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H1 haplotype of the *MAPT* gene is associated with lower regional gray matter volume in healthy carriers

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The microtubule-associated protein Tau (*MAPT*) gene codes for the protein Tau that is involved in the pathogenesis of neurodegenerative diseases. Recent studies have detected an over-representation of the H1 haplotype of the *MAPT* gene in neurodegenerative disorders such as progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), frontotemporal dementia (FTD) and Parkinson's disease (PD), whereas the H2 haplotype has been found to be related to familial FTD. We aimed to investigate the association between *MAPT* haplotype status and brain morphology in healthy adults. A total of 150 healthy subjects underwent 3D high-resolution magnetic resonance (MR). MR images were processed following an optimized protocol to perform the Voxel-based morphometry (VBM) comparisons of the gray matter (GM) in H1 carriers ($n=141$) in contrast to H2H2 homozygous ($n=9$), and H1H1 homozygous ($n=85$) in contrast to H2 carriers ($n=65$). The threshold for statistical significance was 0.005 uncorrected. Opposite comparisons were also carried out. The groups had similar demographic and cognitive features. Compared with H2H2, the H1 carriers showed up to 19% smaller GM volume in the head of the right caudate nucleus, in the right middle frontal gyrus, in the left insula and orbito-frontal cortex, and in the inferior temporal and inferior cerebellar lobes, bilaterally. Compared with all H2 carriers, H1H1 displayed lower GM in the same regions, but the effect was smaller (5%), possibly due to a dilution effect by H1 in the H2 carriers group. The data suggest that H1 haplotype is associated with a particular cerebral morphology that may increase the susceptibility of the healthy carriers to develop neurodegenerative diseases such as sporadic tauopathies.

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Introduction

The microtubule-associated protein Tau (*MAPT*) gene codes for Tau, a phosphorylated protein mainly expressed in neurons where it assists in the stabilization of the cytoskeleton.¹ The cytoskeleton is the major intracellular determinant of neuronal morphology and provides the structural framework for communication between nervous cells; therefore, its integrity is critical for the function and

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survival of neurons. Neurofibrillary tangles of abnormally hyperphosphorylated Tau are a key lesion in Alzheimer's disease and a number of other neurodegenerative disorders such as frontotemporal dementia (FTD), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD),² that are today grouped under the concept of tauopathies.³

Within the *MAPT* gene two conserved haplotypes H1 and H2, comprising eight single nucleotide polymorphisms and a microsatellite marker, extend over the entire gene.⁴ These haplotypes mainly differ in transcriptional activity. *In vitro* studies suggested that the H1 haplotype might be more efficient in the *MAPT* gene expression than the H2 haplotype.^{5,6} Nonetheless, the most common H1 haplotype has also been found to be significantly over-represented in persons affected by sporadic tauopathies such as FTD,^{7,8} CBD,^{9–11} PSP^{4,12–17} and other neurodegenerative disorders such as Parkinson's disease (PD).^{18,19} In contrast, the H2 haplotype seems to be related to familial FTD^{20–22} and it has been reported to anticipate the age at onset of sporadic FTD.^{23,24} However, the role of *MAPT* regarding the fundamental disease process is still unclear.

Putative functions of Tau during normal brain development are unknown, which complicates the understanding of Tau in the disease process.¹ Studies investigating the effect of the *MAPT* haplotypes on normal brain structure are lacking. Aim of this study was to investigate the impact of the *MAPT* haplotypes on brain structure of healthy individuals.

Materials and methods

Subjects

This is a cross sectional study based on data acquired between March 2001 and March 2003 at the Neuroradiology Unit of the 'Città di Brescia' Hospital (Brescia, Italy) and selected by magnetic resonance (MR) and genotyping.

A total of 150 community-dwelling volunteers aged 40 years and older were enrolled in a study on normal brain structure with MRI aiming to capitalize on unnecessary scans in otherwise normal persons undergoing brain MR for reasons unrelated to cognition, as described in detail elsewhere.²⁵ Reasons for MR prescriptions included migraine, headache, auditory (hypoacusia, dizziness, tinnitus) or visual concerns (diplopia), sensory disturbances (paresthesias), syncope, or suspected cerebrovascular or orbital diseases.

Exclusion criteria were based on clinical information prior to scanning and MRI assessment by a neuroradiologist. *A priori* exclusion criteria were: (a) MR scan for memory problems or cognitive impairment; (b) MR scan for clinical suspicion of neurodegenerative diseases (eg PD, PSP, Huntington's disease, multiple system atrophy); (c) MR scan for suspected stroke and (d) history of transitory ischemic attacks (TIA) or stroke, head trauma, alcohol and

substance abuse, corticosteroid therapy and loss of weight greater than 5 kg in the last 6 months. *A posteriori* exclusion criteria included: (a) any abnormal brain mass; (b) white matter hyperintensities in a subject undergoing MR scan for suspected multiple sclerosis; (c) aneurysm larger than 10 mm; (d) arteriovenous malformations (except for developmental venous anomaly); (e) malformations of the central nervous system or (f) cognitive impairment on neuropsychological testing.

All 150 enrolled volunteers had normal MR scan on visual assessment by a neuroradiologist.

In addition to MRI, volunteers underwent a multi-dimensional assessment that included clinical, neurological and neuropsychological evaluations as well as blood sampling.

Volunteers gave informed consent. The study was approved by the local ethics committee.

Genetic analyses

Blood samples were collected from the subjects after obtaining informed consent, as approved by the local ethics committee. DNA was isolated according to standard procedures.

MAPT intragenic polymorphic markers were analyzed by PCR amplification followed by sequencing: sequence reactions were separated on DNA sequencer ABI Prism Genetic Analyzer 310 and analyzed using Sequence Navigator Software (Applied Biosystems, Monza, Italy). *MAPT* haplotypes were reconstructed as previously described.²⁰ Eight intragenic polymorphic markers in exons 1, 7 and 9 and introns 2, 3 and 13 were used to infer *MAPT* haplotypes: polymorphisms of exons 1 (g.75859 G>A), 7 (g.104964 G>A; P176P), 9 (g.109929, A227A; g.110013 T>C, N255N; g.110058 G>A, P270P) and of introns 2 (g.85372 C>T), 3 (g.87889 A>G) and 13 (g.137615 T>C) were analyzed. Genomic polymorphisms numbering (g.) is relative to AC091628.2 starting at nt 1. *APOE* genotyping was also carried out using a standard method.²⁶

MR scan acquisition protocol

3D high-resolution T1-weighted MR scan were acquired on a 1.0 Tesla scanner (Philips Gyroscan, Philips Medical Systems, Best, the Netherlands). MR scan were acquired with gradient echo 3D technique as follows: TR = 20 ms, TE = 5 ms, flip angle = 30°, field of view = 220 mm, acquisition matrix 256 × 256, slice thickness 1.3 mm.

Voxel-based morphometry

The Voxel-based morphometry (VBM) technique allows between-group comparisons of the local (voxel-wise) and global (lobar volumetry) gray and white matter (GM, WM). Briefly, the procedure involves spatial normalization of a high-resolution image to a single reference space and segmentation of brain tissues (GM, WM and cerebrospinal

fluid (CSF)) into classes.²⁷ The detailed procedure for local and global GM measurements is described below.

T1-weighted MR scans were processed with the Statistical Parametric Mapping-2 (SPM-2) software (<http://www.fil.ion.ucl.ac.uk/spm/software/spm2>) following an optimized protocol that included: (a) generation of a customized template representative of the whole population under study; (b) generation of customized prior probability maps; and (c) voxel-wise analysis on the GM images.²⁸ In order to aid normalization algorithm,²⁹ T1-weighted images were pre-processed by manually setting the anterior commissure as the origin of the spatial coordinates with MRIcro (<http://www.sph.sc.edu/comd/rorden/mricro.html>) by an investigator blinded to gene status.

Customized template All study MRs were linearly normalized to the Montreal Neurological Institute template³⁰ with SPM2 using a 12-parameter affine transformation. The linear normalization used a trilinear interpolation algorithm to resample images to a $2 \times 2 \times 2$ mm voxel size, a measure that was used in subsequent processing and analyses. After normalization images were smoothed using an 8 mm isotropic Gaussian kernel and then averaged to create a customized template for the whole group. Normalized MRs were visually checked for quality control.

Customized prior probability maps Normalized MRs created at the previous step were automatically segmented into GM, WM and CSF maps, and then smoothed with an 8 mm isotropic Gaussian kernel and averaged to create customized prior probability maps specific for each tissue class.³¹

Voxel-wise analysis The original MRs were normalized to the customized template through affine and non-linear transformations with a cut-off of 25 mm, medium regularization, 12 iterations and resampling to a $2 \times 2 \times 2$ mm voxel size. The normalized images were segmented into GM, WM and CSF tissue maps using the customized prior probability maps, and cleaned in order to remove extra-brain misclassified tissue. The segmented and cleaned images were modulated to preserve total volumes and then smoothed with a 12 mm FWHM isotropic Gaussian kernel to form tissue concentration maps that served as input to the voxel-wise statistical analysis.

Total intracranial volume (TIV) TIV was obtained by manual tracing of the entire intracranial cavity (the lower boundary being the foramen magnum) on coronal MRs (voxel size $1 \times 1 \times 7$ mm) using the software Display 1.3 (McConnel Brain Imaging Centre, Montreal Neurological Institute, McGill University, Montreal, Canada, <http://www.bic.mni.mcgill.ca/software>). To obtain this voxel size the images were transferred to a SUN Workstation and transformed from Digital Imaging and Communication in

Medicine – DICOM to mnc format. A resampling algorithm, included in the MINC package developed at the McConnel Brain Imaging Centre, was used to set the voxel dimension to $1 \times 1 \times 7$ mm for all images.

Lobar volumes analyses Lobar masks were traced with MRIcro (<http://www.sph.sc.edu/comd/rorden/mricro.html>) along the boundaries of the four main lobes and the cerebellum on the mean of the normalized MR template following a referenced atlas.³² A customized program (<http://www.jiscmail.ac.uk/cgi-bin/wa.exe?A2=ind02&L=spm&P=R176348&I=-1>) implemented in SPM2 (<http://www.fil.ion.ucl.ac.uk/spm/software/spm2>) was applied to the modulated GM and WM images that are 3D matrices in which the intensity of each voxel is proportional to GM and WM volume within each voxel. The program calculates volumes by adding the voxels of the segmented images times voxel volumes. ROI volumes were computed for each lobe and for the cerebellum by applying a binary mask to the segmented images. The volumes were computed as the sum of the voxel intensities within the pertinent masks times voxel volume. Total GM and WM separately and brain volumes have been obtained by summing up GM and WM volume of all lobes and cerebellar region.

Statistics

ANOVA and χ^2 -tests were used for sociodemographic features and lobar volumes, and Bonferroni *post hoc* test was used to underline the differences between groups, that were found using ANOVA. A general linear model was used to compare lobar and total GM, WM and brain volumes between groups using age, sex and TIV as covariates. Smoothed GM images were analyzed with an ANCOVA model ('Single Subjects: conditions and covariates' design) to detect regions with smaller GM volumes. The threshold for statistical significance was set at $P < 0.005$ uncorrected. Age, sex and TIV were included as nuisance variables. Prior to statistical analysis the data were not scaled, neither by a common factor (no grand mean scaling) nor to a same global value (no global normalization).

A default threshold of 0.8 was set to ensure that only GM voxels were included in the analysis by masking out voxels below the threshold. Finally, extracranial voxels were excluded from the analysis by masking out voxels that do not reach a threshold computed based on the mean voxel values. Subjects were grouped by genotype (H1H1, H1H2 and H2H2) and multiple *t*-test were performed: H1 carriers < H2H2 ($-1-12$); H1H1 < H2H2 (-101); H1H2 < H2H2 ($0-11$); H1H1 < H2 carriers (-211); H1H1 < H1H2 ($1-10$); H2H2 < H1H1 ($10-1$); H2H2 < H1H2 ($01-1$). The threshold for statistical significance was set at $P < 0.005$ uncorrected. To reach the aim of the study we considered as main contrasts those between H1 carriers compared to H2H2 and H2 carriers compared to H1H1.

Percent changes Percent changes were computed from the VBM values of the smoothed images by subtracting, in each voxel of the different regions found in the T map, the mean value of the group with more GM (adjusted for age, sex and TIV) minus the mean value of the group with minor GM (adjusted for age, sex and TIV) and dividing this difference by the mean value of the group with more GM. The percent changes have been computed for all positive contrasts.

Results

No differences were observed between the three experimental groups (*MAPT* H1H1, H1H2 and H2H2) in age, gender, education and MMSE (Table 1). The *MAPT* haplotype frequencies in the whole experimental group were as follows: *MAPT* H1H1: 56.7%; H1H2: 37.3%; H2H2: 6.0% (Table 1). No deviation from the Hardy–Weinberg equilibrium was observed ($P = 1.000000$, exact test).

No differences were observed for the $\epsilon 4$ allele distribution across groups ($P = 0.42$): 18 (21.2%) out of H1H1 carriers; 12 (21.4%) out of H1H2 carriers; 1 (11.1%) out of H2H2 carriers.

The total and lobar GM, WM and brain volumes, measures that are sensitive to diffuse changes, did not differ among groups (Supplementary Table 1). The VBM analyses, instead, denoted that even in the lack of diffuse changes H1 carriers did show focal differences, in terms of lower GM, in a number of regions compared with H2 carriers. In Table 2 the two main contrasts are shown. Contrasting H1 carriers to H2H2 we observed that H1 carriers had lower GM volume in the right head of the caudate nuclei, right middle frontal gyrus, left insula and orbital frontal cortex, and in the inferior temporal gyrus and inferior lobe of the cerebellum bilaterally (Table 2a; Figure 1a). GM reductions amounted of up to a 19% difference in the more significant clusters. Exactly the same pattern of local changes was observed in both the homozygous (peak cluster 1256 mm^3 , right head of caudal nuclei, $x 18, y 24, z 6, Z 3.78, P = 0.000$) and heterozygous forms (peak cluster 696 mm^3 , right head of caudal nuclei, $x 18, y 24, z 6, Z 3.65, P 0.000$) when contrasted to H2H2

separately (data not shown). GM reductions in these contrasts amounted of up to a 15% difference. The contrast between H1H1 and H2 carriers showed that H1H1 carriers had lower GM in the left insula, right head of caudate nuclei, orbital frontal cortex and inferior lobe of cerebellum (Table 2b, Figure 1b). This difference presented a maximum percent change of 5%. Moreover, the contrast H1H1 < H1H2 showed small GM regions of differences in the insula (peak cluster 440 mm^3 , $x 36, y 32, z 6; Z 3.62, P = 0.000$ uncorrected) presenting a maximum percentage change of 5%. The opposite comparisons were negative (H2H2 < H1 carriers; H2H2 < H1H1; H1H2 < H1H1; H2H2 < H1H2; H2 carriers < H1H1).

Discussion

In this study we have investigated the effect of the haplotypes H1 and H2 of the *MAPT* gene on normal brain structure. In the light of previous studies observing that these haplotypes are involved in neurodegenerative diseases, our main hypothesis was that they could affect the normal cerebral structure. We found that the H1 haplotype in both homozygous and heterozygous groups was associated with lower GM volume than the H2H2 haplotype in several regions: the head of the right caudate nucleus, the inferior temporal and middle frontal gyrus, the left insula and orbital frontal cortex and the inferior cerebellar lobe bilaterally, as detected by the VBM comparisons. In the main contrast, H1 carriers reached a percentage difference of up to 19% compared to H2H2. When H1 homozygous and heterozygous subgroups were contrasted with H2H2 separately the percentage difference observed was up to 15%. When H1H2 subjects were grouped with the H2H2 sample, H1H1 displayed lower GM in the same regions, but the effect was smaller (5%) and we believe this is due to the presence of H1 in the H2 carriers group. The small differences observed in the contrast between H1H1 and H1H2 (only 440 mm^3 , in the insula) could confirm this interpretation.

Interestingly, total, lobar GM and WM and brain volumetry failed to find significant differences among *MAPT* haplotypes, suggesting that the effect is remarkably focal rather than diffused.

Some of the regions characterized by smaller volume in the H1 carriers match those involved in neurodegenerative conditions where the H1 haplotype represents a risk factor. Specifically, FTD is characterized by cerebral atrophy and reduced glucose metabolism in rostral regions including orbitofrontal cortices^{33–35} and striatum.^{34–37} Reduced glucose metabolism has also been found in the cerebellar cortex³⁸ and cerebral atrophy in the anterior insula.³⁴ The atrophy found in PSP is similar to that found in FTD: frontal^{39–41} and temporal lobes,⁴¹ caudate nuclei^{39–40,42–45} and insula⁴⁶ seem to be affected. Glucose hypometabolism

Table 1 Demographic features of the three experimental groups

Carriers	H1H1 <i>n</i> = 85	H1H2 <i>n</i> = 56	H2H2 <i>n</i> = 9	<i>P</i> -value
Age (years)	53 ± 12	54 ± 13	54 ± 14	0.91
Gender (females) (%)	46 (54)	38 (68)	3 (33)	0.08
Education (years)	10 ± 4	10 ± 4	11 ± 4	0.51
Minimental state exam	29.1 ± 1.0	28.9 ± 1.1	29.0 ± 1.1	0.83

Numbers denote mean (SD) or number (%). *P*-value denotes significance on ANOVA or χ^2 .

Table 2 Regions showing gray matter volume reduction in different contrasts between groups

Cluster size (mm ³)	Region	Local maxima Stereotaxic coordinates (mm)				P<0.005 uncorrected
		x	y	z	Z	
(a) H1 carriers (n= 141) < H2H2 (9)						
1024	Right head of caudate nuclei	18	24	6	3.82	0.000
	Right head of caudate nuclei	14	26	-6	3.25	0.001
696	Left insula	-22	24	4	3.16	0.001
	Left orbital frontal cortex	-20	26	-8	2.81	0.002
	Left orbital frontal cortex	-28	26	-8	2.75	0.003
296	Right inferior temporal gyrus	36	-8	-46	3.08	0.001
224	Right inferior lobe of the cerebellum	46	-72	-48	2.95	0.002
	Right inferior lobe of the cerebellum	36	-76	-56	2.83	0.002
128	Right inferior lobe of the cerebellum	10	-60	-64	2.92	0.002
40	Right middle frontal gyrus	42	8	20	2.84	0.002
112	Right inferior temporal gyrus	20	6	-38	2.79	0.003
16	Left inferior lobe of the cerebellum	-40	-72	-56	2.74	0.003
8	Right head of caudate nuclei	16	10	18	2.59	0.005
8	Left inferior temporal gyrus	-44	20	-38	2.58	0.005
(b) H1H1 (n= 85) < H2 carriers (65)						
1424	Left insula	-24	24	2	3.37	0.000
	Left insula	-28	20	-6	3.18	0.001
512	Right head of caudate nuclei	22	22	6	3.29	0.001
128	Right inferior lobe of cerebellum	8	-60	-62	2.96	0.002
200	Right head of caudate nuclei	6	20	0	2.96	0.002
16	Right inferior lobe of the cerebellum	28	-86	-50	2.68	0.004
32	Right inferior lobe of the cerebellum	36	-74	-58	2.66	0.004
8	Right inferior lobe of the cerebellum	30	-84	-52	2.62	0.004
8	Right orbital frontal cortex	18	26	-10	2.60	0.005

Figure 1 (a) Lower GM in H1 carriers ($n=141$) compared with H2H2 homozygous ($n=9$) (coordinates: $x=18$, $y=24$, $z=6$ in MNI space) and (b) lower GM in H1H1 homozygous ($n=85$) compared with H2 carriers ($n=65$) (coordinates: $x=22$, $y=22$, $z=6$ in MNI space). $P<0.005$ uncorrected.

has been found in PSP in the frontal cortex and caudate nucleus.^{47,48} CBD does not have a pathognomonic pattern of atrophy, although one study did find atrophy in the head of the caudate nuclei.⁴⁹ However, studies with

FDG PET found that CBD presents reduced glucose metabolism in the frontal lobe and in the subcortical structures such as thalamus, caudate and putamen with marked asymmetry between the two hemispheres.^{50–52}

Based on these observations, we may hypothesize that the neurobiological process of these diseases might interact with a brain morphology conferring a specific vulnerability for diseases of the FTD spectrum in the H1 carriers.

With respect to this hypothesis, a very recent study by Hayesmoore and colleagues⁵³ demonstrated a strong correlation between age and relative expression of the H1 haplotype: with increased age, the expression of the *MAPT* H1 haplotype is suppressed as compared with H2, but the physiological significance of this finding, and therefore any possible connection with the neurodegenerative disorder, is still unknown. If this is true, the smaller GM volumes that we found in the H1 carriers may depend on their relatively advanced age (54 years), but further studies investigating the effects of this differential expression of H1 on brain morphology should be carried out.

Finally, six Tau isoforms are expressed in the adult human central nervous system, produced by alternative splicing of exons 2 (+, -), 3 (+, -) and 10 (+, -). Alternative splicing of exon 10 leads to a protein containing either three (3R; exon 10-) or four (4R; exon 10 +) tandem repeats of a microtubule-binding motif.⁵⁴ It has also been shown that in PSP and CBD patients, there is an increase in exon 10 + (4R) *MAPT* mRNA transcripts in regions of the brain most severely affected by neurodegeneration. Caffrey and colleagues⁵⁵ found that the H1 haplotype expresses significantly more exon 10 + (4R) *MAPT* mRNA transcripts than H2. The deviation in expression from the expected 1:1 ratio is greatest in the globus pallidus, a region of the basal ganglia severely affected by neurofilaments-tangles in PSP and CBD, and in frontal lobes. Interestingly towards H1, the authors also observed an increased transcript ratio for exon 10 + (4R) *MAPT* mRNA transcript on these areas in 13 out of 14 control brains.

Moreover, contrasting results in the current literature need to be discussed.

A number of studies found that H1H1 haplotype is over-represented in PSP,^{4,10,16,17} CBD¹⁰ and PD.¹⁹ Two studies showed an over-representation of H1 haplotype in FTD,^{7,8} whereas other studies fail to find any relation with the haplotypes and the risk for the disease.^{56,57} Therefore, H1 haplotype in both homozygous and heterozygous forms have been certainly found to be related to PSP, CBD and PD, but its relation with the risk of developing FTD is still debated. On the other hand, H2 haplotype has been found over-represented in the familial FTD but no studies show a relation between H2 and the risk for developing sporadic FTD. At present, two studies^{23,24} found an over-representation of H2 haplotype in patients presenting FTD at an early age. However, both these studies found similar distribution of H1 and H2 haplotypes so they did not find increased risk of FTD associated with H2 haplotype. This lack of association was supported by the findings of Ghidoni and colleagues²⁰ who reported no association with sporadic FTD. Law and colleagues²⁴ demonstrated that patients who

develop an early onset disease and have an over-representation of H2 are those with more hypometabolism in the frontal regions, but as Laws and colleagues comment the observed hypometabolism did not reflect the clinical symptoms and the severity of the disease, but it could reflect a more aggressive course of the disease without mediating risk for the disease itself. They conclude that H2 could be a modulator of the disease once the neurodegenerative process has started. We can hypothesize that risk and progression factors reflect two separate mechanisms involved in the disease.

However, our data should be considered as explorative, and a number of limitations need to be highlighted. Our conclusions for H2 homozygous carriers are based only on a small number of individuals who were available and therefore it is limited. Moreover, the total group was not sufficiently large to carry out deeper investigation regarding other possible confounding variables, such as an independent effect of the ApoE/ε4 allele. Of particular interest is the hypothesis linking age with a differential expression of H1, but the effect of this interaction on brain morphology definitely requires the availability of larger samples. It will be also interesting to study the age effect in H1 carriers within a longitudinal approach. Moreover, studies using functional neuroimaging could be more sensitive to detect the H1 effect on healthy brains. Finally, the role of the cerebellum in the diseases involving H1 as well as its specific relation to rostral cortices in healthy humans is still unclear. Future studies using diffusion tensor imaging may help to define the fiber tracts joining this region with the rostral cortex and their relevance to the behavioral and cognitive domains.

Conclusions

This is the first study investigating the influence of the haplotypes of the *MAPT* gene on the brain structure of healthy individuals. These data suggest that the H1 haplotype is associated with a particular cerebral morphology that may increase the susceptibility of the healthy carriers to develop neurodegenerative diseases such as sporadic tauopathies. However the results of this study should be considered as explorative; a replication of these data on a larger sample with attention to the influence of other genes, such as ApoE, need to be carried out.

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