.016 and pFWE = 0.001, respectively), and in the left amygdala (pFWE = 0.001).

Finally, we also conducted a direct VBM comparison between subjects with ($n = 37$) and without ($n = 141$) the APOE $\varepsilon 4$ allele, regardless of their degree of cardiovascular risk. This comparison revealed that APOE $\varepsilon 4$ allele (+) subjects in comparison to APOE $\varepsilon 4$ allele (−) subjects presented a significant rGM reduction involving the left precuneus (cluster of 248 voxels, Z = 3.57, PFWE = 0.038).

DISCUSSION

This report describes the results of a community-based study aimed at evaluating the presence of rGM abnormalities in relation to a composite measure aggregating several cerebrovascular risk factors in an epidemiological sample of elderly subjects aged between 66–75 years. First, we showed that elderly individuals even in the absence of clinical disease, once those with symptomatic stroke and dementia were excluded from this analysis, presented vascular-related silent findings. Furthermore, even after excluding those individuals with silent findings, we described significant GM reductions in a population-based non-demented sample in association to the presence of vascular risk factors involving the parietal-temporal cortices. Also we showed that the findings in the parietal cortex presented a linear progression in association

with the presence of cardiovascular risk. We provided further support to the accumulating evidence linking AD with vascular risk factors [23, 27].

Study sampling

The strengths of our study include the large population-based sample and the use of a transcultural protocol [58, 59]. The strategy of recruiting consecutively all subjects from the epidemiological databank led to an excess of male subjects in the high risk subgroup and women in low risk one. This is in consistence with previous epidemiological investigations of vascular risk in elderly populations [60, 61]. However, given that both age [62] and gender [63] could influence rGM distribution in healthy elderly subjects, we have included gender as a covariate in all analyses. Moreover, our approach of limiting the age span for the MRI scanning minimizes the possible bias caused by inter-subject variations in rGM due to the normal aging process [62, 63]. Our exclusion criteria were very strict, and all participants were free of clinically significant cognitive impairment, dementia, stroke, or other neuropsychiatric conditions. Even with applying such stringent exclusion criteria, we were still able to demonstrate the presence of GM abnormalities in the high cardiovascular risk subgroup involving the right superior temporal neocortex.

Silent brain lesions

Among clinically stroke-free participants in this elderly, community-dwelling cohort, almost 20% had at least one SI detected on the brain MRI scan. The identification of gross brain lesions, most of which of vascular origin, in our sample is consistent with the rates previously reported in the literature [64–68]. Evidence has accumulated that vascular risk factors [69], including hypertension [66], hyperlipidemia, diabetes mellitus [70], obesity, and cigarette smoking, play an important role in the etiology of silent brain infarcts [64] and could lead to both future cognitive decline and dementia. Similar to other studies [69, 71–73], the presence of SI in our study steeply increased with increasing cardiovascular risk. The subjects presenting gross brain lesions were excluded from the final VBM analyses, as their inclusion would severely bias the voxel-by-voxel statistical comparisons. On the other hand, their exclusion also introduces a bias in the voxel-based analyses, as subjects with vascular-related gross brain lesions are likely to score high in regard to cardiovascular risk factors. However, the fact that we recruited subjects for this study from a large, epidemiologically ascertained sample, still allowed us to compose the subgroup with high risk even after the exclusion of those who presented gross brain lesions at MRI scanning.

Findings involving the parietal and temporal cortices

In our study, we observed rGM loss involving the right inferior parietal and right superior temporal cortices in association to the presence of vascular risk factors. Furthermore, we also observed that there was a linear progression of GM loss in the right parietal cortex in association with the presence of cardiovascular risk, as assessed by FCHDR score. The temporo parietal cortices are known to be relevant to the pathophysiology of AD [74, 75]. Furthermore, previous animal studies have also implicated the temporal cortex as vulnerable to blood flow reductions [76, 77]. Taking all this together, it was proposed by de la Torre (2000) that during the aging process, the presence of vascular risk factors could converge to both hypoperfusion and energy crises that would trigger regional changes that at the end point might generate a chain of events that would lead to AD pathology [78, 79]. Finally, a previous study from our group has shown the reduction of cerebral blood flow involving the parieto-occipital cortex in subjects with heart failure, as well as an direct association between lower cognitive scores and cerebral blood flow reductions in the posterior cingulated gyrus [80].

We opted to apply a summary index to measure the effect of cardiovascular risk factors, once in the elderly hypertension; diabetes, smoking, and dyslipidemia seldom appear isolated [39, 81, 82]. Studies that examined each risk factor individually often perform an adjustment for other risk factors. However, the comorbidity might be mediate by common pathway, including the development of atherosclerotic disease [81, 83–85]. Furthermore, the adjustment of different vascular risk factors might underestimate the findings and does not take in account the effect of aggregation of those risk factors.

There is a dynamic relation between cerebral circulation and metabolism, with subsequent risk of ischemia. It is plausible that the presence of cardiovascular risk factors might be associated with diminished perfusion of the brain, as previously shown in heart failure subjects [34, 86]. The reduced cerebral perfusion could trigger brain atrophy involving those areas of sudden reduction of vascular caliber at most distal

vascular field [79, 87, 88]. The localization of vascular pathology findings in the right hemisphere is in accordance with some brain aging studies[34, 89, 90], yet it is still difficult to be completely explained. Our previous investigation of rCBF in HF individuals also showed a right sided preference in the parietal cortex [34, 86]. Giannakopoulos et al. (2009) also suggested that the concomitant predominance of AD and vascular pathology in the right hemisphere was associated with significantly higher cognitive impairment [91]. Previous MRI studies investigating GM abnormalities associated with vascular risk factors have identified brain abnormalities involving brain regions other than the lateral temporo-parietal neocortex, such as the posterior cingulate gyrus [31] and the precuneus [92].

Findings involving the hippocampus and parahippocampal gyrus

In our study, we observed unpredicted foci of rGM increases involving the limbic region (hippocampus, amygdala, and parahippocampal gyri) in the high-risk group compared to the low-risk group. This is contrast with previous findings of hippocampal atrophy observed in association to type II diabetes [93] and hypertension [10]. An explanation for these findings could be related to differences in the sample size [93] and influence of social-cultural environment in the cognitive reserve observed in elderly Japanese [10] in comparison to individuals living in a community with low socioeconomical status [59, 94]. Erten-Lyons et al. (2009) observed that subjects with high AD pathology burden lesions in the autopsy but with larger hippocampal volumes presented a preserved cognitive function, even after findings were adjusted for the presence of vascular disease [95]. Also, the presence of cardiovascular risk factors may enhance the normal process of aging-related brain shrinkage particularly if lacunes and stroke are present [96]. In our study, we excluded subjects with both cognitive decline (MCI) and dementia (AD and VaD) from our sample, and after performing the MRI scan, we also excluded from the VBM analyses all individuals with silent brain lesions, including lacunes and silent strokes, which were present more frequently in subjects from the high-risk group. Therefore, it is plausible to suppose that our inclusion criteria for the VBM analyses may have led to the exclusion of individuals from the high-risk group who presented findings of hippocampal atrophy related to AD pathology and/or vascular disease burden. Thus our finding of higher hippocampal and parahippocampal volumes in the high-risk group might reflect the selection of subjects with individual protection for cognitive decline despite the cardiovascular risk. A better understanding of different factors that might lead to the preservation of brain volumes may provide important clues for the discovery of mechanisms that protect elderly individuals from developing AD.

Impact of the presence of APOE ε4 allele

The major genetic risk factor for senile dementia, the ε4 allele of the gene encoding APOE ε4 allele [26], is also associated with cardiovascular disease [97]. Whereas there is agreement about the association of the *APOE* ε4 with AD, the relation between this allele and vascular dementia remains controversial [98–101]. We observed the presence of APOE ε4 allele associated with reduced rGM involving the precuneus; it is interesting that Honea et al. described in healthy individuals APOE ε4 allele carriers the presence of brain abnormalities in both involving the parahippocampal cortex [102], and in those subjects with maternal family history of AD the presence of APOE ε4 allele was associated with reduced rGM in others brain regions including the precuneus [103]. In order to contemplate this possibility we included the presence of APOE ε4 allele as a covariate of interest; however we did not observed any change in the pattern of rGM between-group differences. Given the role of APOE in the pathophysiology of cerebral amyloidopathy, the fact that, after taking in account a possible effect of APOE ε4 status, our findings of regional GM differences retained significance gave further support to our hypothesis of a direct effect of the presence of cardiovascular risk factors in the brain, and a possible risk for development of non-genetic AD [104]. Yet, the effect of the APOE polymorphisms might be complex and mediated by different mechanisms, including a possible protective mechanism mediate by APOE ε2 [102, 105–108] in contrast to the possible acceleration of GM loss associated to APOE ε4 allele[107, 109–111]. Therefore, further studies investigating specifically the effect of different polymorphism of APOE in the brain volume in non-demented elderly would be necessary to further clarify these aspects.

Diabetes and cardiovascular risk assessment

The FCHDR is a measure that was devised to synthesize the combination of different cardiovascular risk factors in the prediction of a major vascular-related event (namely stroke or heart attack) [47, 112, 113]. The inspection of the frequencies of each individ-

ual cardiovascular risk factor in the three subgroups showed only a significant difference in the distribution of diabetes. Because the FCHDR is a composite measure, it would be expected that the high-risk subgroup the presence of multiple cardiovascular risk factors [114, 115]. Previous cross-sectional studies have reported an association between diabetes and dementia [16, 116–118]. Furthermore, the presence of diabetes has been considered a risk factor for both lacunae and stroke [70, 119], as well as brain atrophy involving both the prefrontal [37] and medial temporal [120] cortices [33, 121–124]. Yang and colleagues investigated the presence of diabetes in a population-based sample, and described that both hypertension and dyslipidemia were strong predictors of diabetes [125], therefore in the assessment of individual risk the presence of several cardiovascular risk would be included in higher cardiovascular risk subgroup [42, 115, 126–128]. We cannot entirely exclude the possibility that our results were highly influenced by the presence of diabetes in the high risk group. However, by applying a composite measure rather than assessing each risk individually, our investigation gives us an idea of the cerebrovascular burden, and assisted us in the assessment of the additive nature of cardiovascular risk factors[43].

Limitations

As in most imaging studies, the present investigation will be subject to methodological limitations. For instance, the use of the VBM approach to assess the presence of rGM abnormalities associated with vascular risk factors is also subject to limitations, for instance the increased chance of type one errors due to the large number of statistical comparisons conducted across the entire brain. Therefore, the use of statistical levels corrected for multiple comparisons is of critical importance in studies such as ours. Image analysis methods that involve the manual placement of ROIs on selected portions of key brain structures is associated with a lower risk of type I errors. However, ROI-based methods are labor intensive and present other important limitations, such as the difficulties to reliably define anatomical boundaries, frequently leading to low intra- and inter-rater reliability. Separate measurements of subregions within each brain structure of interest using ROIs are also cumbersome and subject to observer biases. Finally, the placement of ROIs in selected portions of the brain prevents the investigation of volumetric/metabolic abnormalities in other, remote brain regions that also participate in the neural network responsible for the maintenance of cognitive abilities, and which could present neuronal abnormalities due to subtle vascular-related pathological processes.

Conclusion

In conclusion, our study reinforces the possibility of a "vascular hypothesis" related to the pathology of AD. The association of cardiovascular risk factors and GM loss in both right parietal and temporal cortices, in association with the linear progression of regional GM loss in the right parietal cortex, may be another step in providing a rationale of how vascular factors might be associated with the development of non-familial AD. Furthermore, differently from the presence of APOE $\varepsilon 4$ allele, those vascular risk factors are potentially modifiable, leading to possible preventive strategies.

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